

METHOPRENE AS A POULTRY FEED ADDITIVE
FOR HOUSE FLY CONTROL

by

Gary Clinton Breeden

Dissertation submitted to the Graduate Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Entomology

APPROVED:

E. C. Turner, Jr., Chairman

W. H. Robinson

J. L. Eaton

L. T. Kok

A. L. Buikema

J. E. Roberts, Sr.

September, 1976

Blacksburg, Virginia

ACKNOWLEDGMENTS

I would like to extend my appreciation to the following persons for their encouragement and guidance throughout the period of study and in the preparation of this manuscript: Drs. W. H. Robinson, J. E. Roberts, Sr., J. L. Eaton, and L. T. Kok of the Entomology Department; Dr. A. L. Buikema of the Biology Department, and especially to Dr. E. C. Turner, Jr. of the Entomology Department who served as my graduate advisor.

The following persons deserve credit and my thanks for technical assistance in gathering data for this manuscript: Robert A. Barlow, Deborah J. Smith, Peter J. Egan, Lee H. Townsend, Arthur F. Buckman, Cecil H. Kessinger, and especially Stephen W. Bullington who identified almost all of the insects collected. Dr. R. J. Gagne, Systematic Entomology Laboratory, ARS, USDA, deserves credit and my thanks for the identification of several species of Cecidomyiidae. Walter I. Knausenberger also deserves credit and my thanks for the identification of several species of Chironomidae.

I wish to thank the following persons for their cooperation and for providing the necessary facilities and laying hens for the field tests: Paul Wagner of Dutt and Wagner Eggs for the use of the laying hens in 1974; Vann Reynolds of the S & M Milling Co., Inc. for the use of the caged-layer houses in 1974 and for mixing the feed in 1974; Sterling Carter of Grassy Knoll Enterprises for the use of the laying hens in 1975 and for providing the gratifying services of Henry Dodson

of the Elkcar Feed Mill for mixing the feed in 1975; Dan Parrish of the Parrish Egg Farm for the use of a caged-layer house in 1975, and Dave Florey for the use of a caged-layer house in 1975. Dr. Winston L. Beane of the Poultry Science Department also kindly provided the necessary facilities and laying hens for the laboratory bioassays in 1974 and 1975.

I wish to express my special thanks to W. H. Palmer, Eastern Field Research Supervisor, Zoecon Corp., for his encouragement and advice and to Dr. J. McD. Grayson, Department Head of Entomology, for his financial and professional support and guidance throughout the period of study.

I am especially indebted to my wife, Linda, for her help and encouragement and for the typing of this manuscript.

This research was supported in part by grants from the Zoecon Corp., Palo Alto, California.

TABLE OF CONTENTS

	<u>Page</u>
INTRODUCTION	1
LITERATURE REVIEW	5
MATERIALS AND METHODS	
1974 Site Locations and Operations	12
1975 Site Locations and Operations	15
Feed Treatment	22
1974 Season Treatment Schedule	23
1975 Season Treatment Schedule	24
1974 Season Sampling Procedures and Techniques	24
1975 Season Sampling Procedures and Techniques	26
Residue Sampling and Analysis	28
Resistance Bioassays	29
Statistical Analysis	30
RESULTS AND DISCUSSION	
1974 Season Treatment Effect on House Fly Emergence	32
1975 Season Treatment Effect on House Fly Emergence	41
1974 Season Treatment Effect on Adult House Fly Populations	50
1975 Season Treatment Effect on Adult House Fly Populations	66
SUMMARY	82
LITERATURE CITED	83
APPENDIX	89
VITA	122

LIST OF TABLES

<u>Table</u>		<u>Page</u>
1	Total number of house fly adults that emerged and mean emergence percentages \pm SD from the six emergence core samples on each sample date for both treated and untreated houses in 1974.	33
2	Emergence percentages of a susceptible lab-reared and the 1974 field-pressured strain of house flies from manure from chickens fed methoprene treated feed.	39
3	Total number of house fly adults that emerged and mean emergence percentages \pm SD from the six emergence core samples on each sample date for both treated and untreated houses in 1975.	42
4	Emergence percentages of a susceptible lab-reared and the 1975 field-pressured strain of house flies from manure from chickens fed methoprene treated feed.	47
5	Number of adult house flies caught in each UV light trap in both treated and untreated houses for each sample date in 1974	50
6	Number of adult house flies caught in each UV light trap in both treated and untreated houses for each sample date in 1975	65
<u>Appendix</u>		
1	The insects and their seasonal totals caught in the UV light traps in both houses in 1974	90
2	The insects and their seasonal totals collected from the emergence core samples from both houses in 1974.	97
3	The insects and their seasonal totals collected from the Berlese core samples from both houses in 1974.	99
4	The insects and their seasonal totals caught in the UV light traps in both houses in 1975	101

Table

Page

5	The insects and their seasonal totals collected from the emergence core samples from both houses in 1975.	107
6	The insects and their seasonal totals collected from the Berlese core samples from both houses in 1975.	109

LIST OF FIGURES

<u>Figure</u>		<u>Page</u>
1	Skeleton formula of methoprene.	4
2	The chicken houses used in 1974	13
3	The inside of a caged-layer house	14
4	Scraping blade designed to clean two manure pit rows simultaneously	16
5	The back of the untreated house used in 1974.	17
6	The treated house used in 1975.	18
7	The untreated house used in 1975.	19
8	View from the inside of the untreated house used in 1975 showing a row of opened windows along one side of the house	20
9	Mean house fly emergence percentages on each sampling date for the untreated and treated houses in 1974.	36
10	Mean house fly emergence percentages on each sampling date for the untreated and treated houses in 1975.	45
11	Total number of adult house flies caught in the UV light traps on each sampling date in the untreated and treated houses in 1974.	55
12	Mean house fly emergence percentages and total number of adult house flies caught in the UV light traps on each sampling date in the untreated and treated houses in 1974	58
13	Mean temperatures inside and outside the treated house and the total number of adult house flies caught in the UV light traps on each sampling date in the untreated and treated houses in 1974.	62

<u>Figure</u>		<u>Page</u>
14	Total number of adult house flies caught in the UV light traps on each sampling date in the untreated and treated houses in 1975.	70
15	Mean house fly emergence percentages and total number of adult house flies caught in the UV light traps on each sampling date in the untreated and treated houses in 1975.	73
16	Mean temperatures inside and outside the treated house and total number of adult house flies caught in the UV light traps on each sampling date in the untreated and treated houses in 1975.	76

Appendix

1	Total number of <u>Psychoda alternata</u> adults caught in the UV light traps on each sampling date in the untreated and treated houses in 1975.	113
2	Total number of <u>Psychoda satchelli</u> adults caught in the UV light traps on each sampling date in the treated house in 1975	115
3	Total number of <u>Polypedilum scalaenum</u> adults caught in the UV light traps on each sampling date in the treated house in 1975	117

INTRODUCTION

At present, over 65 percent of all commercial laying hens in the U.S. are housed in cages suspended over the floor. In California, the leading egg producing state, over 90 percent of the laying hens are housed in these cages (Card and Nesheim 1972). This allows manure with a moisture content of approximately 70 percent (Hart 1963) to accumulate under the cages and provides an ideal rearing medium for the larva of the house fly, Musca domestica L. (Bailey et al. 1968). Consequently, during warmer months house fly adults usually emerge in such large numbers that some form of control is necessary (Matthysse and McClain 1973).

The average time from egg to adult for the house fly is 10 days (James and Harwood 1969). An obvious method of control would be removal of the manure on a weekly basis. However, this is not often practical because of the time required for weekly manure removal and disposal. Even in a small house containing 15,000 caged layers, approximately 9,525 Kg (10.5 tons) or 9.8 m³ (12.8 cu. yds.) of wet manure will accumulate in 1 week (Hart 1963). Thus, if weekly manure removal is impractical, other methods of control are necessary.

Larviciding with currently recommended insecticides is convenient and frequently practiced by egg producers (Axtell 1968; Wicht and Rodriguez 1970). However, this method is discouraged because of the hazard of increasing the rate of resistance development and because these treatments have a more deleterious effect on the predators and

parasites, particularly the predatory mites, than on the house fly (Anderson et al. 1968; Axtell 1968; Georghiou et al. 1967). Manure removal is also more deleterious to the predators and parasites than to the house fly and, thus, favors house fly development (Peck and Anderson 1970). In addition, the process of manure removal appears to attract additional house fly adults to the premises (Anderson 1965). Therefore, fly control strategies advocated by Anderson (1965) and Axtell (1970) called for letting the manure accumulate throughout the fly season while directing insecticide treatments at only the adults by using residual wall and ceiling sprays and treated baits.

Matthysse and McClain (1973) found that even in houses with large amounts of accumulated manure adulticiding alone was not often effective. They found that larviciding or larviciding plus adulticiding was more effective, but application of these treatments was usually required every 2 weeks. A strategy by Wicht and Rodriguez (1970) also advocated the use of larvicides. They specified the use of selective larvicides which would effectively control the house fly larvae but cause little reduction in the predatory mite populations. In their study, no conventional insecticide was found to satisfy both criteria when tested in the field. Axtell (1968) was also unable to find a conventional insecticide which satisfied both criteria in the field.

In 1967 Williams advocated the use of insect juvenile hormones (JH) and their analogs (JHA) as third-generation pesticides for the control of insects. Since then, continued research and development (Slama 1971; Menn and Beroza 1972; Slama et al. 1974; Staal 1975) have

steadily increased the practicality of using JHA to control insects. The use of effective JHA appears ideally suited for the control of house flies breeding in chicken houses. JHA apparently have no ill effects on mites (Staal 1972). In addition, it was suggested by Williams (1956, 1967) and believed by many that insects would be unable or at least would find it difficult to develop resistance to these compounds. Therefore, it appeared that JHA could safely be applied directly to the manure without causing a direct reduction in the predatory mite populations or a rapid development of resistance.

Methoprene (Fig. 1) is a JHA developed by the Zoecon Corp., Palo Alto, California (Henrick et al. 1973). At the time of this study, it was the only JHA with sufficient commercial development and appropriate experimental registration from the Environmental Protection Agency to allow large scale field testing. The primary objective of this study was to evaluate the efficacy of methoprene as a poultry feed additive for control of the house fly breeding in chicken manure in a commercial caged-layer house.

In addition to the house fly, the populations of the other insects collected were also monitored in an attempt to evaluate the effect of this treatment on non-target insects. These data are presented in the appendix.

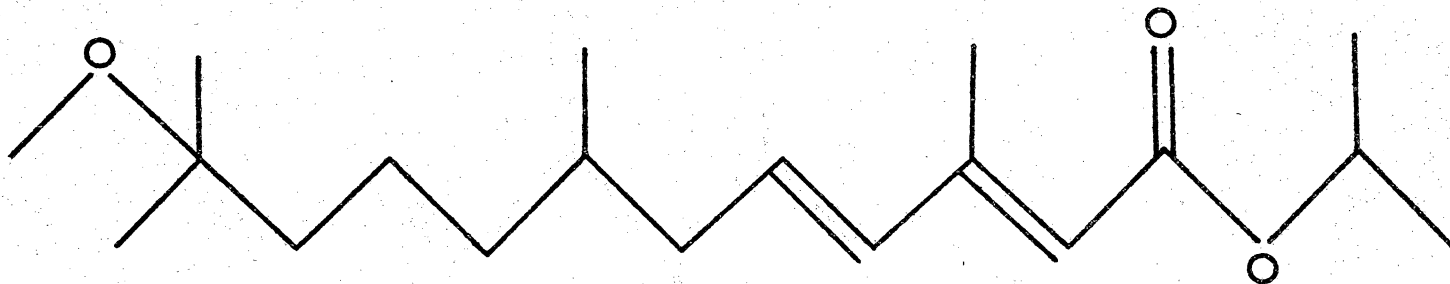


Fig. 1. Skeleton formula of methoprene, Altosid[®], ZR 515, isopropyl 11-methoxy-3,7,11-trimethyl-2 trans, 4 trans-dodecadienoate.

LITERATURE REVIEW

Methoprene has a high degree of JH activity to Diptera (Henrick et al. 1973; Staal 1975). In most cases, methoprene is superior in JH activity to the known natural JH and other JHA against Diptera (Henrick et al. 1973). This superiority is largely a result of greater stability in the field and in vivo. Unlike the known JH and most other JHA, methoprene does not require a terminal epoxide to produce good activity. These terminal epoxides are one of the least stable structural groups in the field and in vivo (Henrick et al. 1973; Staal 1975). In addition, compared to the ester groups of other Alkyl 3,7, 11-trimethyl-2,4-dodecadienoates, the isopropyl ester of methoprene appears also to allow greater stability in vivo (Yu and Terriere 1975).

The use of methoprene and other JHA for insect control involves the substitution for or mimicry of the effect of the insect's own JH at sensitive periods when JH should either not be present or present at a low titer within the insect (Slama 1971; Schneiderman 1972). Exogenous sources of JH or JHA thus applied at appropriate doses to immature insects usually cause a detrimental and often fatal morphogenetic and developmental disruption (Schneiderman 1972; Staal 1972, 1975). Ovicidal effects and disruption of embryogenesis have also been demonstrated with JHA, but these effects have not been especially successful with Diptera (Staal 1975). However, Morgan et al. (1975) have reported some house fly sterility with methoprene.

The exact mode of action of JHA, like that of JH, is not known. However, the basic theories concerning this mode of action were summarized by Slama et al. (1974). These theories all suggest that JH acts during the sensitive periods to inhibit or prevent cells from expressing the genome necessary for the synthesis of the new characters needed for the next instar or stage. The apparent reasons for the occurrence of the disruptive activity and why it is limited to certain sensitive periods have been summarized by Schneiderman (1972). In Endopterygota, the sensitive periods occurring just before and early in the pupal stage coincide with the beginning of the cell division and differentiation initiated by ecdysone for metamorphosis. Once these activated cells have replicated their DNA, they are no longer susceptible to any inhibitory action from JH. However, those activated cells which have not yet replicated their DNA are susceptible. Normally, during this period JH is not present in a titer high enough to cause any interference or inhibition of these cells. However, when appropriate doses of JH or JHA are exogenously applied shortly before or during this process, these susceptible cells are prevented from differentiating into the next stage. It is this combination of unaffected, newly differentiated cells and the affected, old undifferentiated cells which is believed to cause the detrimental and insecticidal effects.

The house fly's most sensitive period begins in the latter half of the last larval stadium, when the larva has ceased feeding and begun migrating, and ends with the tanning of the puparium (Staal

1972, 1975). Srivastava and Gilbert (1968) have shown that this period could be slightly longer if the JHA could penetrate the tanned puparium. When methoprene is exogenously applied at appropriate doses to the house fly during this period, the adult fails to emerge from the puparium and dies (Staal 1975).

Methoprene is an extremely powerful inhibitor of house fly emergence when applied topically during this sensitive period (Cerf and Georghiou 1972, 1974; Plapp and Vinson 1973; Yu and Terriere 1975; Henrick et al. 1975, 1976). Doses as low as 0.0033 and 0.0063 ug/prepupa produced 50 and 95 percent inhibition, respectively, for the most susceptible strain (Cerf and Georghiou 1974). Mixed with rearing media, concentrations as low as 10 ppm (active ingredient = AI) gave almost complete inhibition (Jakob 1973). However, field tests with methoprene as a surface spray for control of house flies breeding in chicken manure yielded only marginal inhibition (personal communication, Zoecon Corp.; Morgan et al. 1975). These failures were attributed to inadequate penetration and distribution of methoprene into the manure, and it was hypothesized by Zoecon personnel that its use as a feed additive might insure adequate distribution (personal communication, Zoecon Corp.). This feed additive method has proved feasible for conventional insecticides with much higher avian and mammalian toxicities (Miller 1970). In addition, there is little need for concern over the safety of methoprene as a feed additive because it is so biodegradable that it is apparently metabolized as another food source by chickens (Quistad et al. 1976) and bovines (Chamberlain

et al. 1975; Quistad et al. 1975a, b). Indeed, in bovines this degradation is so complete that approximately 15 percent of the ^{14}C labeled (C-5 position) oral dose was recovered as CO_2 from the respiration of the animal (Chamberlain et al. 1975).

Morgan et al. (1975) conducted laboratory bioassays on manure collected twice weekly from chickens fed a technical formulation of methoprene for periods of up to 7 weeks. The results from these bioassays showed an overall average of 71 and 99 percent control of house flies with rates of 50 and 100 ppm (AI in feed), respectively. Breeden et al. (1975) conducted laboratory bioassays on manure collected daily from chickens fed a technical formulation of methoprene for 7 days. The results from these bioassays showed that house fly emergence was reduced to 4 percent or less with: 1) a 50 ppm rate (AI in feed) after the chickens were fed treated feed for 3 days or, 2) a 100 ppm rate after the chickens were fed treated feed for only 1 day. Even the 50 ppm feeding rate is not economically feasible for house fly control when compared to currently recommended conventional insecticides. Consequently, an encapsulated, dry premix formulation of methoprene was developed by Zoecon personnel to reduce the amount of methoprene lost to the chicken's metabolism. This loss was found to be quite extensive. Only 6.4 percent of an applied oral dose of technical formulation reached the manure of the chicken intact (Quistad et al. 1976). The encapsulation was accomplished by treating a common carrier particle with methoprene and then coating it with a lignin

complex keyed to release at the alkaline pH encountered in the lower gut of a chicken (personal communication, Zoecon Corp.).

Breeden et al. (1975) conducted laboratory bioassays on manure collected every other day from chickens fed the encapsulated formulation of methoprene for 10 days. The results from these bioassays showed that the encapsulation had apparently reduced the effective treatment rates by a factor of 10. House fly emergence was reduced to 8 percent or less with: 1) 5 ppm rate (AI in feed) after the chickens were fed treated feed for 8 days, or 2) a 10 ppm rate after the chickens were fed treated feed for only 2 days. These rates are considered economically feasible for house fly control when compared to currently recommended conventional insecticides (personal communication, Zoecon Corp.).

All of the doses, concentrations, and rates listed above are from tests on susceptible strains of house flies. Laboratory studies conducted with house fly strains resistant to various conventional insecticides have shown that many of these strains were also cross-resistant to methoprene (Cerf and Georghiou 1972, 1974; Plapp and Vinson 1973; Jakob 1973; Yu and Terriere 1975; Miller and Collins 1975; Morgan et al. 1975). The precise mechanisms for this cross-resistance are unknown. However, the strains which showed the highest cross-resistance to methoprene were organophosphate resistant strains with high levels of microsomal oxidase activity (Cerf and Georghiou 1974; Plapp and Vinson 1973). The dimethoate resistant strain tested by Cerf and Georghiou (1974) imparted the most cross-resistance to

methoprene. This strain imparted 39.4- and 150.8-fold resistances at the LD₅₀ and LD₉₅ values, respectively, over a susceptible strain. The association between high microsomal oxidase activity and cross-resistance to methoprene is understandable because the microsomes apparently play an important part in the degradation of the insect's own JH (White 1972; Ajami and Riddiford 1973; Terriere and Yu 1973; Yu and Terriere 1974a). Also, microsomal oxidase activity in the house fly undergoes a large increase in activity just prior to puparium formation. This increased activity lasts about 15 hours and is preceded and followed by long periods of barely detectable activity (Yu and Terriere 1971). In addition, when microsomal oxidase activity is either induced (Yu and Terriere 1973) or inhibited (Yu and Terriere 1974b) by modifiers, the resulting changes in the insect's development and reproduction are typical of hormone imbalances. The best evidence for a connection between microsomal oxidase activity and cross-resistance to methoprene was reported by Yu and Terriere (1975). They found that the microsomal oxidases of two resistant house fly strains, which had higher microsomal oxidase activity than two susceptible strains, metabolized methoprene 1.7 to 3 times faster than those of the latter. In addition, when a strong inducer of microsomal oxidase activity was added, the oxidative metabolism of methoprene was greatly enhanced. Quistad et al. (1975c) also found that besides degradative detoxification by the house fly, another major mode of detoxification of methoprene is biological isomerization of the 2-ene double bond from trans to cis. The house fly is also apparently unable to

isomerize the 2-ene bond back from cis to trans. The mechanism of the isomerization in the house fly is unknown. However, the resulting cis isomer has approximately 214 times less biological activity in the house fly than the trans isomer (Henrick et al. 1975).

MATERIALS AND METHODS

1974 Site Locations and Operations

Two adjacent controlled-environment chicken houses (Fig. 2) were selected. The houses were located in Montgomery County, Virginia approximately 13 miles southwest of Blacksburg. The larger house (110 m x 14 m, left house in Fig. 2) contained approximately 15,500 caged Leghorn layers and was selected for treatment with methoprene. The adjacent house (91.5 m x 14 m, right house in Fig. 2) contained approximately 12,500 caged Leghorn-Rhode-Island Red layers and served as an untreated check.

In both houses the laying hens were arranged in four long parallel rows of cage systems which extended almost the entire length of the house. The cage-system rows were separated by 1.2 m (4 ft.) wide concrete aisles raised approximately 15.2 cm (6 in.) above the floor immediately under the cage-system rows (Fig. 3). Each cage-system row was divided into two different tiers (Fig. 3). Each tier had two long rows divided into many small three-hen cage units. These tier rows were positioned back to back so that the front of a tier row faced an aisle on one side of a cage-system row (Fig. 3). The cage-system rows were suspended from the ceiling so that the bottom tier of hens was about 1.2 m above the floor (Fig. 3).

Manure accumulated directly under the cage-system rows in shallow pits (15.2 cm below the level of the concrete aisles, Fig. 3). The floor of these pits was dirt in the treated house and concrete in the



Fig. 2. View of the chicken houses used in 1974.

