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Evaluation of apholate chemosterilant against the little housefly Fannia canicularis Linne'.

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EVALUATION OF APHOLATE CHEMOSTERILANT AGAINST THE LITTLE HOUSEFLY FANNIA CANICULARIS LINNE'

BY

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EVALUATION OF APHOLATE CHEMOSTERILANT AGAINST THE LITTLE HOUSEFLY *FANNIA CANICULARIS* LINNE'

A Dissertation Presented

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INTRODUCTION

The annoyance caused by heavy populations of the little housefly Fannia canicularis (Linne') has often been a serious problem to poultrymen. Complaints by Massachusetts residents and poultry farmers were increasing in the early 1960's according to Steve (1960). The situation in homes grows worse as residential areas expand near poultry farms.

In addition to the concern produced by enormous numbers of Fannia canicularis, its disease producing potential as a causitive agent of intestinal and urinary myiasis of man has been noted by Hewett(1912). Lockhead (1919) reported Fannia as a carrier of the typhoid bacillus. Burnett et al (1957) reported developmental stages of the eye worm Thelazia californiensis (Price) in this fly and suspected it as an intermediate host of this parasite, the definitive hosts being man, cat, dog, deer and other mammals.

Insect chemosterilants have recently received increased attention as potential agents of insect control. Sterilization of insects using radioactive materials has been accomplished but requires expensive facilities and techniques as reported by Borkovec (1962).

Knipling (1960) has expressed the idea that chemically

produced sterility has great potential for insect control or eradication when compared to conventional insecticides. The need for new approaches to insect control has become increasingly apparent with the discovery of chlorinated hydrocarbon pesticide residues in food chains, wildlife and man.

This investigation was conducted to observe the effect of apholate chemosterilant on adult Fannia canicularis (Linne'). The information presented should be of value in conducting further experiments concerning actual field uses for control programs.

REVIEW OF LITERATURE

An insect chemosterilant may be defined as a chemical compound which when administered to an insect will deprive it of its ability to reproduce (Borkovec 1962). It has been recognized since 1916 that insects can be sterilized when Runner found that cigarette beetles (Lasioderma serricorne(F.) laid infertile eggs after exposure to ionizing radiation (Bushland et al., 1951). Muller (1928) reported that heavily irradiated male Drosophila flies, when mated to untreated females, produced eggs that failed to hatch (Bushland et al., 1951).

The use of sterilizing techniques to control certain insect populations has received much attention since the eradication of the screw-worm fly (Cochliomyia hominivorax (Coqueral) from Curacao Island and the southeastern United States by the release of male insects made sexually sterile with ionizing radiation (Baumhover et al., 1955, Knipling, 1960). Irradiation techniques require special facilities for rearing, sterilizing and releasing large numbers of irradiated insects whereas sterilization by chemicals has great potential for insect eradication when compared to the use of conventional insecticides (Borkovec 1962).

The term chemosterilant appeared first in 1960 when La Brecque et al. tested 200 compounds for their ability to sterilize or disrupt normal development in house flies (Borkovec 1962). Ten compounds of the 200 chemicals tested affected development with amethopterin the most active causing sterility in female flies only. Sterility was caused by use of the chemical in a single initial feeding. Colchicine, a compound long known for its mutagenic effects on plants and animals, was fed to screw-worm fly larvae but caused high mortality at the 5 ppm concentration in larval media (Chamberlain and Hopkins 1950). Mitlin (1957) found that colchicine when fed to house flies caused sterility, but only in female flies. Goldsmith and Frank (1952) produced sterility in female fruit flies by feeding aminopterin, a folic acid antagonist.

Alkylating agents, commonly called radiomimetic compounds, will replace hydrogen with an alkyl group in genetic material with an effect similar to that of ionizing radiation (Anon. 1959, Alexander 1960). The ethylenimine derivatives of these radiomimetic compounds, including apholate, have been particularly effective in sterilizing both sexes of house flies.

LaBrecque (1961) fed apholate in food to adult flies at concentrations of 0.5-1.0%. The greatest effect from the chemical was observed in flies which were fed one day after

emergence. Male flies fed apholate for five days mated readily with untreated females, their sperm were motile, and zygote formation appeared to occur. Normal females caged first with treated and then with untreated males produced nonviable eggs; whereas females caged first with untreated males and then with treated males laid only viable eggs. In another experiment male houseflies were fed a diet of 1.0% apholate and mating tests showed that these treated males were as successful as normal males in competition for mates La Brecque et al. (1962). Morgan and La Brecque (1962) found that apholate inhibited ovarian development when ovaries of treated flies were compared in size with those of flies not treated with the chemosterilant.

In experiments with stable fly Stomoxys calcitrans (L.) Harris (1962) applied topical one microgram doses of apholate, aphomide, and aphoxide to adult flies. Complete sterility resulted in nearly all cases when treated males were crossed with treated females. Male flies were more susceptible to apholate than females and residual films of the chemosterilant remained effective for 24 weeks when stored indoors. Chamberlain and Hopkins (1962) found no noticeable mortality of adult flies that were treated at sterilizing doses with apholate. Some mortality did result when larvae or pupae were exposed to the chemosterilant and marked changes occurred in the resulting pupae and adults. Morphological examination of

adults showed a decrease in the size of ovaries and testes with distortion and reduction in the number of eggs in the ovaries. Borkovec (1962) reports that the gross histopathological effect on female insects from alkylating agents such as apholate is the retardation or complete cessation of ovarian development while the gross effect on male insects is less distinct in that the testes seldom show drastic morphological changes. Cytological effects in females include clumping of chromatin in nurse cell nuclei, chromosomal aberration and fragmentation, and abnormal mitotic figures. Male germ cells are affected by dosage of the chemosterilant and large ammounts may effect all stages enough to prevent normal reproduction. The compound 5-fluorouracil is incorporated into the RNA molecule of housefly eggs and the result of this "faulty" RNA formation can be called a genetic effect of this chemosterilant (Kilgore and Painter 1965).

Chemosterilant compounds are capable of causing both permanent and temporary sterility. Painter and Kilgore (1964) found apholate to produce permanent sterility in houseflies. The chemical was fed to flies of both sexes with a concentration of 0.25% resulting in normal oviposition with 0-0.1% hatch of eggs. Higher concentrations caused lighter oviposition and no hatching occurred. Gouck et al. (1963) found feeding to be the most effective method of sterilizing screw-worm and house flies. Only apholate caused complete

sterility in both species. Further tests involved dipping of pupae in various concentrations of apholate with sterility resulting (Gouck 1964). Murvosh et al. (1964) obtained wider variations in results from use of chemosterilants than those encountered when testing insecticides. Apholate substantially shortened lifespans although a slight delay in initial male mortality occurred. More than 90% of the males survived for ten days which should allow sufficient time for mating.

In experiments conducted with the screw-worm fly only thiotepa was equally effective in sterilizing both sexes (Crystal 1963). The compound tretamine was most effective for sterilization at dosages which were low in toxicity to the test insect. The range of sterilizing activity and host toxicity should be as wide as possible for best action when chemosterilants are employed. Davis and Eddy (1966) tested the chemosterilants tepa, metepa, apholate, hempa, and hemel on the little housefly and found no great margin between doses causing sterility and those causing mortality. Tepa was the most effective of the compounds tested. Pupae of various ages were unaffected by dipping in ethanol solutions of 0.05%-5.0% metepa. Housefly pupae were dipped in 1+1 volume to volume solutions of acetone and water containing chemosterilants for screening tests by Piquett and Keller (1962). Favorable results were reported using this screening method.

Chamberlain (1962) treated screw-worm flies with apholate in the larval, prepupal, and adult stages but pupal treatments resulted in only partial sterility. The numbers of eggs produced declined in treatments using the chemosterilant and females were more affected than males. Some mortality resulted when larval or prepupal stages were treated but not in adults treated at sterilizing doses. Distortions of ovaries and testes occurred with a reduction in the number of eggs in the ovaries. Treatment of larvae caused morphological changes in prepupae and adults; therefore, this type of exposure to the chemical is not recommended. A single feeding treatment of adult flies resulted in good sterility. Hair and Adkins (1964) sterilized face fly pupae by dipping in 4.0% solutions of apholate for 25 minutes. In tests conducted with adult flies sterility was accomplished by feeding a 1.0% solution for one day or a 0.5% solution for two days. Adult horn flies were fed overnight on food containing 0.01% apholate or 0.05% tepa and complete sterility resulted (Harris and Frazar 1966). Continuous feeding of a 5 ppm tepa solution resulted in 100% sterility, but sterile males were only 60-70% as competitive as normal fertile males. Hair and Turner (1966) fed apholate to adult face flies Musca autumnalis (De Geer) and obtained 78% sterility of males and complete sterility of female flies. Newly hatched males and

females were sterilized at 82% and 86% levels respectively. The adult female face fly imbibes more food than the male resulting in higher sterility levels from a 24 hour 0.5% feeding. In tests comparing amounts of metepa required to sterilize screw-worm flies and stable flies Chamberlain and Barrett (1964) found that the male screw-worm fly required 5.5 times as much chemosterilant as the male stable fly for sterilization. Female screw-worm flies required 18 times as much chemosterilant as female stable flies to cause sterilization. Henneberry et al. (1967) report that apholate appears to induce higher sterility in older adult fruit flies than in those of younger ages. Gamma radiation and apholate produce similar sterilization effects and sperm is affected in or out of male flies similarly. In other experiments comparing radiosterilization vs. chemosterilization apholate equalled or surpassed the radiation effects on mosquitoes (Schmidt et al. 1964). Highly competitive, permanently sterile male mosquitoes were readily obtainable using the chemosterilant apholate. When house flies were treated either method seemed satisfactory but the greater degree of recovery of irradiated insects should be considered.

Tests using chemosterilants on insects other than Diptera have also showed promise. Toppozada et al. (1966) fed apholate at 1.2% concentration to adult Egyptian cotton

leafworms Prodenia litura (L.) and obtained 100% sterility. Tepa was found to be 15 times more effective and sterilized at the 0.08% level. When males were treated the females with which they mated produced fewer eggs. Fourth instar larval treatments were relatively ineffective because only partial sterility resulted even though 50-70% mortality occurred from treatments. Soto and Graves (1967) used apholate and tepa on bollworms and tobacco budworms. The compounds were administered in sugar solutions. Male insects were more affected than females and a reduction in mating frequency and oviposition occurred. Metepa was more effective than apholate.

Banded cucumber beetles Diabrotica balteata (LeConte) were exposed to residual films of metepa and apholate by Creighton et al. (1966). At 4% treatment levels the compounds were very toxic while 2% treatments were only partially effective. Newly emerged Tephritid flies forced to walk on deposits of chemosterilants became sterile (Keiser et al. (1965). Lower concentrations of the compounds were needed for sterilization when flies walked on moist surfaces.