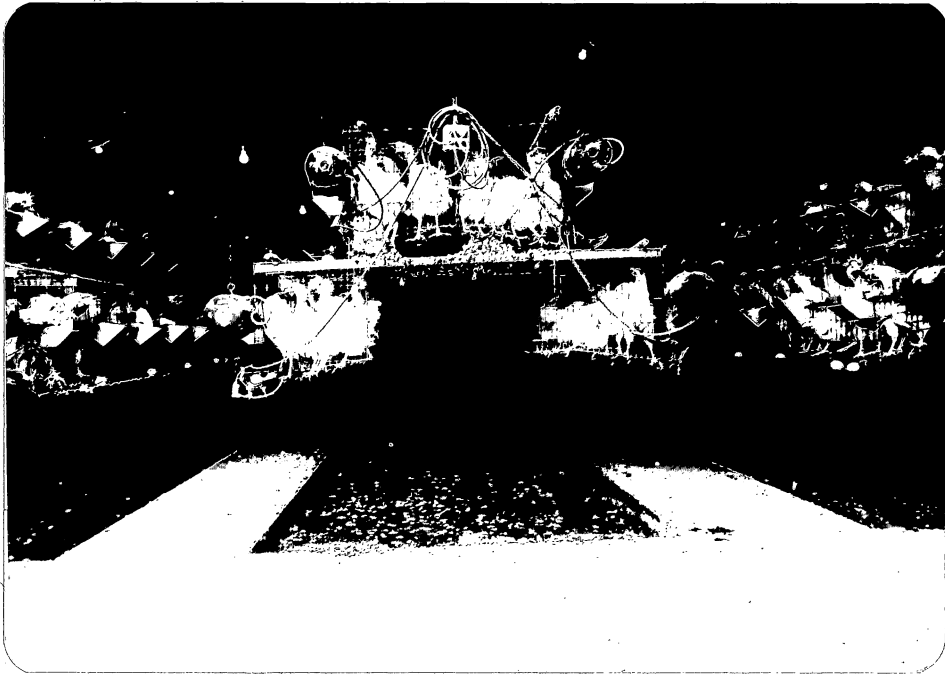


Fig. 3. View of the inside of a caged-layer house showing the end of a cage-system row and the floor structure of the house.

untreated house. Manure was removed from both houses by a small tractor equipped with a blade designed to push manure out while cleaning two pit rows at a time (Fig. 4). The backs of both houses (Fig. 5) were originally designed to allow the manure to be pushed into a waiting truck or manure spreader. However, the present farm manager pushed the manure out the back and let it accumulate. During the test period, manure was removed from the treated house on 9 July and 13 Aug. and from the untreated house on 12 July and 28 Aug. This removal schedule was at the convenience of the farm manager and similar to schedules he has used in previous years.

Both of these houses were taken out of production and permanently closed in Dec. 1974.

1975 Site Locations and Operations

A controlled-environment chicken house (97.5 m x 12.2 m, Fig. 6) located approximately 2 miles northwest of Verona, Virginia was selected for treatment with methoprene. The house contained approximately 17,500 caged Leghorn layers. The hens were housed in four parallel rows of cage systems similar in construction to those in the houses used in the 1974 field trial.

A modified controlled environment house (22 m x 12.2 m, Fig. 7) located approximately 6 miles northeast of the treated house was used as an untreated check. The check house differed from the other houses by having a row of windows on both sides (Fig. 8). These windows were removed in the summer for added ventilation. Screening was placed over

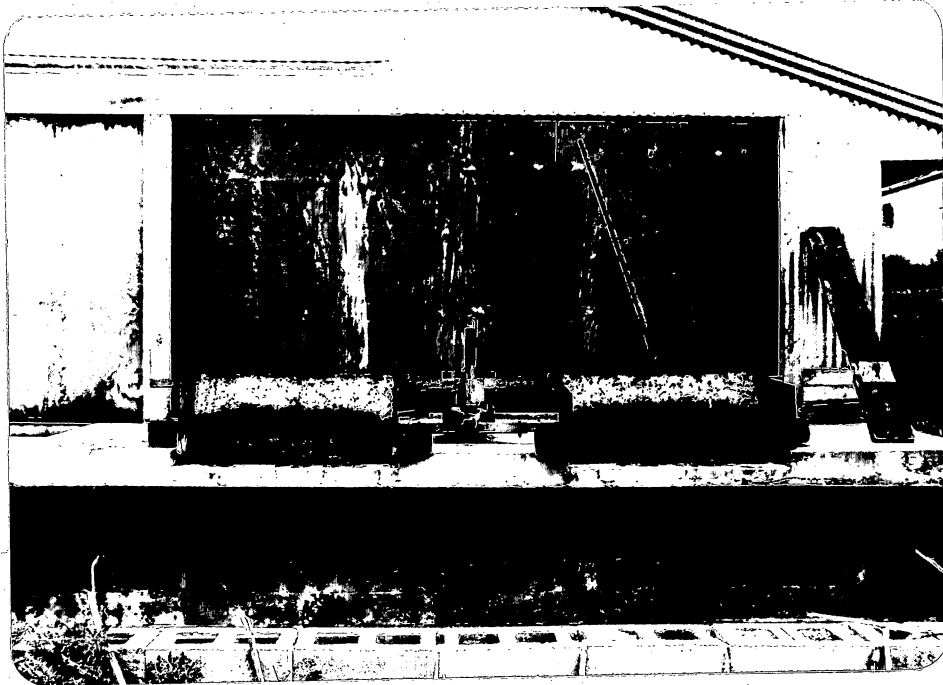


Fig. 4. View of the scraping blade designed to clean two manure pit rows simultaneously.

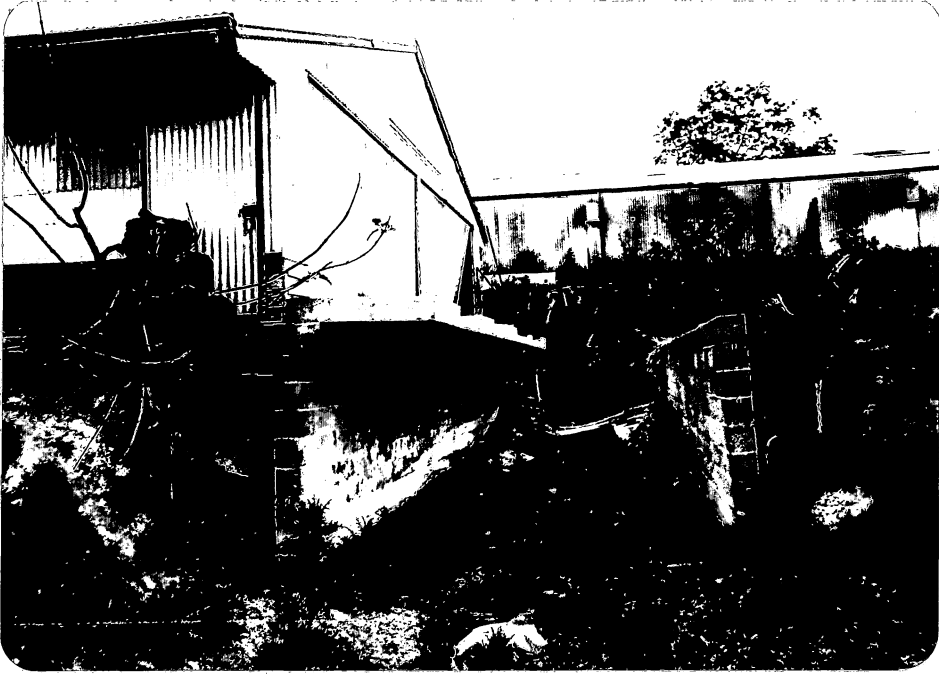


Fig. 5. View of the back of the untreated house used in 1974.

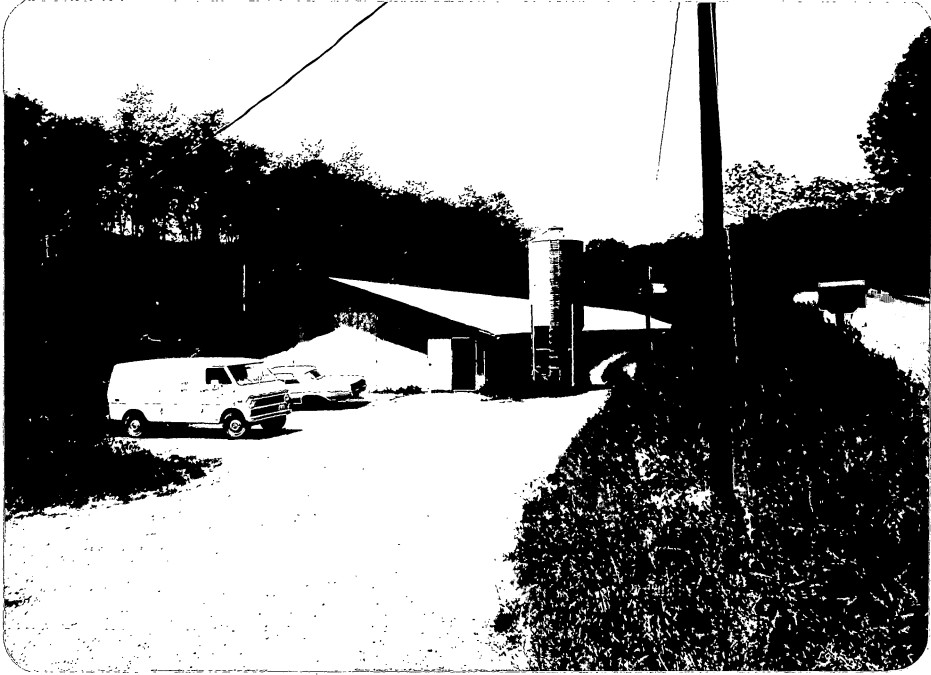


Fig. 6. View of the treated house used in 1975.

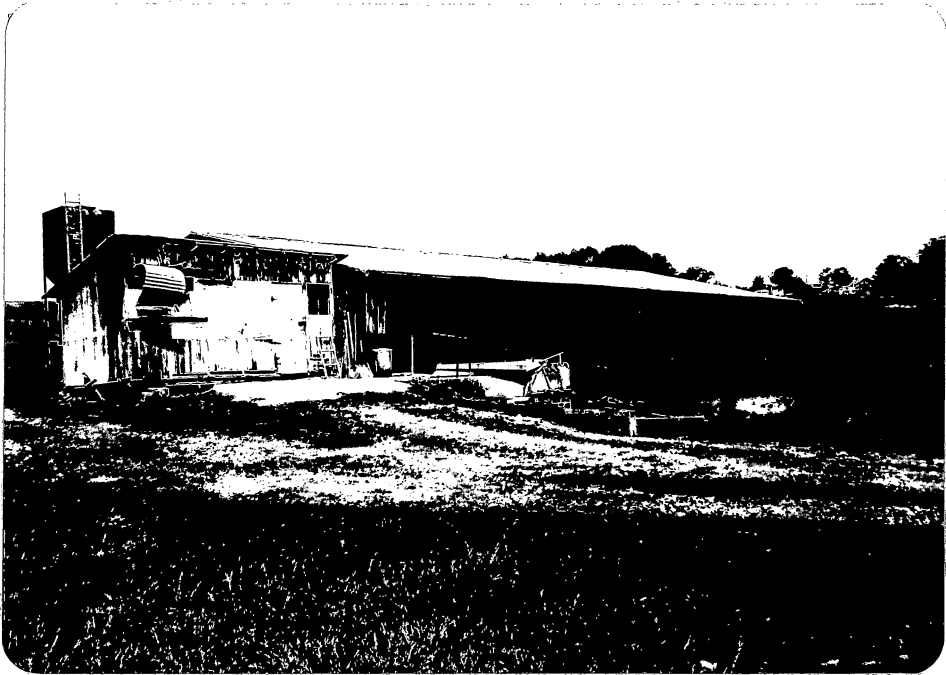


Fig. 7. View of the untreated house used in 1975.



Fig. 8. View from the inside of the untreated house used in 1975 showing a row of opened windows along one side of the house.

the windows to prevent flies from entering or leaving. The house contained approximately 4,500 caged Leghorn layers. The hens were housed in five parallel rows of cage systems similar in construction to those in the houses used for the 1974 field trial.

In both houses, the height of the cage systems off the floor, manure collection pits, and floor construction were similar to those in the untreated house used in 1974. Manure in both houses was removed by tractors and blades similar to the one used in 1974 (Fig. 4). In both houses, manure was pushed to an auger built into the floor in the front of the houses and then into a waiting manure spreader. The manure was then hauled to neighboring farms and used as fertilizer. During the test period, manure was removed from the treated house on 24 June, 15 July, 22 July, 5 Aug., 19 Aug., 26 Aug., and 11 Sept., and from the untreated house on 16 Aug. and 13 Sept. The removal schedule for the treated house is the result of an agreement with the owner. The owner preferred weekly manure removals and was very reluctant to allow the manure to accumulate for more than 2 weeks because of the additional strain on his removal equipment. Therefore, for the purposes of this study, it was agreed to allow the manure to accumulate for an initial pretreatment period of 3 weeks and thereafter for periods of 2 weeks unless I requested a shorter duration. The removal schedule from the untreated house was at the convenience of the owner and similar to schedules he has used in previous years.

Feed Treatment

The feed used in both the 1974 and 1975 seasons was a commercial laying mash similar in mixture to the feed used and described in preliminary laboratory bioassays (Breedon et al. 1975). The feed was treated with the PS-10 formulation of methoprene (Altosid[®]) supplied by the Zoecon Corp., Palo Alto, California. The PS-10 formulation is an encapsulated, dry premix formulation, described previously, containing approximately 10 percent AI.

Treated feed for the 1974 season was prepared by premixing the appropriate amount of methoprene (17.0 g AI) with 100-lb. (45.4 Kg) amounts of laying mash in a model H600 Hobart bakery mixer. These 100-lb. amounts were delivered fresh each week to the S & M Milling Company, Inc. in Christiansburg, Virginia. There, each 100-lb. amount was then mixed with 4,900 lbs. of laying mash in a 5,000-lb. capacity Ford mill. The resulting feed contained 7.5 ppm (AI) of methoprene as prescribed by Zoecon personnel. This treated feed was then delivered to the farm by employees of the feed mill.

Treated feed for the 1975 season was prepared by premixing the appropriate amount of methoprene (27.2 g AI) with 100-lb. amounts of ground corn in a model 4CME Stone cement mixer. These 100-lb. amounts were delivered fresh each week to the Elkcar Feed Mill in Madison, Virginia. There, each 100-lb. amount was then mixed with 5,900 lbs. of laying mash in a 6,000-lb. capacity Circle mill. The resulting feed contained 10.0 ppm (AI) of methoprene. This rate was also prescribed

by Zoecon personnel. This treated feed was then delivered to the farm by employees of the feed mill.

1974 Season Treatment Schedule

Sufficient amounts of the PS-10 formulation of methoprene were not available from the Zoecon Corp. until 1 July. The 7.5 ppm feed was then delivered to the farm on 3 July. Because some untreated feed remained in the feed storage bin and because of the cycle time through the hens, the methoprene probably did not reach full potency in the manure until 9 July. The hens were fed treated feed continuously until 1 Oct.

Some additional insecticide treatments were applied to help reduce the adult house fly populations. These treatments were not part of any design but were applied at the insistence of the farm manager. On 7 July the farm manager, acting on his own, sprayed the manure in the treated house with a 1 percent diazinon larvicide, and on 31 July he spread approximately 6 lbs. (2.7 Kg) of a Purina[®] malathion house fly bait along the concrete aisles of the treated house. The farm manager finally agreed that if he believed further treatments were necessary in the treated house, I would be the one to apply them. Subsequently, on 6 Aug., 23 liters (6 gal.) of a 1.5 percent malathion spray solution mixed with 2 lbs. of sugar was applied to the walls and common night resting areas of the house fly adults in the treated house. No further treatments were applied in the treated house. On 6 Sept. the farm manager spread approximately 6 lbs. of a malathion house fly bait

(Purina) along the concrete aisles of the untreated house. No other treatments were applied in the untreated house.

1975 Season Treatment Schedule

The 10 ppm feed was delivered to the farm on 17 July. However, because some untreated feed remained in the feed storage bin and because of the cycle time through the hens, the methoprene probably did not reach full potency in the manure until 21 July. Treatment was scheduled to be continuous until 23 Sept. Untreated feed was fed to the treated hens from 19 Aug. to 23 Aug. because a local corn shortage required 13,000 lbs. of feed to be delivered to the house from another feed mill. The resulting mixed manure was removed on 26 Aug. No additional insecticide treatments were applied to either house.

1974 Season Sampling Procedures and Techniques

Unavoidable delays in parts needed for the construction of UV light traps prevented sampling before 2 July. Then, before the scheduled pretreatment samples could be taken, the farm manager sprayed the manure in the treated house on 7 July with a 1 percent diazinon larvicide. Because of this, no pretreatment samples were taken, and sampling did not begin until 19 July. Sampling was then continued twice a week every Tuesday and Friday until 1 Oct.

Six pairs of manure core samples were collected from each house on every sampling interval. The core samples were taken by plunging a 0.3 liter (10 oz.) capacity wax cup (model 12SLN3 Tulip[®], Nestrите[®]);

6.5 cm deep with 10 cm diameter at top, and 8.5 cm diameter at bottom) into the manure and then inverting the cup with the aid of a metal spatula. The six collection sites in each house were chosen at random on each sampling date from sites showing signs of larval house fly activity. The core samples were then placed in a box, covered with screening to prevent additional oviposition, and returned to the laboratory.

At the laboratory, one core sample from each pair was placed into a Berlese funnel for collection of insect larvae and adult Coleoptera. The remaining core sample from each pair was placed in a plastic lined emergence carton for collection of adult insects and determination of the percentage of house fly emergence. The emergence cartons were identical to those used and described in preliminary laboratory bioassays (Breedon et al. 1975). Percentage of emergence was determined by comparing the number of empty puparia with the number of unemerged pupae in each emergence sample. All insects collected from the Berlese and emergence samples were preserved in 80 percent ethyl alcohol for later sorting, identification, and counting of pertinent taxa.

Three UV light traps of the same type developed and described by Morgan and Uebel (1974) were placed in each house for sampling adult insects. The light traps were hung about 2.7 m over the middle aisle of the chicken house. One trap was hung in the front near the entrance, another in the middle, and the third at the back of the house. The traps were operated for 6-hour periods from 10 p.m. to 4 a.m. on each sampling interval. Following each trapping, the traps

were returned to the laboratory and placed in a freezer (-18°C) for 30 minutes to immobilize the trapped insects. These insects were then preserved in 80 percent ethyl alcohol for later sorting, identification, and counting of pertinent taxa. These trappings were discontinued on 24 Sept. because of low adult insect populations.

A recording hygrothermograph (Friez Instrument Division, Bendix Aviation Corp., Model 594) was placed in the treated house to monitor temperature and humidity. A similar hygrothermograph was placed in the untreated house the last 2 weeks of the field trial to obtain a standard index of comparison between the two houses.

1975 Season Sampling Procedures and Techniques

Samples were taken once a week. Pretreatment samples were taken from the treated house on 24 June, 29 June, 7 July, and 15 July. No pretreatment samples were taken from the untreated house because it was vacant from 23 June to 14 July. After initiating the treatment, sampling began at both houses on 22 July and continued the first part of every week until 30 Sept.

Materials and methods for the manure core sampling, UV light trap sampling, and determination of the percentage of house fly emergence were similar to those used for the 1974 season. Because of the distance between the chicken houses and the laboratory, the Berlese core samples were not returned to the laboratory but were placed in Berlese funnels located on the farm with the treated house. In addition, the

emergence core samples were placed in the emergence cartons at the chicken houses and then returned to the laboratory.

Each emergence core sample taken after 22 July from the untreated house was seeded with 50 third-instar house fly larvae because of a small natural population of house flies. The seeding took place in the laboratory 2 weeks after each collection so that the naturally occurring house flies could emerge and be separated from the later emerging seeded house flies. The seeded house flies were from a laboratory-reared Stauffer susceptible strain.

The Berlese core samples were also used for determination of the moisture content of the manure samples. This was done to determine if moisture content had any effects on the methoprene treatment. The Berlese core samples were weighed at the chicken house to avoid any weight loss through evaporation using a triple beam balance (model 3201 Ohaus Scale Corp.). The samples were dried by leaving them in the Berlese funnels for a 7-day period. The fauna collected from each Berlese core sample was later weighed and subtracted from the wet weight of that sample.

A recording hygrothermograph was again placed in the treated house. A similar hygrothermograph was placed in the untreated house the last 2 weeks of the field trial to obtain a standard index of comparison between the two houses.

Residue Sampling and Analysis

In both seasons, treated and untreated feed and manure were collected for residue analysis of methoprene.

In 1974, approximately 1 liter of treated feed was collected once a week from 1 Aug. until 27 Sept. Small random subsamples were taken while the feed was being transferred into a truck after mixing at the mill. Three 1-liter samples of treated manure were collected once a week from 27 Aug. until 1 Oct. Four 1-liter samples of both untreated feed and manure were also collected for residue analysis. All samples were collected in glass jars and stored at -18°C .

In 1975, three 0.5-liter samples of treated feed and manure were collected once a week from 22 July until 23 Sept. The treated feed was collected at random in small subsamples from the feed trays in the chicken house. Three 0.5-liter samples each of both untreated feed and manure were also collected for residue analysis. All samples were collected in paper cartons lined with aluminum foil and stored at -18°C .

At the end of each season the frozen samples were packed in "dry ice" and shipped to Zoecon Laboratories in Palo Alto, California for residue analysis. However, because of the high cost of analysis, only some representative feed and manure samples were analyzed in both seasons. In 1974, only the feed samples collected on 1 Aug., 5 Sept., and 27 Sept. were analyzed. The samples were first mixed together to form a single composite sample. Also, only the manure samples collected on 10 Sept., 24 Sept., and 10 Oct. were analyzed. The replicates on each date were first mixed together to form a single

composite sample for each sample date. In 1975, only the feed and manure samples collected on 29 July, 26 Aug., and 23 Sept. were analyzed. Only one replicate from each date was analyzed; these were analyzed separately.

Resistance Bioassays

At the end of each season, the strain of house flies from the treated house (field-pressured) was tested for resistance to methoprene (Altosid, PS-10) by comparing it in laboratory bioassays to a laboratory-reared Stauffer susceptible strain. On 1 Oct. 1974 and 23 Sept. 1975, approximately 8 liters of treated manure were collected in random subsamples from the treated houses. The adult house flies that emerged were used to start laboratory colonies for the comparison bioassays.

The materials and methods used for these comparison bioassays were similar to those used and described in earlier bioassays (Breedon et al. 1975) except that the hens were fed treated feed for 10 days prior to manure collection. Then, the collective manure samples taken on the following days from each of the three replicates per treatment rate were equally divided into pairs. Later, one member of each sample pair was seeded with eggs from the field-pressured strain of house flies. The remaining sample-pair member was seeded with eggs from the susceptible strain of house flies.

The methoprene treatment rates used in 1974 were 0, 1.25, 2.5, 5.0, 7.5, and 15.0 ppm (AI in feed). Those used in 1975 were 0, 2.5,

5.0, 7.5, 10.0, 15.0, and 25.0 ppm (AI in feed). In both seasons, the manure samples used for the bioassays were collected on the 11th and 12th days after treatment began. The results from both treatment days were combined to give a total of six replicates for each treatment rate.

Statistical Analysis

The statistical test used to determine significant difference between two population samples was the two-sample t-test. Linear regression analysis was used to determine the degree of dependence between two variables. The variables compared per sampling date were: 1) moisture content of the 1975 Berlese core samples (independent variable = x) versus the percentage of house fly emergence from the corresponding emergence core sample (dependent variable = y); 2) the mean house fly emergence percentages (x) versus the house fly trap catch totals (y) 3 or 4 (1974) and 7 days (1975) later; 3) the mean of the high temperature during the day and the low temperature during light trap operation for both inside and outside the houses (x) versus the house fly trap catch totals (y); 4) these same temperature means outside (x) versus these same temperature means inside the houses (y); and 5) the mean daily temperature in the treated houses (x) versus the mean daily temperature in the untreated houses (y). The significance of a regression was determined by using the t-distribution to test the linear significance of the regression coefficient. All statistical tests were performed at a 5 percent probability of rejecting a true

null hypothesis. All t-tests and regressions were performed using the programmable Hewlett-Packard HP-25 calculator.

RESULTS AND DISCUSSION

1974 Season Treatment Effect on House Fly Emergence

The percentage of emergence data for both treated and untreated houses are presented in Table 1. The mean emergence percentages obtained on each sampling date from the treated house are significantly lower ($P = 0.05$) than those from the untreated house. However, the lowest mean emergence percentage obtained from the treated house was only 42 percent on 26 July. According to earlier laboratory bioassays (Breedon et al. 1975), the emergence should have been around 5 percent. Another unexpected result can be seen when the mean emergence percentages are presented graphically (Fig. 9). The percentage of emergence in the untreated house remained relatively constant averaging about 95 percent. However, the percentage of emergence in the treated house instead of remaining at a relatively constant low level gradually increased until 30 Aug. Then, for the remainder of the treatment period, the percentage of emergence remained relatively constant averaging about 89 percent.

Part of this gradual increase may have been caused by microbial degradation of the methoprene in the older manure near the sides and on the bottom of the pit rows. This is suggested by the decrease in the percentage of emergence after the house was cleaned on 13 Aug. Schooley et al. (1975) documented the ability of microorganisms to degrade methoprene. In addition, the half-life of methoprene in chicken manure (moisture content 60-80 percent) is around 34 days

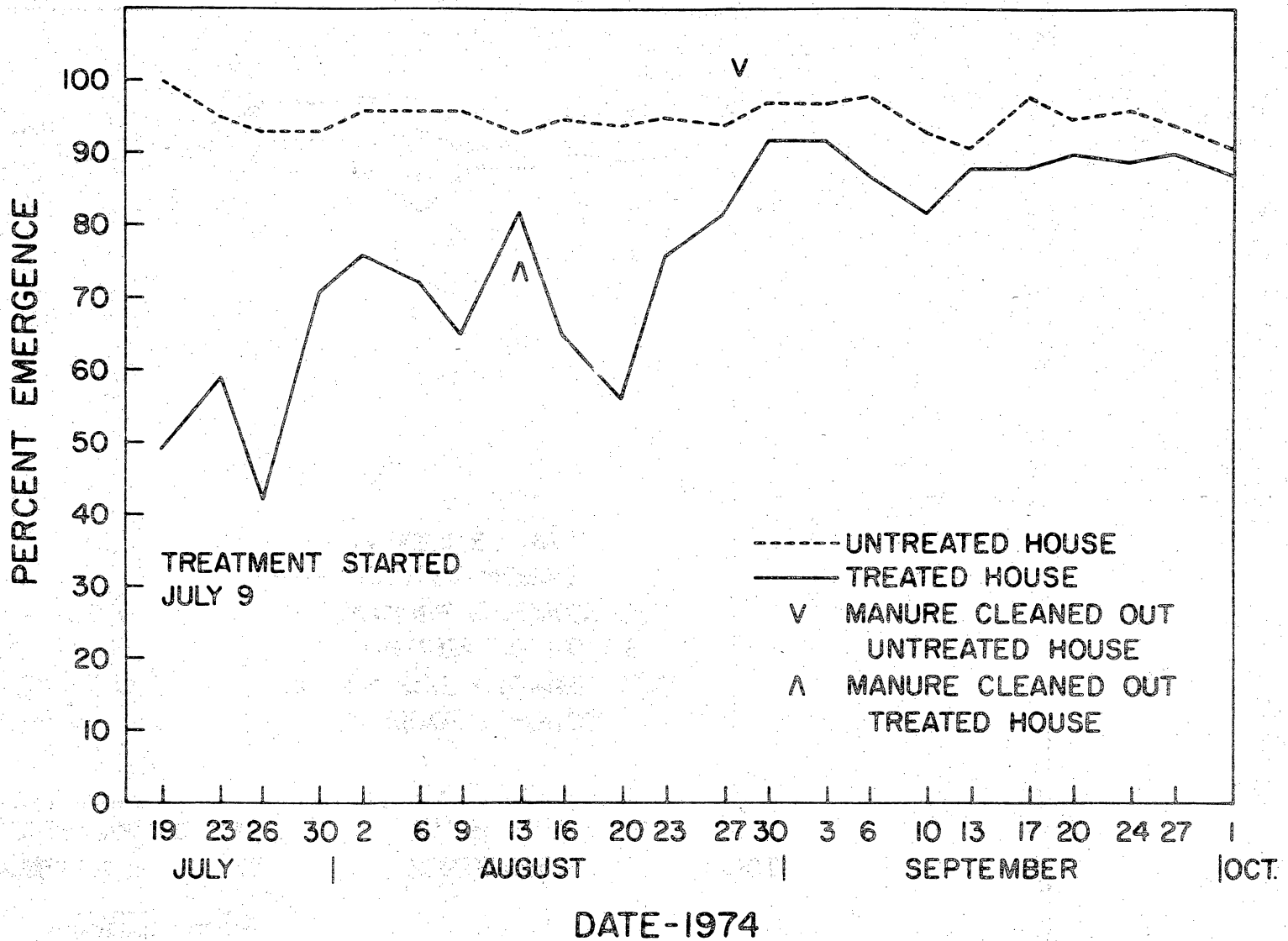
Table 1. Total number of house fly adults that emerged and mean emergence percentages \pm SD from the six emergence core samples on each sample date for both treated and untreated houses in 1974.

Sample date	Treated house		Untreated house	
	Total No. adults emerged	Mean emergence percentages \pm SD	Total No. adults emerged	Mean emergence percentages \pm SD
19 July	1831	49 \pm 29.6	162	100
23 July	1189	59 \pm 25.2	159	95 \pm 3.5
26 July	1577	42 \pm 11.3	130	93 \pm 2.8
30 July	3477	71 \pm 4.9	88	93 \pm 3.3
2 Aug.	1333	76 \pm 12.5	204	96 \pm 3.6
6 Aug.	2389	72 \pm 15.1	673	96 \pm 1.5
9 Aug.	568	65 \pm 18.5	740	96 \pm 2.5
13 Aug.	1604	82 \pm 8.7	1312	93 \pm 4.7
16 Aug.	564	65 \pm 9.8	200	95 \pm 6.4
20 Aug.	1392	56 \pm 15.4	784	94 \pm 6.9
23 Aug.	275	76 \pm 12.2	712	95 \pm 2.9
27 Aug.	1528	82 \pm 8.9	447	94 \pm 6.2
30 Aug.	875	92 \pm 4.8	543	97 \pm 4.6
3 Sept.	741	92 \pm 6.0	1794	97 \pm 1.7
6 Sept.	988	87 \pm 3.3	1275	98 \pm 1.7
10 Sept.	290	82 \pm 11.7	877	93 \pm 3.9
13 Sept.	459	88 \pm 4.2	256	91 \pm 3.9
17 Sept.	663	88 \pm 3.3	367	98 \pm 2.9

Table 1. (continued)

Sample date	Treated house		Untreated house	
	Total No. adults emerged	Mean emergence percentages \pm SD	Total No. adults emerged	Mean emergence percentages \pm SD
20 Sept.	684	90 \pm 5.6	226	95 \pm 5.1
24 Sept.	1020	89 \pm 10.5	753	96 \pm 1.6
27 Sept.	922	90 \pm 4.5	875	94 \pm 3.1
1 Oct.	605	87 \pm 1.9	215	92 \pm 3.9

Fig. 9. Mean house fly emergence percentages on each sampling date for the untreated and treated houses in 1974.



(personal communication, Zoecon Corp.). Because this decrease on 13 Aug. was only temporary and similar to previous decreases on 23 July and 2 Aug. not following manure removals, it seems unlikely that microbial degradation was a significant factor in the overall gradual increase.

A logical hypothesis to explain the unexpected high emergence percentages and gradual increase is an existing cross-resistance to methoprene coupled with an increase in resistance resulting from the continuous exposure to methoprene. The documentation of high degrees of cross-resistance to methoprene from laboratory strains of house flies resistant to conventional insecticides was discussed in the literature review. However, the existence of similar high degrees of cross-resistance in field strains has not been reported, but Cerf and Georghiou (1972) did find a five-fold cross-resistance to methoprene at the ED₉₅ level from a field strain highly resistant to many conventional insecticides. Even this comparatively small amount of cross-resistance could have allowed the initial high emergence percentages shown in Table 1. While no laboratory or field studies have been reported on the induction of house fly resistance to methoprene, Yu and Terriere (1975) found that the mechanisms for such an induction do exist in the house fly.

Since the house flies used in the earlier bioassays (Breedon et al. 1975) were from a susceptible strain, laboratory bioassays were conducted (Nov. 1974) to compare the susceptibility of the field-pressured strain to that of the susceptible strain used in the earlier

bioassays. The results (Table 2) showed no significant difference ($P = 0.05$) between the two strains. However, both strains showed evidence of resistance to methoprene when compared to the earlier bioassays (Breeden et al. 1975) which yielded around 5 percent emergence at 5 ppm (AI in feed).

The lack of difference in the results of the two strains was probably caused by contamination of the susceptible colony by members of the field-pressured strain. Rearing medium containing larvae of the susceptible colony was often left exposed in a room adjacent to the emergence cartons containing the field core samples. House fly escapes from these emergence cartons were minimal but sufficient throughout the season to have caused such a contamination. In addition, large numbers of adults from the field-pressured strain were unavoidably brought back, in the camper area of a pickup truck, to the immediate vicinity of the laboratory after each visit to the treated house.

The results of the comparison bioassays appear to support the hypothesis of resistance to methoprene. However, the results of other field trials in 1974 by Zoecon personnel in California and Texas did not show evidence of cross-resistance or an induction of resistance (personal communication, Zoecon Corp.). Therefore, Zoecon personnel requested that samples of my methoprene premix formulation be sent to them for analysis. The same batch (prepared in July 1974) of premix formulation was used for the field trial and the comparison bioassays. These samples unexpectedly assayed (analysis done in Dec. 1974) an

Table 2. Emergence percentages of a susceptible lab-reared and the 1974 field-pressured strain of house flies from manure from chickens fed methoprene treated feed.^a

Treatment rate ^b	Susceptible	Field pressured
0	98 ± 2.1	96 ± 2.2
1.25	94 ± 1.8	94 ± 2.1
2.5	93 ± 2.3	95 ± 2.4
5.0	94 ± 4.5	96 ± 2.7
7.5	89 ± 9.3	88 ± 5.4
15.0	77 ± 19.8	76 ± 12.6

^a Mean ± SD of six replicates.

^b Active ingredient in feed in ppm.

average of 57 percent subpotent. It is now known that this loss in potency was caused by an antagonistic impurity in the technical formulation which causes a gradual loss of potency (personal communication, Zoecon Corp.). This loss in potency could then also seem to explain the unexpected results from my field trial and the high emergence percentages in the comparison bioassays. However, even with the loss in potency the supposed 15 ppm treatment rate used in the comparison bioassays would still be at least 6.45 ppm. When compared to the earlier bioassays (Breedon et al. 1975), this 6.45 ppm rate should have given only about a 5 percent emergence instead of those shown in Table 2.

The results from the feed and manure samples sent for residue analysis indicated that the loss in potency apparently did not affect my field trial. A composite of three representative treated feed samples collected on 1 Aug., 5 Sept., and 27 Sept. assayed 9.8 ppm instead of the prescribed 7.5 ppm. This was probably caused by overestimation or "short-weighting" of the feed being mixed by the miller at the feed mill. In addition, the treated manure samples collected on 10 Sept., 24 Sept., and 1 Oct. assayed 1.42, 1.37, and 1.26 ppm (AI in manure), respectively. While these levels did show a decrease with time, they were all still comparable to manure residue levels from field trials conducted in California and Texas which achieved emergence percentages of around 5 percent with feeding rates of 10 ppm (AI) (personal communication, Zoecon Corp.). Therefore, since the loss in potency apparently did not affect my field trial, the resistance

hypothesis still offers the most probable explanation for the high emergence percentages and the gradual increase in the percentage of emergence shown in Fig. 9.

1975 Season Treatment Effect on House Fly Emergence

The percentage of emergence data for both treated and untreated houses are presented in Table 3. The mean emergence percentages obtained on each sampling date from the treated house are significantly lower ($P = 0.05$) than those from the untreated house for the period from 22 July until 9 Sept. In addition, the mean emergence percentages obtained after treatment began and up until 9 Sept. are significantly lower ($P = 0.05$) than the pretreatment mean emergence percentages. After 9 Sept., no significant difference ($P = 0.05$) was found. As in 1974, the mean emergence percentages obtained after treatment began were much higher than would be expected when compared to earlier bioassays (Breedon et al. 1975). The lowest mean emergence percentage obtained from the treated house was 53 percent on 12 Aug. This again indicates that a cross-resistance to methoprene existed in the field strain.

Instead of an expected sharp decrease in the percentage of emergence after 22 July, a gradual decrease occurred until 12 Aug. This gradual decrease was probably caused by some untreated manure still remaining after the house was cleaned on 22 July. The tractor blade used for manure removal usually missed about 5 cm of manure on each side and 2 cm of manure on the bottom of each pit row. When this

Table 3. Total number of house fly adults that emerged and mean emergence percentages \pm SD from the six emergence core samples on each sample date for both treated and untreated houses in 1975.

Sample date	Treated house		Untreated house	
	Total No. adults emerged	Mean emergence percentages \pm SD	Total No. adults emerged ^a	Mean emergence percentages \pm SD
24 June	871	91 \pm 5.5		
29 June	1045	96 \pm 4.3		
7 July	3809	89 \pm 10.2		
15 July	1063	96 \pm 2.3		
22 July	634	79 \pm 22.8	(48)	89 \pm 2.8
29 July	1772	65 \pm 23.3	272 (0)	91 \pm 2.2
5 Aug.	334	61 \pm 10.3	264 (1)	95 \pm 2.2
12 Aug.	427	53 \pm 21.1	269 (1)	91 \pm 2.9
19 Aug.	676	76 \pm 7.5	325 (55)	98 \pm 3.7
26 Aug.	1013	65 \pm 36.1	306 (21)	93 \pm 7.5
2 Sept.	1164	83 \pm 7.2	323 (50)	100
9 Sept.	176	90 \pm 9.6	456 (185)	90 \pm 4.9
16 Sept.	188	90 \pm 5.7	268 (0)	93 \pm 4.3
23 Sept.	3553	86 \pm 7.0	266 (0)	90 \pm 5.8
30 Sept.	1958	91 \pm 2.6	284 (0)	94 \pm 4.1

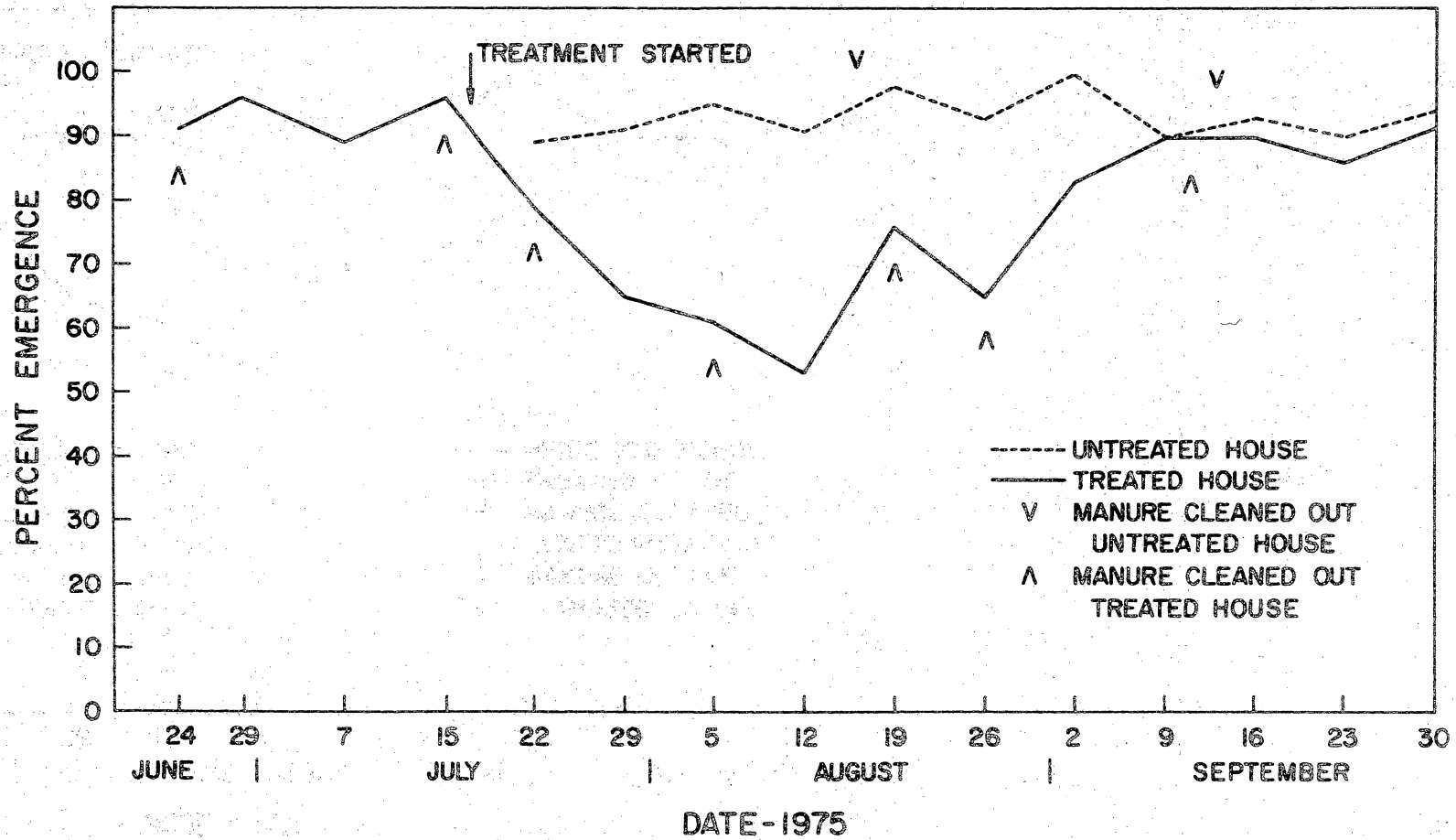
^a Numbers in parentheses indicate emerged flies from natural infestation, and those not in parentheses indicate the total No. of emerged flies from artificial and natural infestations.

mixed manure was removed on 5 Aug., the percentage of emergence decreased further before beginning to increase.

When the mean emergence percentages from both houses are presented graphically (Fig. 10), the overall similarities with the 1974 season (Fig. 9) become apparent. The percentage of emergence in the untreated house remained relatively constant averaging about 93 percent. However, starting on 12 Aug., the percentage of emergence in the treated house, instead of remaining at a relatively constant low level, gradually increased until 9 Sept. Then, for the remainder of the treatment period, the percentage of emergence remained relatively constant averaging about 89 percent. Unlike 1974, manure removal was too frequent to consider that part of the gradual increase was caused by microbial degradation of the methoprene. Indeed, even the unavoidable interruptor in treatment from 19 Aug. to 23 Aug. apparently had little effect on the percentage of emergence from the resulting mixed manure because the percentage of emergence actually decreased from 19 Aug. to 26 Aug. Therefore, as in 1974, the gradual increase in emergence from 12 Aug. to 9 Sept. indicates that an induction of resistance resulted from the continuous exposure to methoprene.