In experiments conducted with the boll weevil Anthonomus grandis (Boherman) using apholate treated males often regained fertility 10-20 days after treatment (Lindquist 1964). Egg hatch was not a good criterion for determining sterility because a dominant lethal gene expressed itself in the young

larvae. Microscopic observations indicated the end effects of apholate had been directed to the spermeogenic transformations and/or II meiotic divisions. Further experiments on boll weevils involved feeding the chemosterilant apholate in diets and on foliage (Hedin et al. 1964). Untreated females when mated with treated males produced eggs with a reduced hatch. At higher dosages of the compound longevity decreased. Another effect of apholate was recorded involving phytotoxicity to cotton where leaf necrosis, stunting, and cessation of square production occurred.

Howland et al. (1965) conducted feeding tests on cabbage loopers Trichoplusia ni (H.) using apholate and tepa. The chemosterilants caused sterility at lower doses in males than in females. Collier and Downey (1965) evaluated tepa, metepa, and apholate on the gypsy moth Porthetria dispar (Linne') and found apholate to be effective when high residual films were used and both sexes treated.

In experiments using Japanese beetles Popillia japonica (Newman) the chemosterilant tepa was four times more effective than apholate and 13.7 times more effective than metepa (Ladd 1966). The same compounds were tested on pea aphids Macrosiphum pisi (Harris) fed an artificial diet and apholate proved best in sterilizing the insect while metepa was too toxic (Bhalla and Robinson 1966). Tepa and apholate are effective as chemosterilants when administered in the diet of the pea aphid, a viviparous and parthenogenic insect.

In certain cases involving the laboratory use of chemosterilants resistance to the chemicals has occurred. A colony of yellow fever mosquitoes Aedes aegypti (Linne') developed a 20 fold increase in resistance to apholate after 43 generations of exposure to 5-25 parts per million larval treatments (Patterson et al. 1967). Adult mosquitoes showed only a 6-7 fold increase in resistance. Morgan et al. (1967) fed 0.01% apholate to house flies in food and the resulting sterility changed from 6% for the F_1-F_5 generations to 69% for the F_{22}-F_{30} and then decreased to 14-22% for the F_{51}-F_{65} generations. Other tests showed no resistance after 58-59 generations at various doses. A fly colony fed 0.05% apholate died out by the F_7 generation and another fed 0.2% metepa died out by the F_{10} generation.

The usefullness of chemosterilants for control of insects in the field is becomming more important as programs of this type are instituted and evaluated. Knipling (1962) has pointed out the advantages of chemosterilants over radiation treatments both in the laboratory and in field use. The use of the sterile male technique in itself presents many advantages over conventional killing procedures. LaBrecque et al. (1962) used cornmeal baits containing 0.5% aphoxide on a Florida dump-site for control of house flies. New applications were made each week for nine weeks except during the second week and populations were reduced from 47 flies per grid to 0 flies

per grid in four weeks. The proportion of egg masses containing one viable egg were reduced from 100% to 10% within four weeks. The percentage hatch among all eggs laid was reduced to 1% within a five week period. In another experiment of field application metepa in 0.5% fly baits was applied to droppings in a Florida poultry house (LaBrecque et al. 1963). Weekly applications for 9 weeks resulted in female fertility below 10%. Gouck et al. (1963) used 0.75% apholate cornmeal baits on an isolated dumping area. Applications were made weekly for 7 consecutive weeks and then 5 times per week for the next 5 weeks. Fly populations dropped from 68 per grid to 5-20 per grid during the first seven weeks and remained between 0-3 per grid for the following 5 weeks. Egg hatch ranged from 81% before treatment to 12-49% during the weekly treatments and from 2-26% for the remaining five week period. Male fertility decreased little with weekly treatments but averaged 22% when bait was available at all times.

Chamberlain (1962) reports that the release of male insects made sterile by chemicals would be effective even if only 90% of the released insects were sterile. Reduction in the wild population would take slightly longer if 90% sterility was the highest level attainable by laboratory rearing procedures. Howland et al. (1965) has indicated that more effective use of chemosterilants for control of insect populations may be possible by combining insect attractants with chemosterilants.

MATERIALS AND METHODS

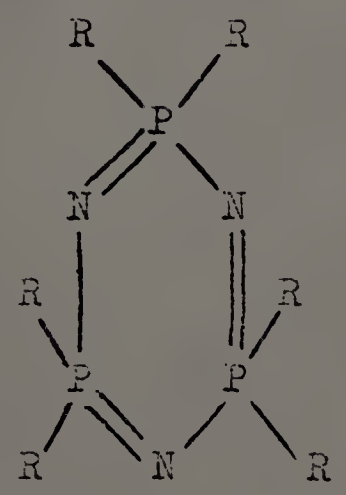
Apholate

The chemosterilant effect of apholate was first reported by LaBrecque et al. (1960). The following technical data on apholate were obtained from the Olin Mathieson Chemical Corporation, 275 Winchester Avenue, New Haven, Connecticut.

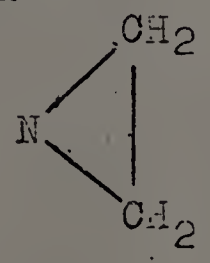
Chemical names: 2,2,4,4,6,6-hexakis(1-aziridinyl)-1,3,5,2,4,6 triazatriphosphorine: 2,2,4,4,6,6-hexa (1-aziridinyl)-2,4,6-triphospha-1,3,5 triazine; hexakis (aziridine)-cyclotriphosphaza-1.3.5-triene; hexakis (1-aziridinyl)- phosphonitrile; and 2,2,4,4,6,6-hexakis (1-aziridinyl)-2,2,4,4,6,6-hexahydro-1,3,5,2,4,6- triazatriphosphorine, the official chemical name.