To test for resistance in the field-pressured strain, laboratory bioassays were conducted to compare the susceptibility of the field-pressured strain to that of the susceptible strain used in the earlier bioassays (Breedon et al. 1975). This season, the susceptible strain was reacquired from J. Sternberg, University of Illinois, in July and carefully maintained in another building to avoid any possible

Fig. 10. Mean house fly emergence percentages on each sampling date for the untreated and treated houses in 1975.



contamination by the field-pressured strain. The results of these comparison bioassays (Table 4) did show a significant difference ($P = 0.05$) between the susceptibility of the two strains. The methoprene apparently had no effect on the emergence of the field-pressured strain even at 25 ppm. Whereas, the results from the susceptible strain showed a decrease in emergence as the treatment rate increased with complete inhibition of emergence at 15 and 25 ppm. These results show that the field-pressured strain was resistant to methoprene. The results from the susceptible strain for the 2.5 and 5 ppm rates were higher than those from earlier bioassays (Breedon et al. 1975). This indicated the possibility that the methoprene formulation (batch prepared in Aug. 1975) used was subpotent. However, any reduction in potency would not have affected the validity of the comparison bioassays in showing resistance by the field-pressured strain to methoprene (see Materials and Methods).

This season, other researchers' results also supported the resistance hypothesis. Similar field trials in California and Texas by Zoecon personnel (personal communication) also resulted in unexpected high emergence percentages; treatment levels in the manure of 1.2 and 1.5 ppm (AI) from feeding rates of 10 ppm (AI) resulted in overall mean emergence percentages of 58 and 61 percent, respectively. In addition, unexpected high emergence percentages were reported in field trials by R. W. Miller, USDA, Beltsville, Maryland. A feeding rate of 10 ppm (AI) resulted in approximately 70 percent emergence. Laboratory bioassays by Miller also showed resistance by his field-pressured strain

Table 4. Emergence percentages of a susceptible lab-reared and the 1975 field-pressured strain of house flies from manure from chickens fed methoprene treated feed.^a

Treatment rate ^b	Susceptible	Field pressured
0	97 ± 2.8	95 ± 1.3
2.5	76 ± 2.4	98 ± 1.8
5.0	21 ± 1.3	96 ± 1.9
7.5	8 ± 2.1	96 ± 2.9
10.0	2 ± 2.4	97 ± 1.5
15.0	0	97 ± 1.7
25.0	0	96 ± 1.6

^a Mean ± SD of six replicates.

^b Active ingredient in feed in ppm.

to methoprene. The emergence of his strain was not reduced to 10 percent or less until treatment levels in the manure reached 50 ppm (AI) or more (personal communication, R. W. Miller).

This season the loss in potency problem apparently did affect my treatment levels for the field trial. The same batch (prepared in April 1975) of premix formulation was used for the entire treatment period. Three representative treated feed samples collected on 29 July, 26 Aug., and 23 Sept. assayed (analysis done in May 1976) 6.6, 5.9, and 7.9 ppm (AI in feed), respectively. Three representative treated manure samples collected on the same dates assayed 0.92, 0.61, and 1.0 ppm (AI in manure), respectively. It seems unlikely, however, that a gradual loss in potency is the only cause of the subpotent treatment levels because the residue levels for the samples collected on 23 Sept. were the highest and not the lowest of the three sample dates. Therefore, part of the subpotency of the treatment levels was probably caused by underestimation or "long-weighting" of the feed being mixed by the miller at the feed mill.

The subpotent treatment levels might be part of the cause for the high emergence percentages obtained in the first part of the treatment period. But, the main cause of these initial high mean emergence percentages (Table 3) is cross-resistance. Even with the subpotent treatment levels (6.6, 5.9, and 7.9 ppm AI in feed), the resulting mean emergence percentages should have been much lower when compared to the results from laboratory bioassays using susceptible strains (Table 4 and Breeden et al. 1975). In addition, the gradual loss in potency

appears to have had little to do with the gradual increase in emergence because the highest residue levels were from 23 Sept., which also had one of the highest mean emergence percentages (Table 3). If the gradual loss in potency was responsible for the increase in emergence, then the mean emergence percentage from 23 Sept. should have been correspondingly lower instead of higher than those on 29 July and 26 Aug.

The possibility that the percentage of moisture in the manure might have an effect on the methoprene treatment and, thus, on the resulting percentage of house fly emergence was also investigated. The percentage of moisture obtained for each treated Berlese core sample was compared to the percentage of emergence from the corresponding emergence core sample. No significant regression ($r^2 = 0.06$) was found. Thus, moisture content apparently had no effect on the ability of the methoprene treatment to inhibit house fly emergence.

Based on all of the above data from both seasons and in spite of the potency problems, the primary causes of the poor inhibition of house fly emergence by methoprene during both seasons were an existing cross-resistance to methoprene followed by an induction of resistance resulting from the continuous exposure to methoprene.

1974 Season Treatment Effect on Adult House Fly Populations

The adult house fly trap catches for both treated and untreated houses are presented in Table 5. These results show that even with the methoprene treatment, the treated house still had a significantly larger population of adult house flies than did the untreated house. This was

Table 5. Number of adult house flies caught in each UV light trap in both treated and untreated houses for each sample date in 1974.

Sample date	Treated house				Untreated house			
	Front trap	Middle trap	Back trap	Total	Front trap	Middle trap	Back trap	Total
19 July	305	380	574	1259	4	10	7	21
23 July	49	68	104	221	23	11	16	49
26 July	108	82	177	367	80	40	13	133
30 July	126	80	89	295	125	32	31	188
2 Aug.	115	80	57	252	88	19	25	132
6 Aug.	435	371	301	1107	20	27	27	74
9 Aug.	443	260	148	851	252	110	129	491
13 Aug.	290	43	86	419	42	67	23	132
16 Aug.	384	46	91	521	123	76	48	247
20 Aug.	228	43	43	314	142	129	60	331
23 Aug.	138	110	23	271	170	195	178	543
27 Aug.	272	100	45	417	206	36	96	338

Table 5. (continued)

Sample date	Treated house				Untreated house			
	Front trap	Middle trap	Back trap	Total	Front trap	Middle trap	Back trap	Total
30 Aug.	429	151	63	643	180	55	27	262
3 Sept.	177	89	76	342	32	7	12	51
6 Sept.	48	56	18	122	13	2	2	17
10 Sept.	382	100	58	540	34	8	6	48
13 Sept.	148	49	27	224	40	8	12	60
17 Sept.	395	47	89	531	208	95	60	363
20 Sept.	337	92	63	492	118	72	46	236
24 Sept.	32	19	15	66	25	5	6	36

unexpected because the houses were only about 15 m apart. This difference in populations can probably be attributed to the difference between the floors of the two houses. The treated house had dirt floors under the cage rows, but the untreated house had concrete floors. Dirt floors would have allowed for overall more moist conditions in the treated house and, thus, the possibility of more potential breeding sites. My observations supported this contention. In addition, the dirt floors probably caused the treated house to be more attractive to gravid females earlier in the season and just after cleaning than the untreated house.

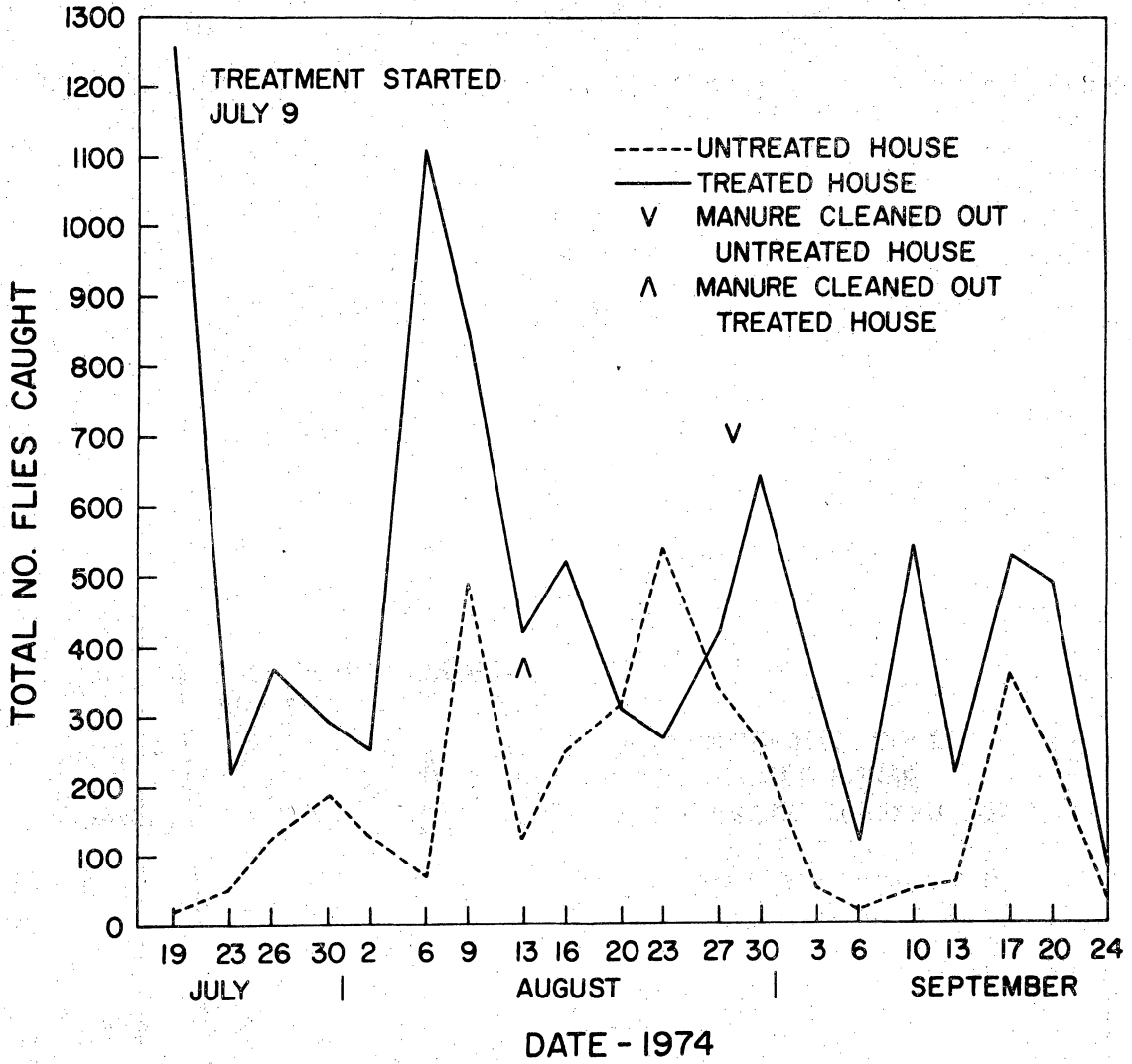
The individual trap catches on each sampling date indicate that an unequal dispersion of adults existed in both houses. Anderson and Poorbaugh (1964), using sticky tapes to sample adult populations, also found unequal dispersions of house fly adults within caged-layer houses. They listed different environmental factors (direct sunlight, light intensity, and different larval fly densities) as probable influences on adult dispersion. In both test houses, the traps in the front usually caught more house flies than the traps in the middle or back. The higher concentration of adults in the front of the houses was probably caused by the higher light intensity in the front because the large double doors in the front were left slightly ajar during the daytime. This also provided a potential entrance for flies from the outside. In addition, the fronts of the houses were the last to be warmed by the sun because they faced west. This might have influenced the trap catches because the traps operated only at night (10 p.m. to 4 a.m.).

However, the trap catches still accurately reflected the daytime dispersion based on my observations of the frequented resting areas of the adults.

To compare the fluctuations in the adult populations of both houses, the trap catch totals for each sampling date are presented graphically in Fig. 11. The larviciding in the treated house on 7 July by the farm manager prevented the sampling of pretreatment populations. However, this treatment had only a temporary effect on the adult population because the treated manure was removed on 9 July. By 15 July, the adult population was back to the pretreatment levels based on my observations of the frequented resting areas of the adults. The decrease in the adult population in the treated house from 19 July to 23 July may be attributed to the methoprene treatment because the adult population in the untreated house increased during that period. From then until 13 Aug., the increases and decreases in the adult population of the untreated house closely followed those in the treated house by 3 or 4 days. This indicated that adults were migrating from the treated house to the untreated house. This was probably helped by the exhaust fans in the treated house which expelled many adults outside toward the ventilation intake areas of the untreated house. After 13 Aug., the populations of both houses apparently functioned independently from one another.

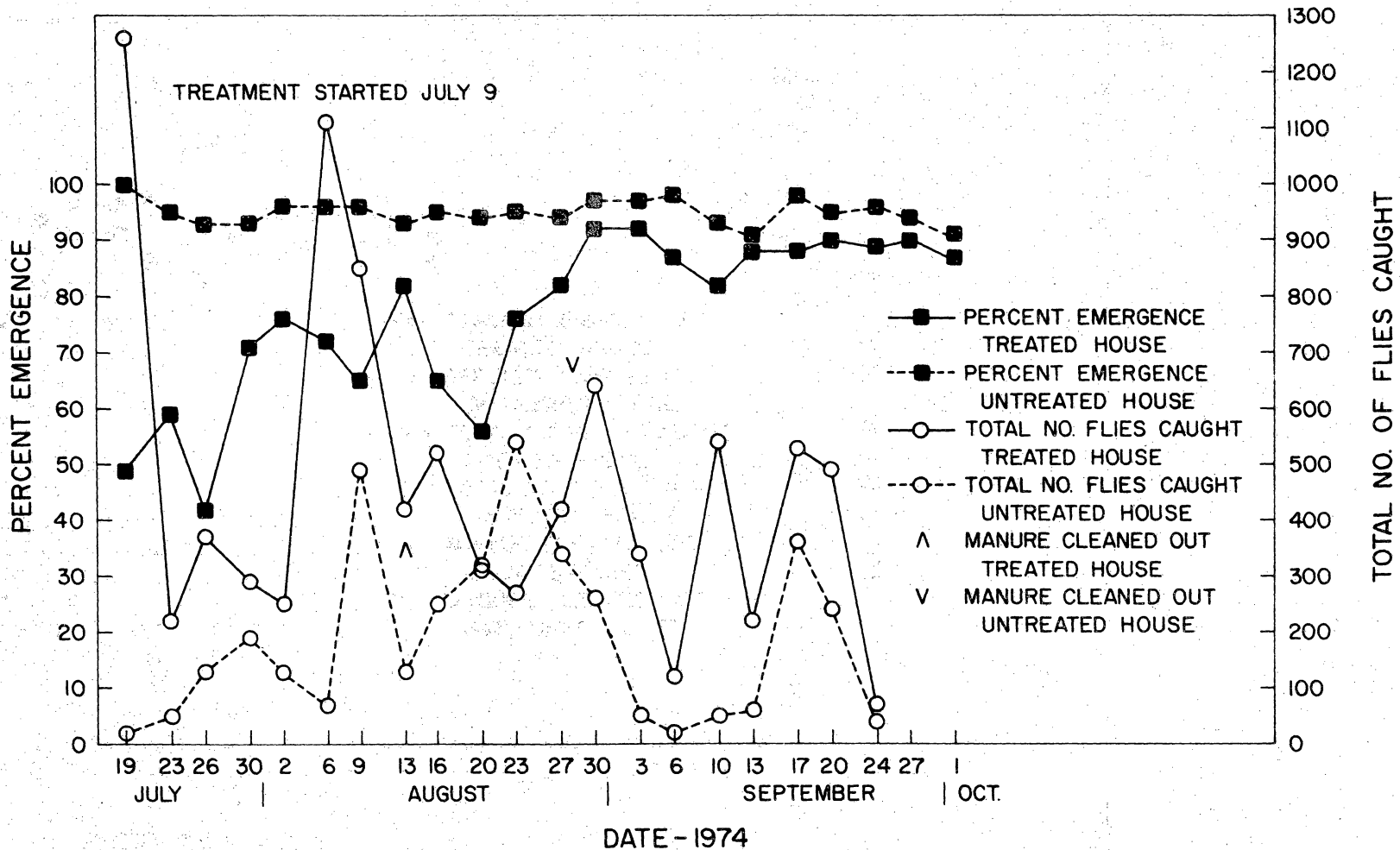
The correlation between the effect of the methoprene treatment and the resulting adult populations can be seen when the mean emergence percentages (Fig. 9) and trap catch totals (Fig. 11) are presented

Fig. 11. Total number of adult house flies caught in the UV light traps on each sampling date in the untreated and treated houses in 1974.



together (Fig. 12). Since methoprene is not effective before the last part of the third instar or after the tanning of the puparium, the resulting effect on the adult population would be delayed. In the field, the pupal stage usually lasts 4 or 5 days (West 1951). Therefore, in the treated house the increases and decreases in the percentage of emergence should have been closely followed by similar changes in the adult population. This appears to be what happened before 30 July and after 13 Aug. Between 30 July and 13 Aug., this issue is confused by the unscheduled additional insecticide treatments. The increase in percentage of emergence from 19 July to 23 July, was followed by an increase in the adult population from 23 July to 26 July. The emergence decrease from 23 July to 26 July was followed by an adult population decrease from 26 July to 30 July. The percentage of emergence increased from 26 July to 30 July; however, the adult population decreased slightly from 30 July to 2 Aug. This slight decrease instead of a proportionally large increase was caused by the effect of the malathion fly bait put out on 31 July. This treatment was only temporary because the bait was removed on 1 Aug. The adult population then underwent a large increase from 2 Aug. to 6 Aug. which probably reflected the large increase in the percentage of emergence from 26 July to 2 Aug. The percentage of emergence subsequently decreased from 2 Aug. to 9 Aug., but the adult population underwent a proportionally larger decrease from 6 Aug. to 13 Aug. The larger size of this decrease was caused by the malathion spray applied on 6 Aug. From 13 Aug. to 30 Aug., variations in the

Fig. 12. Mean house fly emergence percentages and total number of adult house flies caught in the UV light traps on each sampling date in the treated and untreated houses in 1974.



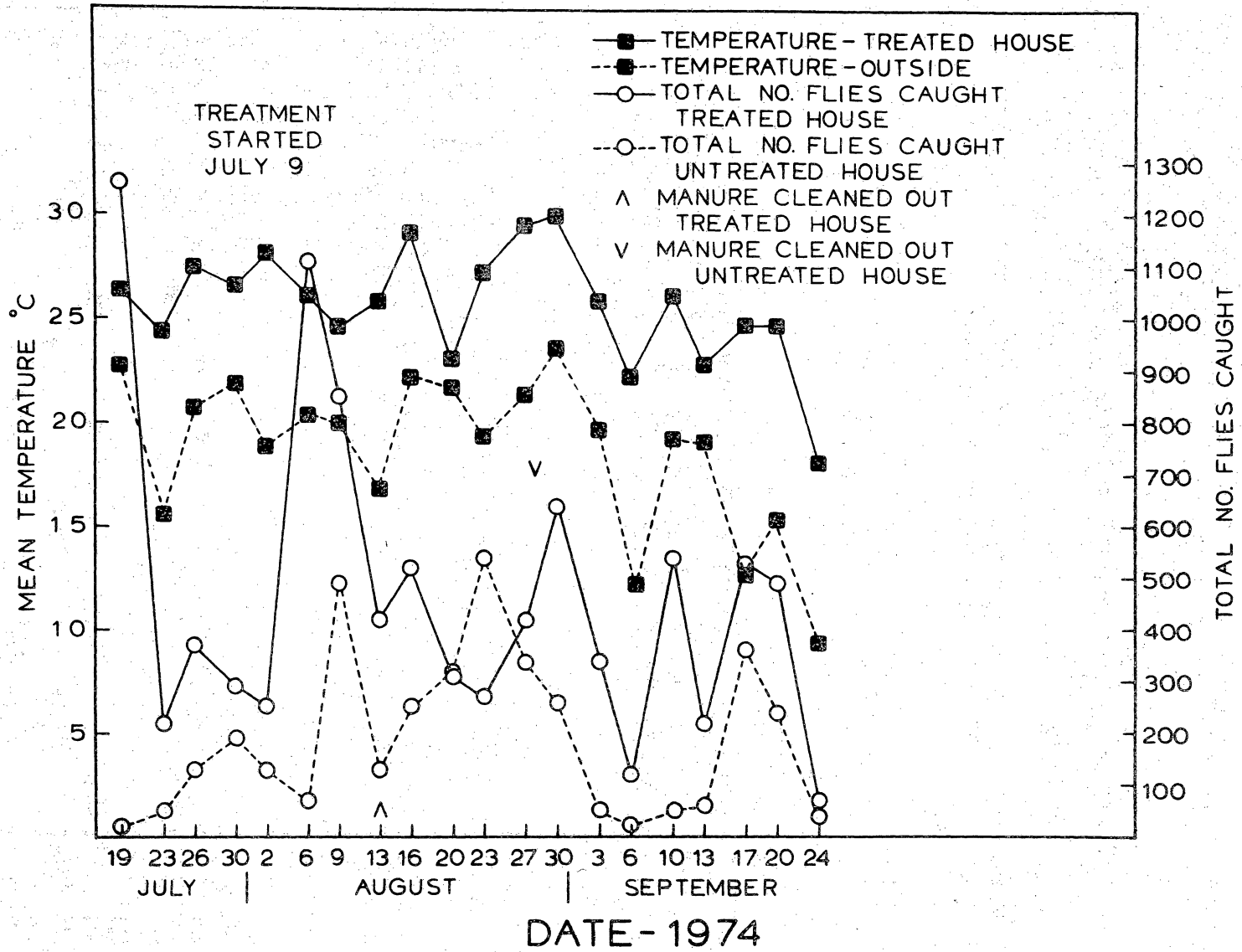
adult population corresponded closely with preceding increases and decreases in the percentage of emergence.