Other designations: Apholate, SQ 8388 and USDA ENT. No. 26,316.

Structural Formula:



Where R is-



Molecular Formula: $C_{12}H_{24}N_9P_3$

Molecular Weight: 387.4

Physical Form: White Powder

Melting Point: Approximately 150°C

Because of polymerization occurring at its melting point the exact temperature of this property has not been obtained.

Approximate Solubility (per cent by weight): Water 33%, methylene chloride 33%, chloroform 31%, methanol 14%, 70% ethanol 15%, acetone, xylene, mineral oil, hexane and carbon tetrachloride less than 1%. All samples of apholate contain from one to seven percent of insoluble materials, most of which form fine dispersions in solution.

Stability: Moisture, high temperature, low pH, proteinaceous materials and highly sorptive carriers such as Attaclay cause polymerization or inactive apholate. Water solutions are reasonably stable for 14 days. Sugar baits are stable to ultraviolet light, sunlight, and high relative humidity. When stored for long periods of time apholate should be kept in a tight container in a cool dry area. Apholate polymers have high melting points, insoluble in solvents tested so far and are biologically inactive.

Patent: U.S. No. 2, 858,306 assigned to Olin Mathieson Chemical Corporation.

Mammalian Toxicity: Acute oral LD_{50} mg/kg is 410 for chickens, 120-180 for mice, 90 for rats, 12 for sheep and 10 for dogs.

Intramuscular- Sheep died after 11 doses of 0.5 mg/kg/day, and cattle died after one injection of 2.5 mg/kg.

Dermal- Rabbits tolerated 200 mg/kg on unabraded skin, while the LD_{50} mg/kg for abraded skin.

Other Data: One of the first signs of apholate poisoning is a reduction in the number of white blood cells. There may also be a reduction in the number of platelets. Except in the case of severe poisoning there is no effect on the red corpuscles or hemoglobin. Dogs that showed a very low white cell count after large oral doses of apholate regained normal blood levels in three weeks when removed from the experiment. There are also some reports of bone marrow depression in experimental animals, but no records for personnel working with apholate.

Handling Precautions: The following precautions are recommended, even though no person is known to

have developed illness during the four years that apholate has been under test in many laboratories: (1) wear rubber gloves, (2) wear respirators when handling large quantities, (3) avoid inhalation of the powder, (4) avoid contact with the skin, and (5) wash thoroughly after using the chemical. Do not use apholate on food crops or animals. As an additional safeguard, personnel using apholate in large quantities over a prolonged period of time should have a white blood cell count taken every 15 to 30 days.

Formulations used in this study:

(1) Technical apholate in dilute malt syrup (non-diastatic).

(2) Impregnated cotton cords 20%.

(3) Various apholate dusts.

The Fly Culture

The culture room was located in the basement of Fernald Hall and measured approximately 18 x 4½ x 11 feet. The walls were painted light green. A sink, shelving, and a large southern window were present. The window was covered to exclude most extraneous light, but some ventilation from outside was possible if it was opened slightly.

The culture cages were constructed by fastening aluminum window screening over a light wooden framework. A black cloth sleeve was fitted over an entrance hole 8 inches square and located at the front. The hole was positioned at the bottom center of each cage and allowed easy transfer of flies from rearing jars to culture cages. The cages measured approximately 24 inches wide, 20 inches high and 18 inches deep.

Four (4) Champion Cool White F 96 T 12/CW fluorescent lamps were used as a light source in the room. An automatic timer was used to turn the lamps on and off providing sixteen (16) hours of light per day.

Temperature and humidity varied considerably in the culture room but only in cases of a high temperature and low humidity combination were the flies adversely affected. Under such an environmental condition most of the flies in a culture

cage would die within forty-eight (48) hours.

The original fly culture was one which had been maintained in Fernald Hall for several years by previous workers. A 6% V/V solution of light non-diastatic malt syrup in water was placed in eight (8) ounce waxed paper cups containing a piece of crumpled paper toweling and was the only food needed to support the population of adult flies. The cups were refilled every other day from a main supply bottle of three quart capacity. It was important to keep the bottle loosely capped because gases from fermentation processes formed excessive pressures if a tight seal was maintained.

When fresh malt syrup was prepared about 100 ml of the old solution was added to the new mixture. Apparently this "inoculum" provided a source of yeasts which grew quite readily in the malt syrup solution. A vigorous culture was easily maintained using this "inoculated" syrup whereas malt syrup prepared just prior to use resulted in flies with shorter life spans and correspondingly low total number of eggs produced. The yeasts may have provided a source of vitamins and other nutrient factors not present in freshly prepared syrup. Food for the flies in the test experiments was prepared in the same manner.

The malt extract was manufactured by Premier Malt Products, Inc.; 1037 W. McKinley Avenue; Milwaukee, Wisconsin. It was designated as "Hop Flavored Light" and has the following composition.

SOLIDS	81.0%
REDUCING SUGARS (as maltose)	54.35%
pH 10% Solution	5.30
ACIDITY (as lactic)	1.30%
ASH	1.12%
PROTEIN	5.50%
Color (Lovibond)	280

Because the viscous concentrated syrup was difficult to measure a 50/50 V/V solution in tap water was prepared as a stock solution, refrigerated, and used to prepare the 6% fly food.