Despite the additional insecticide treatments, a slightly significant regression ($r^2 = 0.34$) was found between the mean emergence percentages in the treated house from 19 July until 27 Aug. and the trap catch totals 3 or 4 days from 23 July until 30 Aug. When the additional insecticide treatment dates are excluded from the regression, a highly significant regression ($r^2 = 0.79$) was found between the mean emergence percentages from 9 Aug. to 27 Aug. and the trap catch totals 3 or 4 days later from 13 Aug. to 30 Aug. However, no significant regression ($r^2 = 0.01$) was found between the mean emergence percentages from 27 Aug. to 20 Sept. and the trap catch totals 3 or 4 days later from 30 Aug. to 24 Sept. This lack of significance is understandable because by 30 Aug., the flies had become so resistant to methoprene that the treatment was no longer substantially inhibiting emergence.

Since the methoprene treatment was no longer a substantial factor, the increases and decreases in the trap catches after 30 Aug. indicated that another factor was possibly affecting the adult population in the treated house. These large fluctuations in the trap catches were at variance with my visual observations. While I did observe the gradual decline indicated in Fig. 11 after 30 Aug., presumably caused by a temperature dependent seasonal decline, I did not notice the large fluctuations indicated by the trap catches. Some factor was apparently biasing the trap catches rather than causing substantial fluctuations in the adult population density. The obvious choice of factors would

be those which have a substantial influence on the activity of the adults and, thus, alter their ability to respond to the light traps. Both temperature and humidity have a substantial effect on the activity of adult house flies. Nieschulz (1935) found that house fly adults were most active around 34°C and that their activity decreased above or below that temperature. Dakshinamurty (1948) found that house fly adults remained reasonably active at tolerably low temperatures (18°C) regardless of the humidity. However, they became very sluggish when both readings were high (35°C and 90 percent R.H.). Since the traps operated only at night, the combination of high temperature and humidity was unlikely. In addition, during the day, the forced ventilation always kept the humidity low. Thus, temperature is the apparent factor most likely to be the dominant influence on the ability of the adults to respond to the light traps. Morgan and Pickens (1968) and Pickens et al. (1969) found that changes in the temperature caused similar changes in the adult house fly catches of UV light traps. When this is applied to my data, the most significant regressions occurred using the mean of the high temperature which occurred during the day and the low temperature which occurred during the operation of the light traps. These temperature means for the inside of the treated house and the outside of both houses are presented graphically with the trap catch totals in Fig. 13. The inside temperature means for the treated house can also serve for the untreated house because the temperatures inside the two houses were practically identical. The outside temperature means were

Fig. 13. Mean temperatures inside and outside the treated house and the total number of adult house flies caught in the UV light traps on each sampling date in the treated and untreated houses in 1974.



calculated from data taken at the V.P.I. & S.U. Agronomy Experiment Station located in Blacksburg.

In the treated house, no significant regression ($r^2 = 0.01$) was found between the inside temperature means and the trap catch totals from 19 July to 30 Aug., but a highly significant regression ($r^2 = 0.78$) was found between these two variables from 30 Aug. to 24 Sept. These regressions combined with the earlier regressions indicate that the methoprene treatment was the determining factor in the increases and decreases in the adult population and the corresponding trap catches taken before 30 Aug. After 30 Aug., when methoprene resistance was high enough to allow the flies to have a relatively normal percentage of emergence, the influence of the temperature on the adult population densities caused the increases and decreases in the trap catches.

In the untreated house, no significant regression was found between the inside temperature means and the trap catch totals either before ($r^2 = 0.001$) or after ($r^2 = 0.23$) 30 Aug. The lack of significance before 30 Aug. is not unusual because the population was still increasing and until 16 Aug. was apparently largely influenced by migrating flies from the treated house. The lack of significance after 30 Aug. was probably caused by the effect of the malathion fly bait put out on 6 Sept.

No significant regressions were found between the outside temperature means and the trap catch totals for either the treated house before ($r^2 = 0.17$) or after ($r^2 = 0.38$) 30 Aug. or the untreated house before ($r^2 = 0.02$) or after ($r^2 = 0.01$) 30 Aug. A highly significant

regression ($r^2 = 0.56$) was found between the outside and inside temperature means from 19 July to 24 Sept. Inside the houses, however, temperature fluctuations were effectively controlled and modified by thermostatically controlled forced ventilation. This would account for the lack of significance between the outside temperature means and the trap catch totals in the treated house after 30 Aug. The lack of significance in the treated house before 30 Aug. and in the untreated house before and after 30 Aug. is explained not only by the controlled inside temperature but also by the same reasons mentioned earlier for the regressions with the inside temperature.

1975 Season Treatment Effect on Adult House Fly Populations

The adult house fly trap catches for both treated and untreated houses are presented in Table 6. This season the adult house fly population in both houses was much smaller than those in the houses used in 1974. This was not typical of either of these houses. In the past, both houses have always had much larger numbers of house flies. Indeed, inspections of both houses in Dec. 1974 revealed the presence of large numbers of house flies. The small numbers in 1975 can probably be attributed to the house fly population starting atypically late in the season. Other poultry house operators in the area also stated that their house fly populations were late in building up and in much smaller numbers than in previous years.

As in 1974, even with the methoprene treatment, the treated house had a significantly larger population of house flies than did the

Table 6. Number of adult house flies caught in each UV light trap in both treated and untreated houses for each sample date in 1975.

Sample date	Treated house				Untreated house			
	Front trap	Middle trap	Back trap	Total	Front trap	Middle trap	Back trap	Total
24 June	49	16	24	89				
29 June	7	8	10	25				
7 July	3	5	12	20				
15 July	39	13	19	71				
22 July	29	17	15	61	0	0	1	1
29 July	18	15	6	39	0	0	0	0
5 Aug.	18	11	4	33	3	1	3	7
12 Aug.	14	10	6	30	4	3	1	8
19 Aug.	12	6	0	18	13	4	1	18
26 Aug.	10	7	10	27	7	6	0	13
2 Sept.	2	1	14	17	2	5	3	10
9 Sept.	2	12	11	25	5	1	2	8

Table 6. (continued)

Sample date	Treated house					Untreated house			
	Front trap	Middle trap	Back trap	Total		Front trap	Middle trap	Back trap	Total
16 Sept.	1	2	6	9		0	0	2	2
23 Sept.	4	4	12	20		3	2	1	6
30 Sept.	2	1	1	4		0	0	0	0

untreated house. This was probably due to the untreated house being vacant from 23 June to 14 July. Also, during the first few days of that vacancy, the house was thoroughly cleaned and treated with a 1 percent dichlorvos spray.

As in 1974, the individual trap catches on each sampling date indicated an unequal dispersion of the adults within both houses. The traps in the front usually caught more house flies than the traps in the middle or the back of the houses. These trap catches again accurately reflected the daytime dispersion of adults based on my observations of the frequented resting areas of the adults. In the treated house, the higher concentrations of adults in the front were probably caused by the higher light intensity resulting from the front door being left partially open during the daytime. This also provided a potential entrance for flies from the outside. After 26 Aug. more house flies were caught in the back trap than in the front. This shifting in the adult dispersion was probably in response to the seasonal decline in temperature because the back of the house faced west and was the last to be warmed by the sun. In the untreated house, the higher concentrations of house flies in the front were probably caused by the front of the house being warmer and protected from drafts. The house was oriented so that the front faced south. In addition, all the windows on both sides of the house had been removed (Fig. 8). This created a situation in which the front of the house would be the warmest area and the only area consistently protected from the natural

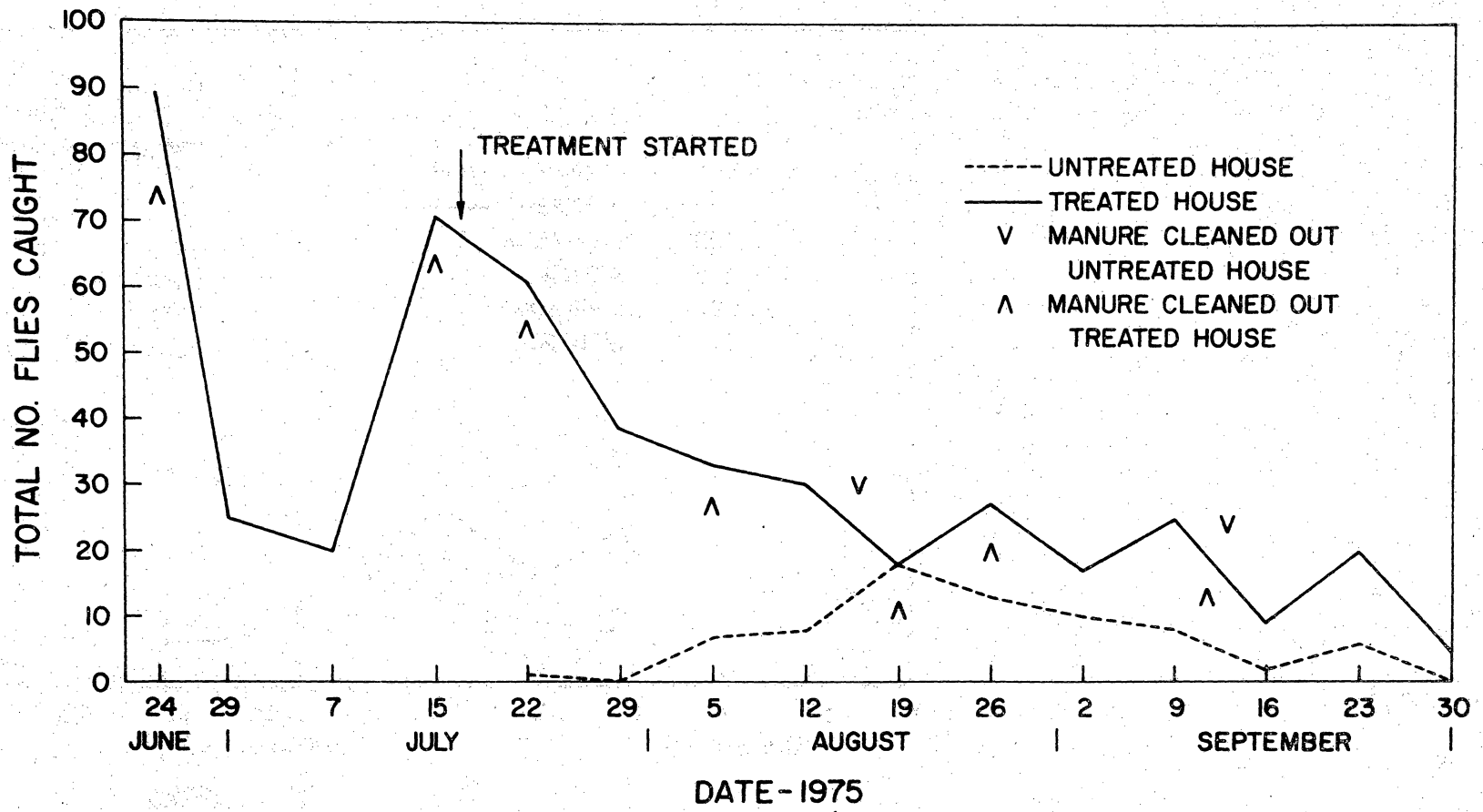
side-to-side drafts at night and the forced ventilation during the daytime caused by the exhaust fans.

To compare the fluctuations in the adult populations of both houses, the trap catch totals for each sampling date are presented graphically in Fig. 14. No additional insecticide treatments were applied this season, and the houses were well isolated (6 miles apart) from one another. A 3-week vacancy prevented taking pretreatment samples from the untreated house, but pretreatment samples were taken from the treated house from 24 June to 15 July.

A large decrease in the adult population of the treated house occurred during the pretreatment period from 24 June to 7 July due to the manure being removed on 24 June. This type of decrease is common after manure removal during the early part of the fly season. Later in the season when the fly population becomes established, decreases after manure removal are much less substantial if they occur at all. Even with the methoprene treatment, no other large decreases occurred after the manure was removed. In addition, the adult population in the untreated house actually increased after the manure was removed on 16 Aug.

After treatment began on 17 July, the adult population in the treated house gradually decreased until 19 Aug. The adult population in the untreated house gradually increased until 19 Aug. After 19 Aug., the adult populations in both houses gradually decreased presumably from a temperature dependent seasonal decline. It is doubtful that the frequent manure removals contributed to the decrease in the adult

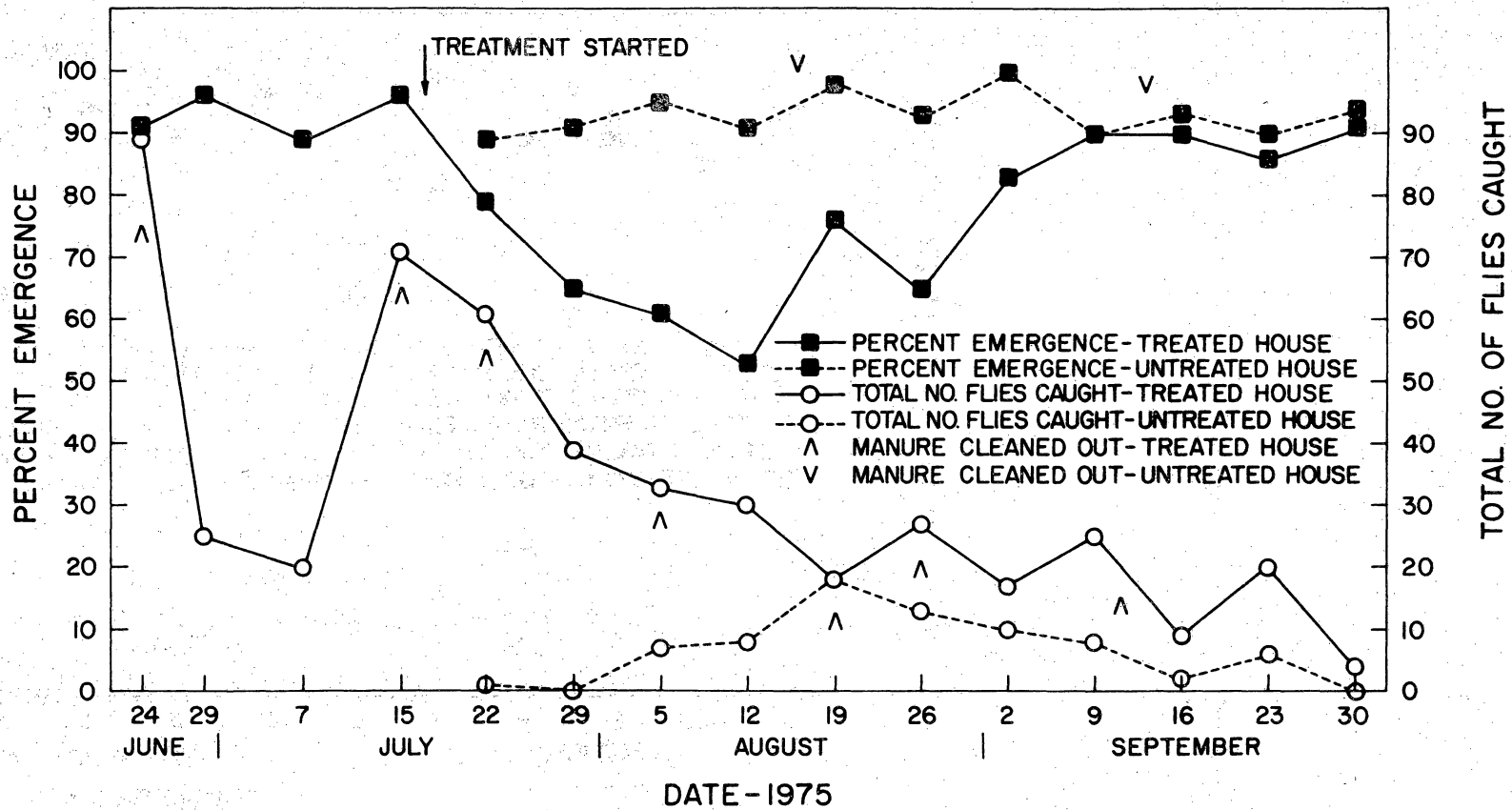
Fig. 14. Total number of adult house flies caught in the UV light traps on each sampling date in the untreated and treated houses in 1975.



population in the treated house from 15 July to 19 Aug. Anderson (1965) found that during the fly season the process of manure removal usually attracted additional house flies to the premises. This, added to the increased attractiveness and greater habitat suitability of the following fresh manure for house flies (Legner et al. 1973), ultimately causes the house fly population to increase after each manure removal (Anderson 1965). Since the adult population in the untreated house increased from 22 July to 19 Aug., the decrease in the adult population of the treated house during that period can be attributed to the methoprene treatment.

The correlation between the effect of the methoprene treatment and the resulting adult population can be seen when the mean emergence percentages (Fig. 10) and the trap catch totals (Fig. 14) are presented together (Fig. 15). As in 1974, the resulting effect of the treatment on the adult population should have shown a delayed response. Therefore, the increases and decreases in the percentage of emergence should have been closely followed by similar changes in the adult population. This is what happened from 15 July until 9 Sept. A significant regression ($r^2 = 0.51$) was found between the mean emergence percentages from 15 July to 2 Sept. and the trap catch totals 7 days later from 22 July to 9 Sept. But, no significant regression ($r^2 = 0.25$) was found between the mean emergence percentages from 2 Sept. to 23 Sept. and the trap catch totals 7 days later from 9 Sept. to 30 Sept. As in 1974, this lack of significance can be accounted for because by 9 Sept., the flies

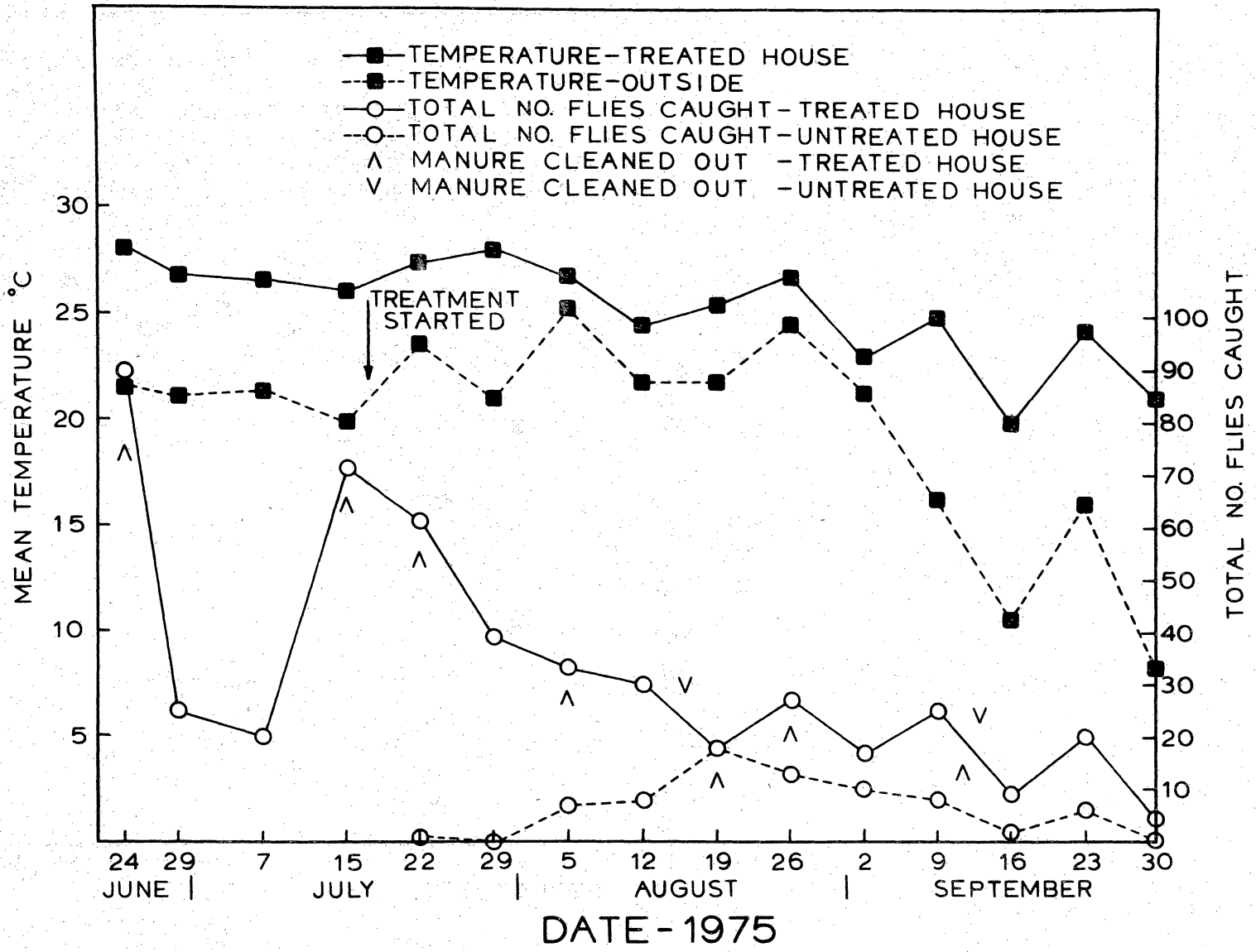
Fig. 15. Mean house fly emergence percentages and total number of adult house flies caught in the UV light traps on each sampling date in the treated and untreated houses in 1975.



had become so resistant to methoprene that the treatment was no longer substantially inhibiting emergence.

Since the methoprene treatment was no longer a substantial factor after 9 Sept., the possibility that temperature was affecting my trap catches, for the same reasons as mentioned earlier, was investigated. As in 1974, the most significant regressions occurred using the mean of the high temperature which occurred during the day and the low temperature which occurred during the operation of the light traps. These temperature means for the inside of the treated house and the outside of both houses are presented graphically with the trap catch totals in Fig. 16. Unlike 1974, the inside temperatures of the two houses were quite different. In addition, no significant regression ($r^2 = 0.42$) was found between the daily temperature means of the two houses for the 2 weeks that readings were taken in the untreated house. A highly significant regression ($r^2 = 0.97$) was found between the daily temperature means outside the untreated house and those inside. This can be attributed to the modified structure of the untreated house because both sides of the house contained a row of opened windows (Fig. 8). Therefore, even though the daily temperature means inside the house averaged 6.1°C warmer than those outside, the outside temperature means in Fig. 16 can also serve as an accurate index of the inside temperature for the untreated house. Outside temperature means were calculated from data taken at the Staunton Sewage Plant located northeast of Staunton. The sewage plant is located approximately 4 miles southwest of the treated house and 10 miles southwest of the untreated house.