Fresh or previously frozen poultry manure was used as the oviposition site in the culture cages. About 50 grams of manure was spooned into a 4 oz. paper cup and a cone shaped depression was made sloping from the sides of the cup one inch into the manure. Eggs were deposited on the manure by adult flies. Every day eggs were transferred to one gallon laboratory battery jars containing a larval rearing substrate. The top of the jar was covered with cheese cloth secured by a strong rubber band. The media was prepared by mixing 500 cc of Chemical Specialties Media Association fly larval media with 325 ml of tap water containing approximately 5 grams of sodium propionate. The propionate was used to retard mold formation in the moist medium. Eggs hatched within 48 hours and larvae fed on the medium until pupation. Pupation occurred in the

dryer upper layers of the medium.

Adult flies emerged 18-22 days after egg laying and were released into the culture cages. When culture cages become overcrowded rearing jars were occasionally cooled to 45°F. This allowed adults to be held for several days before release into the culture.

Northern fowl mites were troublesome at times in rearing jars and if they became well established were very difficult to control. In cases of serious infestations the mites could be seen in great numbers on flies and jar walls. Many methods of control were tried but simple sanitary procedures such as thorough washing of the jars proved most effective. When large numbers of mites were noticed in a rearing jar the entire contents were discarded. To assure a mite free culture it was important not to include any manure in the egg transfer from cups to jars as this was the source of mites and their eggs. Clusters of eggs could be gently transferred to battery jars using a small chemical spatula without including manure.

EXPERIMENTAL PROCEDURES

Environmental Chamber

An environmental chamber was constructed to provide the necessary conditions of temperature and humidity for sterilization tests (fig. 1). Temperatures in excess of 80°F combined with a relative humidity of less than 35% prove detrimental to the life of this insect when reared in the laboratory. The chamber insured life spans of sufficient length to allow egg collections for periods up to 8 weeks from any single cage of flies.

The chamber was constructed using 3/4" x 3/4" square wood strips to form a framework 80 inches long, 30 inches wide, and 36 inches high. Two (2) mil black polyethylene plastic sheeting was used to cover the framework except for a center strip eight inches wide on the top. This top center strip ran the length of the chamber and was covered with transparent plastic to allow passage of light into the interior from the external light source. Two (2) Champion F 40 Cool White fluorescent lamps provided sufficient lighting and were connected to an automatic timer set for sixteen (16) hours of light per day. A three foot square section of one side of the chamber was covered by a removable flap of 2 mil black plastic

sheeting to allow access to the interior. Two small air vents measuring 3/4 x 8 inches were left open at the top on each end of the chamber to provide limited ventilation. This was necessary because the decomposition of the poultry manure used for oviposition produced small amounts of ammonia gas.

The chamber was located in a basement room of West Experiment Station on the University campus. Heating was accomplished by a conventional steam radiator with a blower for circulation. Cool air was drawn from outside the building by a blower system. Thermostats were used to effect a balance of the two systems and although the room temperature fluctuated rapidly between extremes the interior of the chamber did not. An average temperature of $75^{\circ}F \pm 3^{\circ}F$ was desired and for the most part was sustained. Occasionally seasonal variations caused temperatures within the chamber to reach $\pm 6^{\circ}F$ of the intended 75° but this occurred rarely and for only a few hours during any one test.

Adult Collection Methods

CO_2 method. A carbon dioxide unit consisting of a gas cylinder, hoses, and two Büchner funnels was originally used to anesthetize adult flies for sexing, counting, and final testing.

A pint sized cardboard container with a screened bottom was inverted and placed over a hole cut in the cheese cloth covering of the culture jar. As adults emerged from the medium they flew up into the pint container. When a sufficient number accumulated within the container the cover was quickly slipped into place. Tapping the sides of culture jars with the hands helped move flies into the screened cartons. The container could then be placed, screen down, into the Büchner funnel for anesthetizing the flies with carbon dioxide.

All flies collected for testing by this method were kept anesthetized for 15 minutes. Mortality was low using this procedure but results of some tests indicate that mating may have occurred within the culture jars before sexing was accomplished. A method of collecting pupae was developed to eliminate the chance of mating in culture jars before sexing of the flies for tests.

Pupae collecting method for obtaining adult flies. Culture jars containing mature pupae were opened and the darkest appearing pupae were separated from the medium particles with forceps. These pupae were transferred to individual 6 inch test tubes and a loose fitting aluminum cap used to cover the mouth of the tube. The caps were one inch long with 4 small lobes protruding from the rim of the opening. The lobes were pressed inward slightly to grip the sides of the test tube thus securing the cap in place. Emerged flies could not escape and the loose fitting caps allowed an air exchange within the test tube. Flies which emerged in the individual pupal tubes lived up to 48 hours without food or water and could be counted and sexed very easily.

This method was preferred over CO_2 anaesthesia because no mating could occur, counting and sexing was easier, and many flies could be held up to 48 hours at room temperature until sufficient numbers had emerged to make up a battery of tests.

Individual test cages. Aluminum cages measuring nine (9) inches square and eighteen (18) inches long were used within the environmental chamber to contain individual test groups of flies (fig. 2). The bottom and both ends of the cages were formed of heavy gauge sheet aluminum. The back of the cage had a sliding plate of thin aluminum 8½ inches wide. The front had a circular hole 6 inches in diameter surrounded by an aluminum ring which held a cloth sleeve in place by means of bolts. The sleeve could be knotted or twisted to prevent the escape of flies.

Angle aluminum braces running from front to back formed the top of the cage and secured #18 mesh aluminum screening between the angle components of each brace. Conver aluminum strips with bolts secured the screening on the bottom sides, and ends of the cage.

In tests where only one sex was being treated with chemosterilant the cage was divided in half using aluminum foil. The foil was secured to the bottom, top and sides of the cage with one inch wide masking tape. The tape was then sewn to the screening with needle and thread to assure a tight seal. Flies of either sex could be released into respective sides of the cage and treated appropriately. When treatment times had elapsed the separating foil could be torn from the cage by means of the cloth entry sleeves. The flies could

then mix for mating and subsequent sterility determination.

Apholate was fed to test flies in freshly prepared 6% malt syrup. Weight to volume solutions were prepared and served in 50 ml beakers containing a crumpled piece of paper toweling. When dividing foils were removed the flies were restored to the routine culture diet of 6% malt syrup contained in waxed paper cups with crumpled paper toweling as a resting surface.

Seven days after the flies had been introduced into the test cages petri dishes containing poultry manure covered with filter paper circles were placed within the test cages. These oviposition dishes were removed every 48 hours and replaced by new ones. Malt syrup was similarly exchanged at this time and mortality counts made when necessary.

Techniques of egg collection and incubation. Standard size petri dishes were filled with 40 grams of fresh or previously frozen poultry manure. The manure was spread with a spatula using care not to get excess manure on the upper half of the sides of the dishes. When the surface of the manure was smooth, a piece of nine (9) centimeter Schleicher and Schuell #595 filter paper was fitted into the dish and gently brought into contact with the entire surface of the manure.

Adult flies will lay eggs on the surface of the filter paper and on the clean sides of the dish (fig. 3). The eggs can be counted and transferred easily using this method. If fly eggs are oviposited directly onto manure they often become hidden or stuck and counting or transfer without damaging the fragile eggs is very difficult. Transfer of eggs to clean petri dishes for incubation is necessary because large quantities of ammonia gas are produced by decomposing manure. If concentrations of ammonia become too high egg hatch is adversely affected. The following method was used to avoid this problem.

Random samples of eggs were removed from the oviposition dishes with a moistened camel hair brush and transferred to clean dishes. The clean dishes contained a circular piece of nine (9) centimeter blue blotting paper. Pencil lines were drawn on the blotter parallel to each other and spaced 3/8 inches

apart to facilitate egg counting. The blotting paper was moistened with 5 ml of distilled water to provide a humid atmosphere within the closed dish during incubation (fig. 4). A small pan of water was maintained within the incubator to provide additional moisture. When a sufficient number of eggs had been transferred they were counted using a 15 power binocular dissecting stereoscope. The dishes containing the eggs were covered and incubated at 30°C for 48 hours. The percent sterility was computed by counting the number of eggs unhatched at the end of incubation.

EXPERIMENTS AND RESULTS

The primary objective of this project was to determine a feasible method of using apholate to chemosterilize adult Fannia canicularis for potential use in control programs. Preliminary tests were run to find the most practical procedure applicable to this particular insect.

Preliminary Tests

Dusting. Adult flies 24 hours old or less were anaethetized with carbon dioxide and dusted with pure apholate or dilutions of apholate in Pyrax A B B dust. Flies were introduced into test tubes containing measured amounts of dust and gently tumbled to cause thorough dusting.

Mortality was extremely high in all the tests performed and further experiments involving dusting were not carried out.

Larval feeding. Apholate was incorporated into the larval medium at various concentrations. Results showed that apholate in concentrations exceeding .025% by weight acted as a larvicide. Concentrations below .025% by weight produced adult flies that laid fertile eggs. The higher concentrations of apholate may have been lethal to larvae because they need eat only a small amount of the medium to ingest large amounts of the chemosterilant. The lower concentrations of apholate may have been

ineffective because of their weak concentration within the medium or metabolism to non-sterilizing compounds by larvae or pupae.

It seemed unusual to find no range of concentration which produced sterile adult flies.

Apholate impregnated cords. Thin cotton cords containing 20% apholate by weight were suspended in test cages where adult flies could land on them. Two cords extended 6" into the cages one at either end and exposure time was limited to 24 or 48 hours.

Sterility resulted from this contact cord treatment but no definite percentages were determined because a more standardized method was needed. The possibility exists that some flies would receive sterilizing doses of apholate while others would not, depending on how long they were in contact with the cord.

Further tests indicated that feeding adult flies low concentrations of apholate in their liquid diet for relatively short periods of time would be a more practical approach to sterilizing large numbers of flies with a minimum of difficulty. This method proved to be easily standardized using common laboratory facilities and was adopted for the primary tests of this project.

Adult Feeding Tests

Trial runs were performed to determine concentration ranges of apholate in adult liquid diet that would not cause adverse side effects or excessive mortality.

A concentration of .5% weight to volume of apholate when fed to adult flies caused excessive initial mortality as compared to untreated flies. This indicated a safe upper limit range of apholate to be well below .5% as it was highly desirable to produce sterile flies that were normal in other respects such as life span, activity, and mating competition. The highest percentage of apholate used in the tests was .2% weight to volume.

Preliminary test I - The effect of feeding various concentrations of apholate for 24 hours. This test was designed primarily to determine percentages of apholate most applicable for adult sterilization, number of flies needed for sufficient egg collections, and permanence of sterility induced by the treatments.