Fig. 16. Mean temperatures inside and outside the treated house and total number of adult house flies caught in the UV light traps on each sampling date in the treated and untreated houses in 1975.



In the treated house, a highly significant regression ($r^2 = 0.84$) was found from 9 Sept. to 30 Sept. between the inside temperature means and the trap catch totals. A significant regression ($r^2 = 0.80$) was also found between these two variables from 19 Aug. to 30 Sept. This regression overlaps with the emergence and trap catch regression from 15 July to 9 Sept. Although this overlap suggests the possibility that the effect of the methoprene on emergence and temperature were acting in unison to influence the trap catches after 19 Aug., it is doubtful that the effect of temperature was significant because no such unison occurred from 15 July to 19 Aug. No significant regressions were found between the inside temperature means and the trap catch totals from 24 June to 9 Sept. ($r^2 = 0.28$) or from 15 July to 9 Sept. ($r^2 = 0.25$). Therefore, the effect of the methoprene treatment on emergence was still the dominant influence on the adult population and the resulting trap catches between 15 July and 9 Sept. After 9 Sept., when methoprene resistance was high enough to allow the flies to have a relatively normal percentage of emergence, the influence of temperature on the adult activity and not substantial changes in the adult population densities caused the increases and decreases in the trap catches.

As in 1974, a highly significant regression ($r^2 = 0.67$) was found between the temperature means outside the treated house and those inside from 24 June to 30 Sept. Unlike 1974, however, a highly significant regression ($r^2 = 0.98$) was found for the last part of the trial period, from 9 Sept. to 30 Sept., between the outside temperature means

and the trap catch totals in the treated house. No significant regression ($r^2 = 0.1$) was found between these two variables from 15 July to 9 Sept. Thus, the temperature inside the treated house was not as independent of the influence of the outside temperature as were the temperatures in the houses used in 1974.

In the untreated house, highly significant regressions were found between the outside temperature means and the trap catch totals from 26 Aug. to 30 Sept. ($r^2 = 0.98$) and from 9 Sept. to 30 Sept. ($r^2 = 0.96$). No significant regression ($r^2 = 0.01$) was found between these two variables from 22 July to 26 Aug. because the population was still increasing until 19 Aug.

The primary objective of this study was to evaluate the efficacy of methoprene at the prescribed feeding rates of 7.5 (1974) and 10 ppm (1975) (AI) in inhibiting house fly emergence and, thus, in reducing the adult populations. The methoprene treatments had a definite effect on the adult populations. In both seasons, the trap catch totals fluctuated in response to previous increases or decreases in the mean emergence percentages. However, because of the differences in environment, maintenance, and house fly population densities between the treated and untreated houses, an accurate estimate of the percentage of control could not be determined from the trap catch totals. It is highly unlikely that reasonably similar house fly population densities could have been found between any two houses. In addition, Pickens et al. (1972) found that UV light traps and sticky tapes were too inconsistent and the other methods of sampling adult house fly

populations (on-site resting counts, Scudder grill, 30 cm cord counts, and spot counts on white cards) were too insensitive for accurate estimates of actual adult population densities. UV light traps were recommended as the preferred method because they were the most sensitive to relative changes in and differences between adult house fly population densities. Fortunately, the mode of action of methoprene allowed the percentage of inhibition or control of house fly emergence to be directly assessed. Therefore, the mean emergence percentages in Tables 1 and 3 did provide a reasonable accurate estimation of the percentage of control.

In 1974, the average percentage of emergence for the untreated house during the test period was approximately 95 percent. By using 95 percent as the emergence standard, an estimate of the percentage of inhibition or control can be determined. The lowest mean emergence percentage, 42 percent, occurred on 26 July. This corresponds to a 53 percent inhibition or control which is clearly an unacceptable level of control. At no time in 1974 was the adult population acceptably controlled even with the additional insecticide treatments.

In 1975, the average percentage of emergence in the untreated house and in the treated house during the pretreatment period was approximately 93 percent. Using 93 percent as the emergence standard, the maximum average percentage of inhibition or control of house fly emergence was 40 percent on 12 Aug. This is also an unacceptable level of control, but because of the atypical fly season (late in starting and low numbers), the adult house fly populations in both houses never

achieved unacceptable or pestiferous levels. However, the methoprene treatment was instrumental in preventing the initial small population from increasing to pestiferous levels. The treatment steadily reduced the adult population until 19 Aug. After 19 Aug., the population was apparently kept at low levels by a temperature dependent seasonal decline. However, during a normal season, it is highly unlikely that this treatment would have been as successful even if it had begun before the population became established to pestiferous levels.

The light traps removed adults from each house at each sampling. However, it is doubtful if this method of sampling without replacement contributed significantly to any suppression or decrease in adult numbers. Thimijan et al. (1972) released known numbers of house fly adults into a barn containing three UV light traps equipped with 40-watt lamps. After 24-hour periods of continuous operation, the three traps combined caught an average of only 17 percent of the released population. My traps were probably considerably less efficient because they used only 6-watt lamps and operated for only 6 hours at night.

In spite of the loss of potency problems from the formulation and improper mixing, the failure of methoprene at feeding rates of 7.5 and 10 ppm (AI) to adequately inhibit house fly emergence can be attributed to an existing cross-resistance to methoprene in the field strains followed by an induction of resistance resulting from the continuous exposure to methoprene. The logical response to such a resistance would be to increase the treatment rates accordingly until acceptable levels of inhibition or control (above 90 percent) are attained.

However, the higher rates required in response to cross-resistance or induction of resistance would probably be economically unfeasible when compared to currently recommended insecticides. Zoecon personnel (personal communication) estimate the cost to the consumer for the 10 ppm feeding rate to be from \$1 to \$2 per ton of treated feed. This is quite expensive by current market standards considering that for thorough treatment, the feed-through application should be continuous throughout the majority of the fly season. Even if a susceptible field strain was encountered, my data indicates that resistance to methoprene would probably soon develop. Therefore, considering all the above data and the rather limited market for methoprene in the poultry industry, I doubt that commercial registration of methoprene as a poultry feed additive to control house flies would prove economically feasible.

SUMMARY

The primary objective of this study was to evaluate the efficacy of the juvenile hormone analog, methoprene, as a poultry feed additive for control of the house fly breeding in chicken manure in a commercial caged-layer house. Methoprene was evaluated at 7.5 ppm (AI in feed) in the 1974 fly season and at 10 ppm (AI in feed) in the 1975 fly season. In both seasons, the methoprene treatment caused an inhibition of house fly emergence. However, the average percentage of inhibition did not exceed 53 percent in 1974 or 40 percent in 1975. Both levels of inhibition were too low to result in acceptable control of the resulting adult populations of house flies. In addition to this problem, the level of inhibition gradually decreased throughout the latter part of both seasons until the methoprene treatment had little if any significant inhibition of house fly emergence. In both seasons, evidence from the field trials and laboratory bioassays showed that the primary causes of these problems were an existing cross-resistance to methoprene in the field strains followed by an induction in resistance resulting from the continuous exposure to methoprene. Considering the resistance problem, the already high expense of the methoprene treatment even at 10 ppm, and the limited market for methoprene in the poultry industry, I doubt that commercial registration of methoprene as a poultry feed additive for control of house flies in chicken houses would prove economically feasible.

LITERATURE CITED

- Ajami, A. M., and L. M. Riddiford. 1973. Comparative metabolism of the *Cecropia* juvenile hormone. *J. Insect Physiol.* 19:635-45.
- Anderson, J. R. 1965. A preliminary study of integrated fly control on northern California poultry ranches. *Proc. Calif. Mosquito Control Assc.* 33:42-4.
- Anderson, J. R., and J. H. Poorbaugh. 1964. Observations on the ethology and ecology of various Diptera associated with northern California poultry ranches. *J. Med. Entomol.* 1:131-47.
- Anderson, J. R., A. S. Deal, E. F. Legner, E. C. Loomis, and M. H. Swanson. 1968. Fly control on poultry ranches. *Calif. Agric. Ext. Serv. Bull. AXT-72 Rev.* 13 p.
- Axtell, R. C. 1968. Integrated house fly control: populations of fly larvae and predaceous mites, *Machrocheles muscadomesticae*, in poultry manure after larvicide treatment. *J. Econ. Entomol.* 61:245-9.
- Axtell, R. C. 1970. Integrated fly-control program for caged-poultry houses. *J. Econ. Entomol.* 63:400-5.
- Bailey, D. L., D. W. Meifert, and P. M. Bishop. 1968. Control of house flies in poultry houses with larvicides. *Fla. Entomol.* 51:107-11.
- Breeden, G. C., E. C. Turner, Jr., and W. L. Beane. 1975. Methoprene as a feed additive for control of the house fly breeding in chicken manure. *J. Econ. Entomol.* 68:451-2.
- Card, L. E., and M. C. Nesheim. 1972. *Poultry production.* Lea & Febiger, Philadelphia, Pa. 392 p.
- Cerf, D. C., and G. P. Georghiou. 1972. Evidence of cross-resistance to a juvenile hormone analogue in some insecticide-resistant house flies. *Nature* 239:401-2.
- Cerf, D. C., and G. P. Georghiou. 1974. Cross resistance to juvenile hormone analogues in insecticide-resistant strains of *Musca domestica* L. *Pestic. Sci.* 5:759-67.
- Chamberlain, W. F., L. M. Hunt, D. E. Hopkins, A. R. Gingrich, J. A. Miller, and B. N. Gilbert. 1975. Absorption, excretion, and metabolism of methoprene by a guinea pig, a steer, and a cow. *J. Agric. Food Chem.* 23:736-42.

- Dakshinamurty, S. 1948. The common house-fly Musca domestica L. and its behaviour to temperature and humidity. *Bull. Entomol. Res.* 39:339-57.
- Darby, R. E. 1962. Midges associated with California rice fields, with special reference to their ecology. *Hilgardia* 32:1-206.
- Fagan, E. B., and W. R. Enns. 1966. The distribution and biology of aquatic midges in Missouri lagoons. *Proc. Entomol. Soc. Wash.* 68:277-89.
- Gelbic, L., and F. Sehnal. 1974. Activity of some juvenoids in chironomid larvae. *Experientia* 30:1250-2.
- Georghiou, G. P., M. E. Hawley, and E. C. Loomis. 1967. A progress report on insecticide resistance in the fly complex of California poultry ranches. *Calif. Agric.* 21:8-11.
- Hart, S. A. 1963. Fowl fecal facts. *World's Poultry Sci. J.* 19:262-72.
- Henrick, C. A., G. B. Staal, and J. B. Siddall. 1973. Alkyl 3,7,11-trimethyl-2,4-dodecadienoates, a new class of potent insect growth regulators with juvenile hormone activity. *J. Agric. Food Chem.* 21:354-9.
- Henrick, C. A., W. E. Willy, B. A. Garcia, and G. B. Staal. 1975. Insect juvenile hormone activity of the stereoisomers of ethyl 3,7,11-trimethyl-2,4-dodecadienoate. *J. Agric. Food Chem.* 23:396-400.
- Henrick, C. A., W. E. Willy, and G. B. Staal. 1976. Insect juvenile hormone activity of alkyl (2E,4E)-3,7,11-trimethyl-2,4-dodecadienoates. Variations in the ester function and in the carbon chain. *J. Agric. Food Chem.* 24:207-18.
- Jakob, W. L. 1973. Insect development inhibitors: tests with house fly larvae. *J. Econ. Entomol.* 66:819-20.
- James, M. T. and R. F. Harwood. 1969. *Herm's medical entomology.* Macmillan Co., Collier-Macmillan Canada, Ltd., Toronto, Ontario. 484 p.
- Legner, E. F., W. R. Bowen, W. D. McKeen, W. F. Rooney, and R. F. Hobza. 1973. Inverse relationships between mass of breeding habitat and synanthropic fly emergence and the measurement of population densities with sticky tapes in California inland valleys. *Environ. Entomol.* 2:199-205.

- Lehmann, J. 1971. Die chironomiden der Fulda. Arch. Hydrobiol., Suppl. 37:466-555.
- Mason, W. T., Jr. 1974. Chironomidae (Diptera) as biological indicators of water quality. Symposium on organisms as indicators of environmental quality. 25 Mar. 1974. Center for Tomorrow. Ohio State Univ., Columbus, Ohio.
- Matthysse, J. G., and D. McClain. 1973. House fly control in climate-controlled caged-hen layer houses. J. Econ. Entomol. 66:927-33.
- Menn, J. J., and M. Beroza, eds. 1972. Insect juvenile hormones. Academic Press, New York. 341 p.
- Miller, R. W. 1970. Larvicides for fly control--a review. Bull. Entomol. Soc. Am. 16:154-8.
- Miller, S., and J. M. Collins. 1975. The nature of the changes in the pattern of RNA synthesis by the juvenile hormone analogue Altosid. J. Insect Physiol. 21:1295-1303.
- Miura, T., and R. M. Takahashi. 1973. Insect development inhibitors. 3. Effects on nontarget aquatic organisms. J. Econ. Entomol. 66:917-22.
- Morgan, N. O., and L. G. Pickens. 1968. Influence of air temperature on the attractiveness of electric lamps to house flies. J. Econ. Entomol. 61:1257-9.
- Morgan, N. O., and E. C. Uebel. 1974. Efficacy of the assateague insect trap in collecting mosquitoes and biting flies in a Maryland salt marsh. Mosq. News 34:196-9.
- Morgan, P. B., G. C. LaBrecque, D. E. Weidhaas, and A. Benton. 1975. The effect of methoprene, an insect growth regulator, on Musca domestica (Diptera: Muscidae). Can. Entomol. 107:413-7.
- Mulla, M. S., W. L. Kramer, and D. R. Barnard. 1976. Insect growth regulators for control of chironomid midges in residential-recreational lakes. J. Econ. Entomol. 69:285-91.
- Mulla, M. S., R. L. Norland, T. Ikeshoji, and W. L. Kramer. 1974. Insect growth regulators for the control of aquatic midges. J. Econ. Entomol. 67:165-70.
- Nieschulz, O. 1935. Uber die temperaturabhangigkeit der aktivitat und die vorzugstemperatur von Musca domestica und Fannia canicularis. Zool. Anz. 110:225-33.

- Peck, J. H., and J. R. Anderson. 1970. Influence of poultry-manure-removal schedules on various Diptera larvae and selected arthropod predators. *J. Econ. Entomol.* 63:82-90.
- Pickens, L. G., N. O. Morgan, and R. W. Miller. 1972. Comparison of traps and other methods for surveying density of populations of flies in dairy barns. *J. Econ. Entomol.* 65:144-5.
- Pickens, L. G., N. O. Morgan, and R. W. Thimijan. 1969. House fly response to fluorescent lamps: influence by fly age and nutrition, air temperature, and position of lamps. *J. Econ. Entomol.* 62: 536-9.
- Plapp, F. W., Jr., and S. B. Vinson. 1973. Juvenile hormone analogs: toxicity and cross-resistance in the house fly. *Pest. Biochem. Physiol.* 3:131-6.
- Quate, L. W. 1955. A revision of the Psychodidae (Diptera) in America north of Mexico. *Calif. Univ., Pubs., Entomol.* 10: 103-273.
- Quistad, G. B., L. E. Staiger, and D. A. Schooley. 1975c. Environmental degradation of the insect growth regulator methoprene. V. Metabolism by house flies and mosquitoes. *Pestic. Biochem. Physiol.* 5:233-41.
- Quistad, G. B., L. E. Staiger, and D. A. Schooley. 1975b. Environmental degradation of the insect growth regulator methoprene. VIII. Bovine metabolism to natural products in milk and blood. *J. Agric. Food Chem.* 23:750-3.
- Quistad, G. B., L. E. Staiger, and D. A. Schooley. 1976. Environmental degradation of the insect growth regulator methoprene. X. Chicken metabolism. *J. Agric. Food Chem.* 24:644-8.
- Quistad, G. B., L. E. Staiger, B. J. Bergot, and D. A. Schooley. 1975a. Environmental degradation of the insect growth regulator methoprene. VII. Bovine metabolism to cholesterol and related natural products. *J. Agric. Food Chem.* 23:743-9.
- Saether, O. A., and M. P. McLean. 1972. A survey of the bottomfauna in Wood, Kalamalka, and Skaha lakes in the Okanagan valley, B.C. *Fish. Res. Bd. Can. Tech. Rept. No.* 342.
- Schneiderman, H. A. 1972. Insect hormones and insect control, p. 3-27. In J. J. Menn and M. Beroza, eds. *Insect juvenile hormones.* Academic Press, New York. 341p.

- Schooley, D. A., B. J. Bergot, L. L. Dunham, J. B. Siddall. 1975. Environmental degradation of the insect growth regulator methoprene (isopropyl (2E,4E)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate). II. Metabolism by aquatic microorganisms. *J. Agric. Food Chem.* 23:293-8.
- Slama, K. 1971. Insect juvenile hormones analogues. *Ann. Rev. Biochem.* 40:1079-1102.
- Slama, K., M. Romanuk, and F. Sorm. 1974. Insect hormones and bioanalogs. Springer-Verlag, New York. 477 p.
- Spielman, A., and V. Skaff. 1967. Inhibition of metamorphosis and ecdysis in mosquitoes. *J. Insect. Physiol.* 13:1087-95.
- Srivastava, U. S., and L. I. Gilbert. 1968. Juvenile hormone: effects on a higher dipteran. *Science* 161:61-2.
- Staal, G. B. 1972. Biological activity and bioassay of juvenile hormone analogs, p. 69-94. In J. J. Menn and M. Beroza, eds. *Insect juvenile hormones*. Academic Press, New York. 341 p.
- Staal, G. B. 1975. Insect growth regulators with juvenile hormone activity. *Ann. Rev. Entomol.* 20:417-60.
- Terriere, L. C., and S. J. Yu. 1973. Insect juvenile hormones: induction of detoxifying enzymes in the house fly and detoxification by house fly enzymes. *Pestic. Biochem. Physiol.* 3:96-107.
- Thimijan, R. W., L. G. Pickens, N. O. Morgan, and R. W. Miller. 1972. House fly capture as a function of number of traps in a dairy barn. *J. Econ. Entomol.* 65:876-7.
- Yu, S. J., and L. C. Terriere. 1971. Hormonal modification of microsomal oxidase activity in the house fly. *Life Sci.* 10:1173-85.
- Yu, S. J., and L. C. Terriere. 1973. Phenobarbital induction of detoxifying enzymes in resistant and susceptible house flies. *Pestic. Biochem. Physiol.* 3:141-8.
- Yu, S. J., and L. C. Terriere. 1974a. A possible role for microsomal oxidases in metamorphosis and reproduction in the house fly. *J. Insect Physiol.* 20:1901-12.
- Yu, S. J., and L. C. Terriere. 1974b. Bimodal effect of methylenedioxyphenyl compounds on detoxifying enzymes in the house fly. *Pestic. Biochem. Physiol.* 4:160-9.

- Yu, S. J., and L. C. Terriere. 1975. Microsomal metabolism of juvenile hormone analogs in the house fly, Musca domestica L. Pestic. Biochem. Physiol. 5:418-30.
- Weber, C. I., ed. 1973. Biological field and laboratory methods for measuring the quality of surface waters and effluents. National Environmental Research Center, Cincinnati, Ohio. 186 p.
- West, L. S. 1951. The housefly. Comstock Publishing Co., Inc., New York. 584 p.
- White, A. F. 1972. Metabolism of the juvenile hormone analogue methyl farnesoate 10,11-epoxide in two insect species. Life Sci. 2:201-10.
- Wicht, M. C., Jr., and J. G. Rodriguez. 1970. Integrated control of muscid flies in poultry houses using predator mites, selected pesticides and microbial agents. J. Med. Entomol. 7:687-92.
- Williams, C. M. 1956. The juvenile hormone of insects. Nature 178: 212-13.
- Williams, C. M. 1967. Third-generation pesticides. Scientific American 217:13-17.

APPENDIX

Treatment Effect on Non-Target Insects

The insects collected and their seasonal totals (when counted) are presented for both houses in 1974 in Table 1 for the light trap catches, Table 2 for the emergence core sample collections, and Table 3 for the Berlese core sample collections. The larviciding by the farm manager on 7 July prevented my taking of any pretreatment samples in 1974. Also, the insect fauna of the two houses often did not contain the same species or adequate densities to compare the same species if they occurred in both houses. These factors and the fact that there was no interruption in the methoprene treatment, negates any possibility of making valid decisions about the effect of the treatment on non-target insects.

In 1975, however, pretreatment samples were taken from the treated house. The insects collected and their seasonal totals (when counted) are presented for both houses in 1975 in Table 4 for the light trap catches, Table 5 for the emergence core sample collections, and Table 6 for the Berlese core sample collections. Since the primary objective of this study was to evaluate the effect of the methoprene treatment on house fly emergence and the resulting adult populations, the emergence and Berlese core samples were collected from sites showing signs of larval house fly activity. As a result, the emergence and Berlese core samples were of little value in quantitative evaluation of the effect of the methoprene treatment on non-target insects. In addition, as a result of this bias, some of the insect species breeding in the manure

Table 1. The insects and their seasonal totals caught in the UV light traps in both houses in 1974.^a

Taxa	Seasonal totals	
	Untreated house	Treated house
Coleoptera	57	72
Scarabaeidae	36	9
Lepidoptera	358	733
Diptera	7,373	16,781
Tipulidae	348	293
<u>Limonia</u>	11	11
<u>distincta</u>	7	6
<u>domestica</u>	3	1
<u>globithorax</u>		1
<u>macateei</u>		1
<u>pudica</u>		1
<u>shannoni</u>		1
<u>Dicranota</u>	1	
<u>Limnophila</u>		1
<u>subtenuicornia</u>		1
<u>Neolimnophila</u>		1
<u>Gnophomyia</u>	2	6
<u>tristissima</u>	2	6
<u>Erioptera</u>	326	272
<u>calipteri</u>	232	189
<u>parva</u>	36	44
<u>septemtrionis</u>	58	38
<u>vespertina</u>		1
<u>Ormosia</u>	3	1
Psychodidae	903	888
<u>Pericoma</u>	*	*
<u>scotiae</u>	*	*
<u>Psychoda</u>	*	*
<u>alternata</u>	*	*
Culicidae	96	98
<u>Aedes</u>		*
<u>trivittatus</u>		*
<u>Culex</u>		*
<u>salinarius</u>		*
Ceratopogonidae	712	1,161
<u>Atrichopogon</u>		*
<u>levis</u>		*
<u>Forcipomyia</u>		*
Chironomidae	218	174

Table 1. (continued)

Taxa	Seasonal totals	
	Untreated house	Treated house
Simuliidae	3	34
<u>Simulium</u>		*
<u>vittatum</u>		*
Anisopodidae	52	89
<u>Sylvicola</u>	52	89
<u>alternatus</u>	2	75
<u>marginatus</u>	50	14
Mycetophilidae	178	531
<u>Orfelia</u>	146	447
<u>discoloria</u>	3	7
<u>elegans</u>	138	439
<u>genualis</u>	1	
<u>mendosa</u>	1	
<u>mimula</u>	3	1
<u>Exechia</u>	2	13
<u>absurda</u>		1
<u>bifurcata</u>		1
<u>nativa</u>	1	1
<u>perspicua</u>	1	10
<u>Rhymosia</u>		1
<u>triangularis</u>		1
<u>Mycetophila</u>	12	6
<u>extincta</u>		1
<u>fungorum</u>	10	1
<u>sigmoides</u>		1
<u>unipunctata</u>	2	3
<u>Zygomia</u>	1	
<u>ornata</u>		1
<u>Mycomya</u>	1	7
<u>Acnemia</u>	3	
<u>flaveola</u>	3	
<u>Megalopeima</u>	2	
<u>glabanum</u>	2	
<u>Leia</u>	12	52
<u>bivittata</u>	6	18
<u>decora</u>	3	11
<u>oblectabilis</u>	3	23
<u>Tetragoneura</u>		2
<u>pimpla</u>		2

Table 1. (continued)

Taxa	Seasonal totals	
	Untreated house	Treated house
Sciaridae	129	86
<u>Trichosa</u>	1	
<u>hebes</u>	1	
<u>Sciara</u>	51	15
<u>ochrolabis</u>	3	2
<u>psittacus</u>	1	
<u>sciophila</u>	33	9
<u>Bradysia</u>	77	69
<u>falcata</u>	1	
<u>diluta</u>		1
<u>ocellaris</u>	21	10
<u>prolifera</u>		4
<u>silvestrii</u>	14	22
Scatopsidae	22	19
<u>Scatopse</u>		19
<u>fuscipes</u>		19
Cecidomyiidae	485	85
<u>Porricondyla</u>		*
<u>borealis</u>		*
<u>Rhabdophaga</u>		*
Xylomyiidae	12	26
<u>Solva</u>	12	26
<u>pallipes</u>	12	26
Stratiomyiidae	2	14
<u>Sargus</u>	1	4
<u>elegans</u>	1	4
<u>Microchrysa</u>		3
<u>polita</u>		3
Tabanidae		2
Rhagionidae	4	7
Asilidae		2
<u>Psilonyx</u>		1
<u>annulatus</u>		1
<u>Cerotainia</u>		1
<u>albipilosa</u>		1
Empididae	7	50
<u>Syneches</u>	7	49
<u>Platypalpus</u>		1
<u>aequalis</u>		1

Table 1. (continued)

Taxa	Seasonal totals	
	Untreated house	Treated house
Dolichopodidae	120	121
<u>Mesorhaga</u>		1
<u>Condylostylus</u>	12	28
<u>flavipes</u>		27
<u>nigrofemoratus</u>		1
<u>Sciapus</u>		1
<u>unicoiensis</u>		1
<u>Dolichopus</u>	103	81
<u>Gymnopternus</u>		1
<u>flavus</u>		1
<u>Thinophilus</u>	1	
<u>Rhaphium</u>	1	
<u>Neurigona</u>	1	7
<u>disjuncta</u>		4
<u>floridula</u>		1
<u>Chrysotus</u>	2	
Lonchopteridae	1	2
<u>Lonchoptera</u>		2
Pipunculidae	1	2
Syrphidae	1	4
Micropezidae		1
Otitidae	1	
Platystomatidae	1	2
<u>Rivellia</u>		*
<u>viridulans</u>		*
Tephritidae		1
Sepsidae	9	44
<u>Meroplius</u>	6	42
<u>stercorarius</u>	6	42
<u>Sepsis</u>		1
<u>Themira</u>	3	
Lauxanidae	1	5
<u>Lyciella</u>		*
<u>pictiventris</u>		*
<u>Sapromyza</u>		*
<u>rotundicornis</u>		*
Chamaemyiidae	1	
Piophilidae	1	
Sphaeroceridae	69	154
<u>Leptocera</u>		*

Table 1. (continued)

Taxa	Seasonal totals	
	Untreated house	Treated house
Milichiidae	12	16
<u>Desmometopa</u>	*	*
Ephydriidae	5	29
<u>Hydrellia</u>	1	
<u>Brachydeutera</u>	4	26
<u>argentata</u>	4	26
<u>Scatella</u>		1
<u>Scatophila</u>		2
Curtonotidae		1
Drosophilidae	14	41
<u>Drosophila</u>	8	2
<u>Scaptomyza</u>	2	38
<u>Chymomyza</u>	4	1
Chloropidae	13	31
<u>Hippelates</u>	3	3
<u>pallipes</u>	3	3
<u>Elachiptera</u>	5	2
<u>Oscinella</u>	3	2
<u>Dicraeus</u>		6
<u>Olcella</u>	3	10
<u>parva</u>	2	10
<u>Tricimba</u>	2	6
<u>Chaetochlorops</u>		1
<u>Meromyza</u>		1
Agromyzidae	4	76
<u>Japanagromyza</u>		*
<u>viridula</u>		*
Anthomyziidae		1
Opomyzidae	1	
Anthomyiidae	6	24
<u>Chiastocheta</u>		1
<u>Hylemya</u>	3	2
<u>Pegomyia</u>		3
<u>Hydrophora</u>	1	1
<u>subpellucida</u>	1	1
<u>Anthomyia</u>	1	17
<u>pluvialis</u>	1	17
Muscidae	3,923	12,626

Table 1. (continued)

Taxa	Seasonal totals	
	Untreated house	Treated house
<u>Coenosia</u>	14	62
<u>alticola</u>	1	
<u>atrata</u>	1	
<u>basalis</u>	1	
<u>humilis</u>	9	42
<u>ovata</u>	2	14
<u>rufitibia</u>		5
<u>Lispe</u>		2
<u>sociabilis</u>		2
<u>Helina</u>	*	7
<u>punctata</u>	1	*
<u>procedens</u>	*	*
<u>Hebecnema</u>		1
<u>fumosa</u>		1
<u>Fannia</u>	93	179
<u>canicularis</u>	6	15
<u>manicata</u>		1
<u>scalaris</u>	7	59
<u>snyderi</u>	83	104
<u>Hydrotaea</u>		4
<u>occulta</u>		4
<u>Neohydrotaea</u>	1	
<u>Ophyra</u>	5	20
<u>leucostoma</u>	5	20
<u>Bigotomyia</u>	29	1
<u>houghii</u>	29	1
<u>Musca</u>	3,755	9,254
<u>domestica</u>	3,755	9,254
<u>Haematobia</u>	9	17
<u>irritans</u>	9	17
<u>Stomoxys</u>	15	6
<u>calcitrans</u>	15	6
Calliphoridae	2	8
Sarcophagidae	7	4
<u>Helicobia</u>		*
Tachinidae	7	38
<u>Melanophora</u>		1
<u>roralis</u>		1
<u>Gymnosoma</u>		1

Table 1. (continued)

Taxa	Seasonal totals	
	Untreated house	Treated house
<u>Alophora</u>		2
<u>Apachemyia</u>		1
<u>Pseudosiphona</u>	5	20
<u>brevirostris</u>	5	20
<u>Pseudapinops</u>		1
<u>niger</u>		1
Hymenoptera	57	162
Braconidae	40	90
Ichneumonidae	13	61
Figitidae		*
Other insect orders	415	564

^a The collective species totals under a particular genus may not add up to the total for that genus. The same may be true for the collective genera totals under a particular family. This indicates that some specimens were not or could not be identified past the family or genus level. An asterisk denotes that that taxa was present but not counted, and a blank space indicates that that taxa was not present.

Table 2. The insects and their seasonal totals collected from the emergence core samples from both houses in 1974.^a

Taxa	Seasonal totals	
	Untreated house	Treated house
Psocoptera	1	
Psocidae	1	
Coleoptera	13	667
Histeridae	12	665
<u>Carcinops</u>	12	665
<u>quatuordecimstriata</u>	12	665
Staphylinidae	1	2
Lepidoptera	6	10
Tineidae	6	10
<u>Tinea</u>	6	10
<u>fuscipunctella</u>	6	10
Diptera	12,816	23,895
Psychodidae	1	
Chironomidae	1	1
<u>Glyptotendipes</u>	1	1
Sciaridae		1
<u>Bradysia</u>		1
Scatopsidae	2	1
<u>Scatopse</u>	2	1
<u>fuscipes</u>	2	1
Sepsidae	6	1
<u>Meroplius</u>	3	1
<u>stercorarius</u>	3	1
<u>Sepsis</u>	1	
<u>biflexuosa</u>	1	
<u>Themira</u>	2	
<u>flavicoxa</u>	2	
Sphaeroceridae	*	*
Milichidae	129	147
<u>Neophyllomyza</u>	3	
<u>Leptometopa</u>	8	1
<u>Desmometopa</u>	118	146
<u>mnigrum</u>	118	146
Drosophilidae	9	4
<u>Drosophila</u>	9	4
<u>busckii</u>	*	*
Muscidae	12,668	23,738

Table 2. (continued)

Taxa	Seasonal totals	
	Untreated house	Treated house
<u>Fannia</u>	38	19
<u>canicularis</u>	2	1
<u>snyderi</u>	36	18
<u>Ophyra</u>	6	1
<u>leucostoma</u>	6	1
<u>Bigotomyia</u>	19	
<u>houghii</u>	19	
<u>Musca</u>	12,599	23,712
<u>domestica</u>	12,599	23,712
<u>Stomoxys</u>	6	6
<u>calcitrans</u>	6	6
Calliphoridae		2
<u>Phormia</u>		1
<u>regina</u>		1
<u>Calliphora</u>		1
<u>vicina</u>		1
Hymenoptera	7	1
Braconidae		1
Mymaridae	1	
Formicidae	6	

^a An asterisk denotes that that taxa was present but not counted, and a blank space indicates that that taxa was not present.

Table 3. The insects and their seasonal totals collected from the Berlese core samples from both houses in 1974.^a

Taxa	Seasonal totals	
	Untreated house	Treated house
Immatures		
Hemiptera	1	24
Anthocoridae	1	24
Coleoptera	*	*
Histeridae	*	*
Staphylinidae	2	12
Cryptophagidae	7	
Colydiidae		4
Lepidoptera		2
Pyralidae		2
Diptera	*	*
Psychodidae		2
Stratiomyiidae	1	7
Sphaeroceridae	*	*
Drosophilidae	1	
Muscidae	*	*
<u>Fannia</u>	57	20
<u>Musca</u>	*	*
<u>domestica</u>	*	*
Adults		
Coleoptera	12	822
Histeridae	5	790
<u>Carcinops</u>	5	790
<u>quatuordecimstriata</u>	5	790
Hydrophilidae		2
<u>Cercyon</u>		2
Staphylinidae	4	18
<u>Nudobius</u>		1
<u>Oxyporus</u>		12
<u>Staphylinus</u>		5
<u>Gyrophæna</u>	1	
<u>Ontholestes</u>	1	
<u>Falagria</u>	1	
<u>Lobrathium</u>	1	
Erotylidae	1	
Cucujidae	2	1

Table 3. (continued)

Taxa	Seasonal totals	
	Untreated house	Treated house
Nitidulidae		4
Colydiidae		5
Meloidae		1
Tenebrionidae		1
Lepidoptera	*	*
Diptera	*	*
Tipulidae	*	*
Psychodidae	*	*
Ceratopogonidae	*	*
Chironomidae	*	*
Sciaridae	*	*
Scatopsidae	*	*
Cecidomyiidae	*	*
Sphaeroceridae	*	*
Drosophilidae	*	*
Muscidae	*	*
Calliphoridae	*	*
Hymenoptera	*	*
Braconidae	*	*
Mymaridae	*	*

^a The collective species totals under a particular genus may not add up to the total for that genus. The same may be true for the collective genera totals under a particular family. This indicates that some specimens were not or could not be identified past the family or genus level. An asterisk denotes that that taxa was present but not counted, and a blank space indicates that that taxa was not present.

Table 4. The insects and their seasonal totals caught in the UV light traps in both houses in 1975.^a

Taxa	Seasonal totals	
	Untreated house	Treated house
Coleoptera	4	42
Scarabaeidae	*	*
Lepidoptera	56	489
Diptera	1,107	3,151
Tipulidae	2	40
<u>Limonia</u>		6
<u>distincta</u>		6
<u>Antocha</u>		4
<u>opalizans</u>		*
<u>Dicranota</u>		1
<u>Gnophomyia</u>		5
<u>tristissima</u>		5
<u>Erioptera</u>	2	24
<u>calipteri</u>	1	8
<u>cana</u>		1
<u>parva</u>		7
<u>septemtrionis</u>	1	8
Psychodidae	55	270
<u>Pericoma</u>	16	6
<u>scotiae</u>	16	6
<u>Psychoda</u>	38	242
<u>alternata</u>	22	130
<u>satchelli</u>	16	112
Culicidae	1	4
Ceratopogonidae	39	252
Chironomidae	22	241
<u>Orthocladus</u>		2
<u>Cricotopus</u>	1	17
<u>infuscatus</u>	1	6
<u>varipes</u>		6
<u>Metriochemus</u>		2
<u>Chironomus</u>		4
<u>attenuatus</u>		1
<u>Cryptochironomus</u>	4	15
<u>fulvus</u>	4	14
<u>Polypedilum</u>	6	108
<u>scalaenum</u>	6	108
<u>Stenochironomus</u>	1	3
<u>hilaris</u>	1	3

Table 4. (continued)

Taxa	Seasonal totals	
	Untreated house	Treated house
<u>Tanytarsus</u>		1
Simuliidae		5
Anisopodidae	1	3
<u>Sylvicola</u>	1	3
<u>marginatus</u>	1	3
Mycetophilidae	12	277
<u>Keroplatus</u>		1
<u>cressoni</u>		1
<u>Macrocera</u>		7
<u>formosa</u>		6
<u>inconcinna</u>		1
<u>Orfelia</u>	8	210
<u>discoloria</u>		9
<u>elegans</u>	8	192
<u>genualis</u>		2
<u>mendosa</u>		5
<u>pellita</u>		2
<u>Exechia</u>	1	1
<u>quadrata</u>	1	1
<u>Rhymosia</u>		1
<u>inflata</u>		1
<u>Mycetophila</u>		3
<u>fungorum</u>		1
<u>luctuosa</u>		1
<u>unipunctata</u>		1
<u>Phronia</u>		1
<u>venusta</u>		1
<u>Mycomya</u>		14
<u>dentata</u>		2
<u>obliqua</u>		8
<u>onusta</u>		1
<u>Monoclona</u>		2
<u>elegantula</u>		2
<u>Sciophila</u>		2
<u>habilis</u>		1
<u>nugax</u>		1
<u>Leia</u>	3	29
<u>bivittata</u>	1	14
<u>oblectabilis</u>	2	15

Table 4. (continued)

Taxa	Seasonal totals	
	Untreated house	Treated house
<u>Tetragoneura</u>		2
<u>pimpla</u>		2
Sciaridae	4	140
<u>Sciara</u>		12
<u>ochrolabis</u>		2
<u>sciophila</u>		8
<u>Lycoriella</u>		1
<u>agravia</u>		1
<u>Zygoneura</u>		2
<u>flavicoxa</u>		2
<u>Bradysia</u>	4	123
<u>diluta</u>		3
<u>fumida</u>		6
<u>ocellaris</u>	4	26
<u>prolifera</u>		1
<u>silvestrii</u>		14
<u>Peyerimhoffia</u>		1
Scatopsidae	4	39
Cecidomyiidae	79	970
Xylomyiidae		39
<u>Solva</u>		38
<u>pallipes</u>		38
Stratiomyiidae		19
<u>Sargus</u>		5
<u>elegans</u>		1
<u>lucens</u>		4
<u>Ptecticus</u>		14
<u>sackenii</u>		1
<u>trivittatus</u>		13
Tabanidae		1
Rhagionidae		6
<u>Chrysopilus</u>		6
Asilidae		2
Empididae		15
<u>Hybos</u>		1
<u>Syneches</u>		1
<u>Hormopeza</u>		2
<u>Oreogeton</u>		1
<u>Hilara</u>		1
<u>Rhamphomyia</u>		1

Table 4. (continued)

Taxa	Seasonal totals	
	Untreated house	Treated house
<u>Proclinopyga</u>		3
<u>Hemerodromia</u>		3
<u>Platypalpus</u>		2
<u>aequalis</u>		2
Dolichopodidae	1	179
<u>Condyllostylus</u>	1	13
<u>calcaratus</u>		1
<u>flavipes</u>	1	3
<u>sipho</u>		9
<u>Dolichopus</u>		117
<u>cuprinus</u>		1
<u>longipennis</u>		22
<u>reflectus</u>		2
<u>scapularis</u>		12
<u>vittatus</u>		2
<u>Gymnopternus</u>		7
<u>vernaculus</u>		2
<u>Tachytrechus</u>		12
<u>protervus</u>		12
<u>Neurigona</u>		1
<u>deformis</u>		1
<u>Diaphorus</u>		19
<u>leucostoma</u>		1
<u>spectabilis</u>		3
<u>variabilis</u>		1
<u>Chrysotus</u>		4
<u>pallipes</u>		*
<u>Campsicnemus</u>		6
<u>americanus</u>		4
<u>hirtipes</u>		1
Lonchopteridae		1
Phoridae		9
<u>Diplonevra</u>		*
<u>Megaselia</u>		*
Syrphidae	2	11
Otitidae	1	2
Tephritidae		1
Sepsidae		5
<u>Mercopilus</u>		3
<u>stercorarius</u>		3
<u>Sepsis</u>		2

Table 4. (continued)

Taxa	Seasonal totals	
	Untreated house	Treated house
Lauxanidae		3
Chamaemyiidae		1
Piophilidae		2
Lonchaeidae		2
Sphaeroceridae		53
Milichiidae	1	
Ephydriidae	1	1
<u>Brachydeutera</u>	1	1
<u>argentata</u>	1	1
Drosophilidae	3	30
<u>Rhinoleucophenga</u>		2
<u>Drosophila</u>	1	16
<u>Scaptomyza</u>	2	11
<u>Chymomyza</u>		1
Chloropidae		26
<u>Hippelates</u>		10
Agromyzidae		14
Clusiidae		1
Chryomyidae		1
Anthomyiidae	1	5
<u>Scatophaga</u>	1	1
<u>Pegomyia</u>		1
<u>Hydrophoria</u>		1
<u>Anthomyia</u>		2
<u>pluvialis</u>		2
Muscidae	846	469
<u>Coenosia</u>		4
<u>ovata</u>		2
<u>rufitibia</u>		2
<u>Helina</u>	4	1
<u>obscurata</u>	4	1
<u>Fannia</u>	749	7
<u>canicularis</u>	10	2
<u>scalaris</u>	1	2
<u>snyderi</u>	738	3
<u>Bigotomyia</u>	7	8
<u>houghii</u>	7	8
<u>Musca</u>	73	448
<u>domestica</u>	73	448

Table 4. (continued)

Taxa	Seasonal totals	
	Untreated house	Treated house
<u>Stomoxys</u>	9	
<u>calcitrans</u>	9	
Calliphoridae	29	1
Sarcophagidae	1	6
Tachinidae		5
<u>Pseudosiphona</u>		2
<u>brevirostris</u>		2
Hymenoptera	16	176
Braconidae	13	132
Ichneumonidae		26
Other insect orders	101	5,488

^a The collective species totals under a particular genus may not add up to the total for that genus. The same may be true for the collective genera totals under a particular family. This indicates that some specimens were not or could not be identified past the family or genus level. An asterisk denotes that that taxa was present but not counted, and a blank space indicates that that taxa was not present.

Table 5. The insects and their seasonal totals collected from the emergence core samples from both houses in 1975.^a

Taxa	Seasonal totals	
	Untreated house	Treated house
Lepidoptera	1	1
Diptera	4,703	18,792
Chironomidae	2	1
<u>Cricotopus</u>	2	
<u>elegans</u>	2	
<u>Polypedilum</u>		1
<u>scalaenum</u>		1
Scatopsidae		1
<u>Scatopse</u>		1
<u>fuscipes</u>		1
Sphaeroceridae	*	*
Milichidae	5	20
<u>Leptometopa</u>	4	9
<u>Desmometopa</u>	1	11
<u>mnigrum</u>	1	11
Drosophilidae		4
<u>Drosophila</u>		4
<u>melanogaster</u>		*
Muscidae	4,618	18,752
<u>Fannia</u>	1,518	68
<u>canicularis</u>	3	
<u>snyderi</u>	1,515	67
<u>scalaris</u>		1
<u>Ophyra</u>	42	
<u>leucostoma</u>	42	
<u>Bigotomyia</u>	32	1
<u>houghii</u>	32	1
<u>Musca</u>	3,023	18,683
<u>domestica</u>	3,023	18,683
<u>Stomoxys</u>	3	
<u>calcitrans</u>	3	
Calliphoridae	78	14
<u>Phormia</u>	25	14
<u>regina</u>	25	14
<u>Phaenicia</u>	53	
<u>sericata</u>	53	

Table 5. (continued)

Taxa	Seasonal totals	
	Untreated house	Treated house
Hymenoptera	49	
Braconidae	49	
<u>Aphaereta</u>	49	
<u>muesebecki</u>	49	

^a An asterisk denotes that that taxa was present but not counted, and a blank space indicates that that taxa was not present.