In each of the individual test cages apholate in the normal 6% malt syrup food was fed to 10 pairs of adult flies less than 24 hours old. The syrup was placed in 100 ml glass beakers containing a piece of crumpled paper toweling which served as a landing place for the flies. Exposure time of the treatment was 24 hours at a temperature

of 72°F using 16 hours of artificial lighting. Eggs were either sampled or completely collected for comparison of percent hatch. The treatments were as follows:

CAGE 1	.025% apholate		all eggs collected
CAGE 2	.2% apholate		all eggs collected
CAGE 3	.025% apholate		eggs sampled
CAGE 4	.2% apholate		eggs sampled
CAGE 5	CONTROL		all eggs collected

Results of the test are shown in Table I. Examination shows that the .2% treatments were more effective in sterilizing adult flies but even these highest treatments show somewhat erratic results during the duration of the test. No correlations could be made between the variations in percent sterility among the various tests. Further tests were run and the data presented within this paper showing the increases in exposure time to similar concentrations of the chemosterilant resulted in higher and more consistent sterility of eggs.

The permanence of sterility caused by apholate was verified by Painter and Kilgore (1965) and the data in Table I would appear to be in agreement with their report. In those cages where sterilization was erratic and somewhat cyclic the possibility exists that some flies were not

sterilized by the treatment. Egg collections showing low sterility could be partially composed of eggs from these particular flies which escaped chemosterilization. This is a possible explanation for the cyclic pattern shown for sterility in the test results.

In future tests 25 pairs of flies were used rather than 10 pairs as greater numbers of flies would insure sufficient oviposition for egg sampling. If any of the test flies were to die during the experiment the overall effect upon egg numbers would be reduced when using 25 pairs of adults as compared to 10 pairs.

Fly mortality was recorded during the eight weeks of the test duration and in no case was the mortality greater in treatments than in the control. The chart of egg production totals indicates a decrease of total eggs laid with increasing concentrations of apholate. This result is similar in further experiments where both sexes were also treated with the chemosterilant.

Table I

The effect of feeding various concentrations of apholate for 24 hours to adult Fannia canicularis.

Date of egg collection	Percent of eggs failing to hatch: Control all eggs	.025% all eggs	.025% eggs sampled	.2% all eggs	.2% eggs sampled
October					
10	2	2	7	97	100
12	7	3	16	66	97
14	1	---	3	99	93
16	0	10	6	95	81
18	---	---	11	---	71
20	18	11	18	98	65
22	7	18	11	95	84
24	11	13	41	96	74
26	15	---	54	94	92
28	17	30	43	85	79
30	71	78	94	96	92
November					
1	25	24	78	85	85
3	30	33	75	95	40
5	36	58	75	86	85
7	42	88	100	93	95
9	54	47	67	91	100

November					
11	15	51	93	96	87
14	26	37	86	91	73
16	36	86	68	100	84
22	18	83	92	91	90
December					
2	43	25	31	95	83

---- Insufficient # of eggs for collection.

Eggs collected every 48 hours whenever possible.
10 pairs of flies per cage.

Preliminary test II - The effect of feeding various concentrations of apholate for 24 and 48 hours. The results in Table I suggest the need for a longer period of feeding apholate treated food to achieve higher sterility. Twenty-five pairs of flies were used in this test. Two cages were duplicates of treatments in Table I for comparison with those cages in which 48 hour exposures were used. The treatments were as follows:

CAGE 1	.025%	Apholate fed for 24 hours.
CAGE 2	.2%	Apholate fed for 24 hours.
CAGE 3	.025%	Apholate fed for 48 hours.
CAGE 4	.2%	Apholate fed for 48 hours.
CAGE 5	CONTROL.	

Egg samples were used from all cages and incubated to determine percent sterility. The results of this experiment are shown in Table II. The highest concentration of .2% apholate fed for 48 hours produced 100% sterility for the full duration of the test. This concentration and time of exposure was used for the third experiment (Table III) in which male flies were treated with the chemosterilant and crossed with untreated female flies. Fly mortality was greater in the highest treatments than in the control cage, but the .025% 48 hour cage had less mortality than the .025% 24 hour cage. Mortality figures for this experiment are shown in Table IIa.

The total number of eggs laid in each cage during the test period are presented in the chart of egg production. Comparison of the concentration of apholate with the indicated exposure times shows a great reduction in eggs produced in the highest levels of treatment. This egg reduction is especially true in tests where females and males are both fed apholate. In the third experiment (Table III) only males were fed the chemosterilant and egg numbers were not reduced to such an extent.

Table II

The effect of feeding various concentrations of apholate for 24 and 48 hours to adult Fannia canicularis.

Percent of egg sample failing to hatch

Date of egg collection	Control	.025% 24 hours	.2% 24 hours	.025% 48 hours	.2% 48 hours
March					
18	1	23	97	95	100
20	1	26	100	92	100
22	1	42	100	94	100
24	6	40	96	91	100
27	5	53	100	94	100
29	6	41	92	94	100
31	2	39	89	95	100
April					
1	21	---	---	91	100
3	4	53	88	100	100
5	3	54	87	89	100
7	21	61	95	94	100
9	19	61	85	85	100
11	50	67	86	92	100
13	21	84	96	96	100
15	48	74	100	97	100

April 17	34	79	95	84	100
19	64	90	100	82	100
21	42	94	100	84	100
23	41	97	100	84	100

--- Insufficient # of eggs for collection.

Eggs collected every 48 hours.
25 pairs of flies per cage.

Table IIa

Mortality of Fannia canicularis when fed apholate for 24 and 48 hours in 6% malt syrup.

Total number of dead flies at end of period shown

	Control	.025% apholate 24 hours	.2% apholate 24 hours	.025% apholate 48 hours	.2% apholate 48 hours
1st week	6	5	12	5	13
2nd week	8	10	17	8	13
3rd week	8	14	17	8	15
4th week	12	19	20	9	19
5th week	14	20	24	14	22
6th week	14	21	24	16	24
7th week	17	23	27	17	25
8th week	17	26	30	18	25

Cages initially contained 25 one day old males and 25 one day old females.