Table 6. The insects and their seasonal totals collected from the Berlese core samples from both houses in 1975.^a

Taxa	Seasonal totals	
	Untreated house	Treated house
Immatures		
Diptera	*	*
Drosophilidae		1
Sphaeroceridae	*	*
Muscidae	*	*
<u>Fannia</u>	1,393	59
<u>Musca</u>	*	*
<u>domestica</u>	*	*
Calliphoridae	*	*
Adults		
Coleoptera	4	3
Histeridae	1	
<u>Saprinus</u>	1	
Staphylinidae	2	3
<u>Sunius</u>		1
<u>Oxyporus</u>		2
<u>Aleochara</u>	2	
Lepidoptera	*	*
Diptera	*	*
Ceratopogonidae	*	*
Scatopsidae	*	*
Sphaeroceridae	*	*
Drosophilidae	*	*
Muscidae	*	*
Calliphoridae	*	*
Hymenoptera	*	*
Mymaridae	*	*
Encyrtidae		*
Pteromalidae	*	*

^a An asterisk denotes that that taxa was present but not counted, and a blank space indicates that that taxa was not present.

and surrounding water in the chicken house were not included in the emergence or Berlese core samples. Many of the non-target insects would have been displaced by competition from the house fly larvae. Also, the core samples were taken from a relatively narrow range of moisture content (58 to 83 percent for both houses in 1975). The houses in both seasons contained manure ranging from relatively dry (less than 40 percent) to areas where the manure was completely submerged under water. Neither of these extremes showed any signs of larval activity of the house fly; therefore, these areas were never sampled. In 1975, the mean moisture content of the manure core samples was 76 ± 3.2 percent from the untreated house and 72 ± 5.3 percent from the treated house.

The possibility of this sampling bias was anticipated. Therefore, it was hoped that the use of UV light traps to sample the adult populations would offset any quantitative or qualitative deficiencies which might result from the sampling bias. In 1975, of all the insects either collected from the emergence or Berlese core samples or known from the literature to be capable of breeding in this environment, only three non-target species were consistently caught in the light traps in numbers sufficient for evaluation. These were Psychoda alternata Say, Psychoda satchelli Quate (Diptera: Psychodidae), and Polypedilum scalaenum (Schrank) (Diptera: Chironomidae).

Neither of the two psychodids were recovered from the emergence or Berlese samples. However, both are well known, particularly P. alternata, as breeders in aquatic and semiaquatic habitats contaminated

with manure or other organic wastes (Quate 1955). It is not surprising that the psychodids were not recovered from the emergence or Berlese core samples because samples were not taken from collection sites with a moisture content above 83 percent. Adults of both species were caught in the light traps in both houses in 1975. However, P. satchelli was not caught consistently in the untreated house in numbers sufficient for evaluation.

P. scalaenum was reared from the emergence samples in the treated house (Table 5). In addition, P. scalaenum is known to breed in diverse aquatic habitats (Mason 1974) and in those habitats heavily contaminated with organic matter (Saether and McLean 1972; Lehmann 1971; Weber 1973). The adults of P. scalaenum were caught in the light traps in both houses in 1975, but they were not caught consistently in the untreated house in numbers sufficient for evaluation.

The trap catch totals are presented graphically for P. alternata in Fig. 1, P. satchelli in Fig. 2, and P. scalaenum in Fig. 3. The increases in the trap catch totals from the treated house of all three species were remarkably similar. All three species were apparently affected by the methoprene treatment. Although the response of these three species to appropriate doses of exogenously applied methoprene has not been previously documented, it is probably similar to that of the house fly i.e. failure of the adult to emerge from the puparium. Miura and Takahashi (1973) confirmed this for another psychodid (Pericoma sp.). Among chironomids, this was confirmed for several species by Miura and Takahashi (1973), Mulla et al. (1974), and Gelbic

Fig. 1. Total number of Psychoda alternata adults caught in the UV light traps on each sampling date in the treated and untreated houses in 1975.

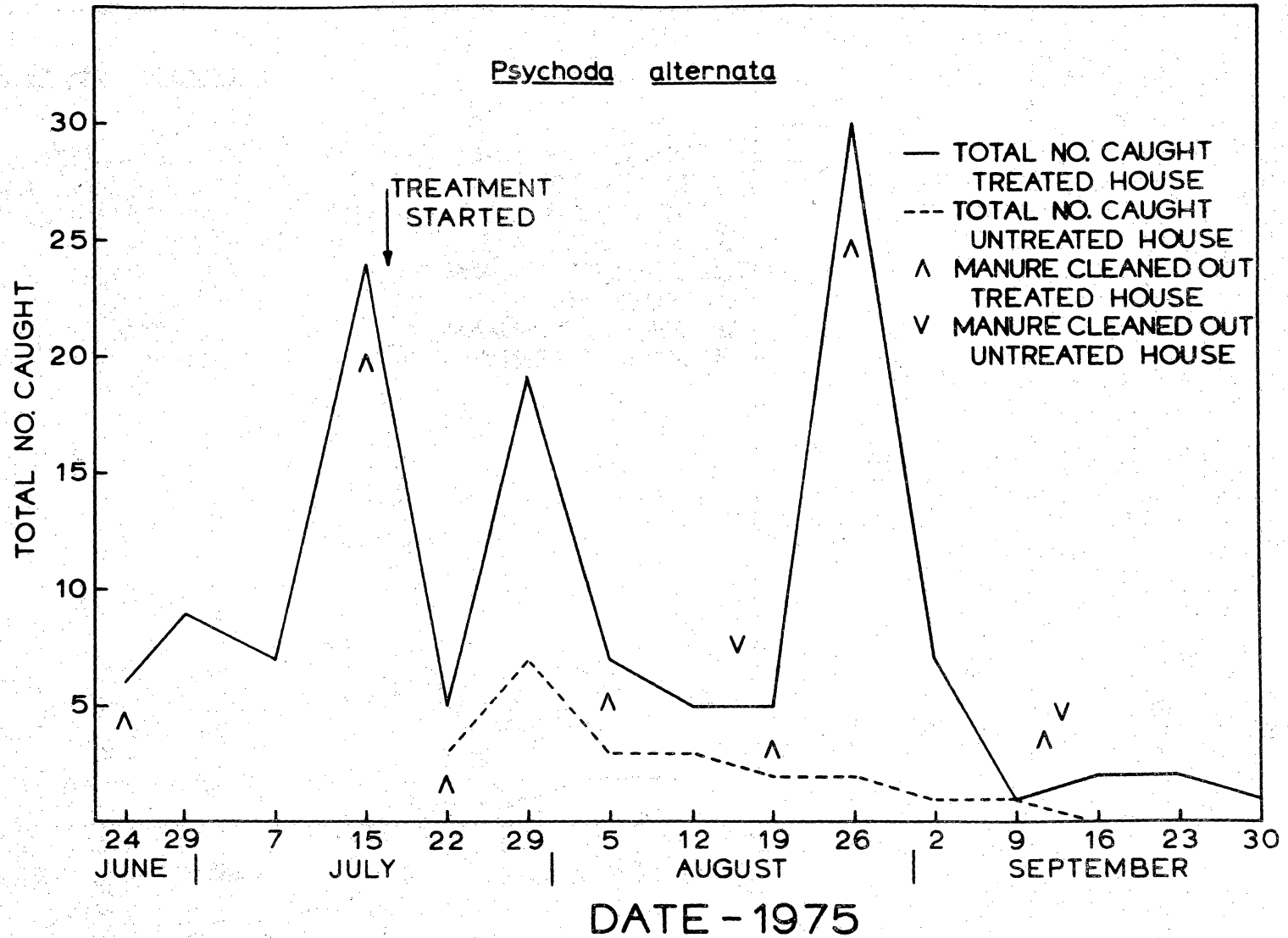


Fig. 2. Total number of Psychoda satchelli adults caught in the UV light traps on each sampling date in the treated house in 1975.

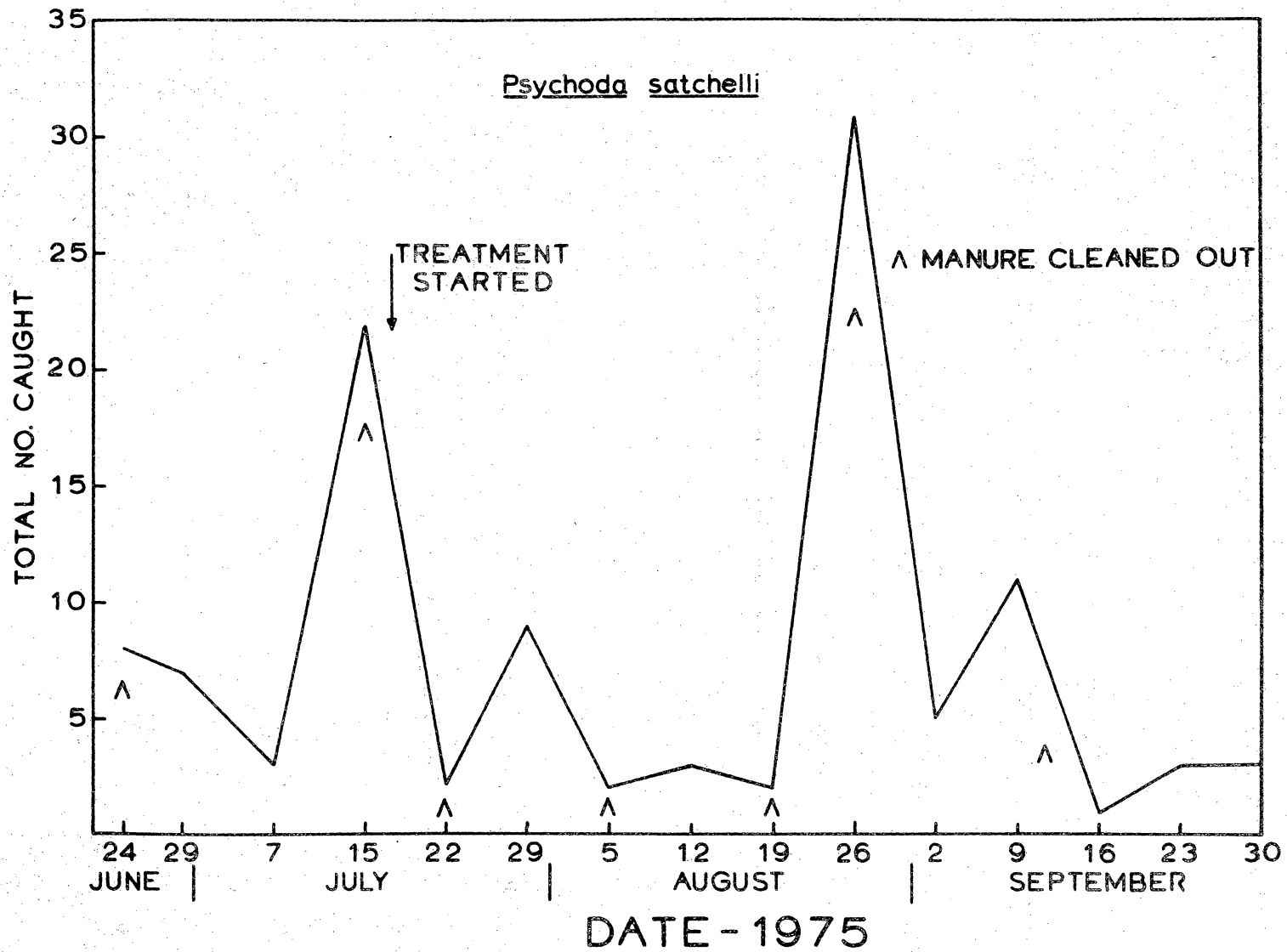
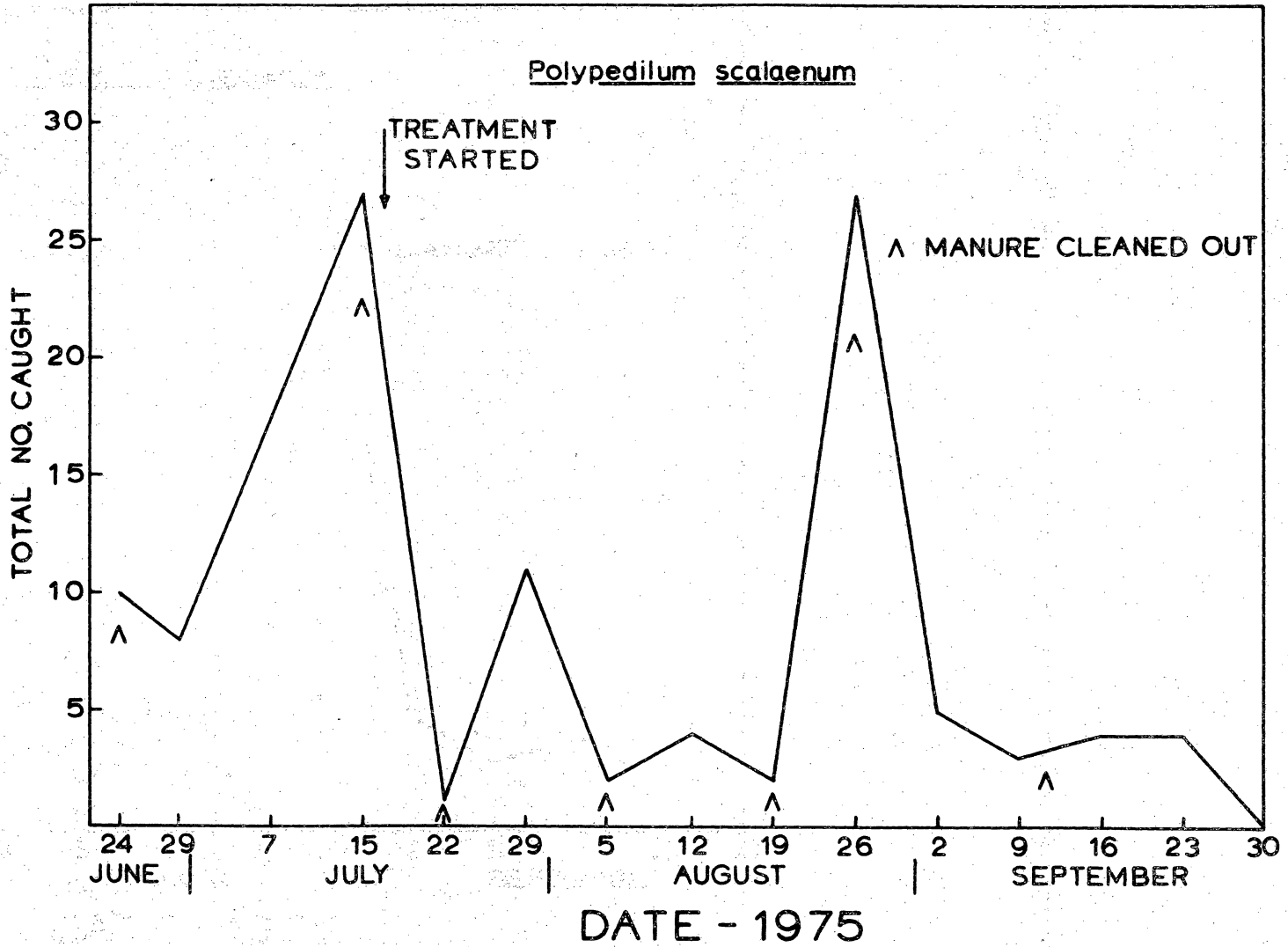


Fig. 3. Total number of Polypedilum scalaenum adults caught in the UV light traps on each sampling date in the treated house in 1975.



and Sehna1 (1974). Similarly, these three species' period of sensitivity to methoprene is probably the latter part of the last larval instar up until pupation. This sensitivity period is apparently shared by all Diptera in response to exogenous applications of JH or JHA (Staal 1972). Among the Nematocera, this was confirmed with methoprene for culicids by Spielman and Skaff (1967) and chironomids by Gelbic and Sehna1 (1974).

The adult population of all three species generally increased until 15 July in the treated house. Then, after treatment began, they decreased sharply until 22 July. Since treated feed did not reach the birds until 20 July, the methoprene treatment probably did not cause this decrease in the adult populations. Quate (1955) found that the length of the pupal stage was about 5 days for P. alternata and 6 days for P. satchelli. The length of the pupal stage for P. scalaenum has apparently not been published. However, Darby (1962) found that for several other chironomid species the average length of the pupal stage was around 2.5 to 3 days. Since the pupal stage is insensitive to methoprene, any individuals of these three species which were ready to emerge between 20 July and 22 July could not have been inhibited by the methoprene treatment. Consequently, the decrease in the adult populations from 15 July to 22 July was probably caused primarily by trapping and to a lesser extent by the house being cleaned on 15 July. Some of the immature stages of these three species must have been removed by the cleaning process. The increase in the adult populations after the cleaning on 22 July and the decrease after 29 July without a cleaning

indicate the minor role of the cleaning process in reducing the adult populations. This is understandable because, as mentioned before, the cleaning process was not thorough. After cleaning, there was usually about 5 cm of manure left on each side and about 2 cm of manure left on the bottom of each pit row. Also, much of the accumulated water in each pit row was left behind after cleaning. Often, this water completely covered the manure remaining on the bottom of the pit rows. In addition, the biology and behavior of these three species may have helped prevent their removal during cleaning. P. scalaenum is a tube building bottom dweller (Lehmann 1971), and the larvae of the two psychodids collapse their caudal fans and sink to the bottom when disturbed in an aquatic habitat (Quate 1955).

The adult populations of all three species increased from 22 July to 29 July and then decreased and remained at low levels until 19 Aug. The relatively smaller size of the peak on 29 July was probably caused by the methoprene treatment. However, the increase from 22 July to 29 July indicates that some individuals avoided effective treatment either by already being in the pupal stage by 20 July or by being in areas where the methoprene had not yet reached effective levels. The sharp decrease in adult populations from 29 July to 5 Aug. was probably caused by the previous trapping.

The adult populations of all three species increased from 19 Aug. to 26 Aug. and then decreased sharply, possibly as a result of the previous trapping, until 2 Sept. This peak equaled or surpassed the pre-treatment levels. No such peak occurred in the adult population of

P. alternata in the untreated house. This third peak is the strongest indicator that these species were affected by the methoprene treatment because it corresponded closely with the interruption in the treatment from 19 Aug. to 23 Aug. A local shortage of corn required bringing in 13,000 lbs. of feed from another feed mill. Since the birds in the treated house had run out of feed on 18 Aug., the feed was delivered to the house on 19 Aug. untreated. This untreated feed ran out on 23 Aug., and later that day, 9,000 lbs. of treated feed was delivered. This allowed an actual interruption of around 7 days because the cycle time for methoprene through the birds is at least 1 day. Therefore, this interruption allowed ample time for these three species to have avoided effective treatment and, thus, caused the resulting increases in the adult populations.

After 2 Sept., the adult populations of P. alternata and P. scalaenum decreased further and remained at low levels for the remainder of the test period. The adult population of P. satchelli peaked again on 9 Sept. This was caused by some individuals which avoided effective treatment in areas where the methoprene had not yet reached effective levels. After 9 Sept., the adult population decreased and remained at low levels for the remainder of the test period.

Even though the trap catch totals of P. satchelli and P. scalaenum from the untreated house were too low and infrequent to use for comparisons, it is clear that all three species were affected by the methoprene treatment. The remarkable similarity between the increases and decreases in the trap catch totals from these three different

species in the treated house and the common peak in the adult populations on 26 Aug. confirm that the methoprene was substantially inhibiting the emergence of these species. Although an accurate estimate of the percentage of inhibition cannot be determined from the light trap catches, the adult populations of all three species were reduced by 80 percent or more based on pretreatment levels.

These results for P. scalaenum are not surprising because methoprene was proven in the field to provide excellent control of nuisance chironomids arising from lakes and ponds in California (Mulla et al. 1974, 1976). However, no such tests have been published for psychodids. Psychodids often become a nuisance, particularly P. alternata (Quate 1955), in the warmer months by emerging in great numbers from sewage disposal plants (James and Harwood 1969). This is also true for several species of chironomids (Fagan and Enns 1966). Therefore, field tests using methoprene for control of psychodids and chironomids arising from sewage beds and oxidation lagoons appear warranted.

**The vita has been removed from
the scanned document**

METHOPRENE AS A POULTRY FEED ADDITIVE
FOR HOUSE FLY CONTROL

by

Gary Clinton Breeden

(ABSTRACT)

Methoprene was evaluated in commercial caged-layer houses as a poultry feed additive at 7.5 ppm (AI in feed) in the 1974 fly season and at 10 ppm (AI in feed) in the 1975 fly season. In both seasons, the methoprene treatment caused an inhibition of house fly emergence. However, the average percentage of inhibition did not exceed 53 percent in 1974 or 40 percent in 1975. Both levels of inhibition were too low to result in acceptable control of the resulting adult populations of house flies. Also, the level of inhibition gradually decreased throughout the latter part of both seasons until the methoprene treatment had little if any significant inhibition of house fly emergence. In both seasons, it was concluded that the primary causes of these problems were an existing cross-resistance to methoprene in the field strains followed by an induction in resistance resulting from the continuous exposure to methoprene.