Male Feeding Test

The effect of feeding various concentrations of apholate to male flies for 48 hours. Treated males mated with untreated females. This experiment was performed to determine the sterility effect of apholate when fed to male flies which were then crossed with untreated females. The treatments and exposure times were as follows;

.05% apholate was fed for 48 hours to males only
.1% apholate was fed for 48 hours to males only
.2% apholate was fed for 48 hours to males only
CONTROL-no treatment.

Twenty-five pairs of flies were used in each test cage and 3 repetitions of each treatment were made. The primary interest in the test was to determine if males could be sterilized without extreme "side effects". Such sterile males could be used in potential control programs for this insect.

All treatments were of 48 hour duration and 3 egg collections were taken from each test cage. Results of the test are shown in Table III.

The test results show considerable variation among treatments. In two of the .1% concentrations sterility is

higher than in one of the .2% treatments. One .05% treatment results in sterility much higher than another test where .1% was used. From these variations the conclusion can be drawn that within certain test cages the amount of apholate consumed by the flies varies considerably and results in erratic sterility. The overall average percent sterility of the .2% treatment is 88.3% which may indicate its potential use for control programs involving release of sterile males. A prerequisite for such programs is that control of the natural population must be possible by mass releasing males of which 88% are sterile.

Egg production totals are shown on the egg production chart and vary considerably from those tests in which both sexes were treated. In general egg production was not decreased as much in male treatments when compared to treatments where both sexes were treated by feeding apholate.

During the test period mortality counts were made to determine if the highest treatments had any effect upon the test insects. Meaningful counts of dead male flies were not possible because many of the dead flies were found in the food cups and decomposed beyond the point of routine sexual identification. There was, however, no initial "knockdown" effect observed in any of the treated cages. The test results show that .2% was the optimum treatment level needed to cause maximum sterility without acute toxicity effects.

Table III

The effect of feeding various concentrations of apholate to adult male Fannia canicularis for 48 hours. Treated males mated with untreated females.

Test group "A"	No. of eggs in egg sample	Percent of eggs failing to hatch	Successive 48 hour collection intervals
Control	86	4.7	1st collection
	131	3.8	2nd "
	145	2.1	3rd "
.05% apholate in food	47	42.6	1st collection
	154	44.8	2nd "
	178	56.2	3rd "
.1% apholate in food	93	96.8	1st collection
	103	97.1	2nd "
	99	97.0	3rd "
.2% apholate in food	0	----	1st collection
	61	100.0	2nd "
	96	100.0	3rd "
Test group "B"			
Control	181	1.1	1st collection
	218	0.9	2nd "
	279	1.8	3rd "
.05% apholate in food	237	80.6	1st collection
	280	87.8	2nd "
	395	87.3	3rd "
.1% apholate in food	116	88.8	1st collection
	212	99.1	2nd "
	218	92.6	3rd "
.2% apholate in food	105	77.1	1st collection
	245	92.2	2nd "
	107	49.5	3rd "

Test group "C"

Control	163	3.1	1st collection
	195	2.6	2nd "
	271	3.3	3rd "
.05% apholate in food	206	21.8	1st collection
	160	35.0	2nd "
	160	28.7	3rd "
.1% apholate in food	97	44.3	1st collection
	137	48.9	2nd "
	246	54.1	3rd "
.2% apholate in food	125	89.6	1st collection
	196	98.5	2nd "
	221	98.2	3rd "

25 pairs of flies per test cage.

Table IV

Egg production totals from Tables I, II, and III.

Table I Treated males X treated females, 10 pairs of flies 24 hour exposure, totals of 21 egg collections.

Control	5,758
.025%	4,321
.2%	2,507

Table II Treated males X treated females, 25 pairs of flies exposure time as indicated, totals of 19 egg collections.

Control		13,697
.025%	24 hours	12,718
.2%	24 hours	5,948
.025%	48 hours	10,737
.2%	48 hours	1,531

Table III Treated males X untreated females, 25 pairs of flies, 48 hours exposure, totals of 3 egg collections.

Control	1,491	2,655	3,685
.05%	2,222	3,160	2,617
.1%	813	2,339	2,410
.2%	374	1,590	3,083

SUMMARY AND CONCLUSIONS

Laboratory experiments were performed using the chemosterilant apholate on different stages of the little housefly Fannia canicularis (Linne'). Hatchability of eggs produced by the treated flies was the criterion for determining sterilizing effect of the chemical.

Preliminary tests indicated that feeding apholate to adult flies was the most practical means of exposing Fannia to the chemosterilant. Tests were conducted wherein both sexes were treated and eggs collected for extended periods of time. A concentration of .2% weight to volume of apholate when fed for 48 hours to both sexes resulted in 100% sterility of eggs collected from the treated adults.

The primary experiment was conducted to determine the feasibility of sterilizing male Fannia only and crossing them with untreated female flies. Such chemosterilized males might provide a potential source of flies for use in control programs involving mass release of sterile male Fannia. The data obtained indicate somewhat erratic results among replicate experiments but overall average sterility was 88.3% in the highest treatments. Mortality counts were made to determine if the treatment had any initial adverse effects upon the flies but no such effects were noticed.

The experimental results indicate that apholate could be effective as a chemosterilant for the little housefly if incorporated into a suitable bait upon which adults could feed. The experiments conducted here do not indicate criteria for control programs such as is needed in poultry houses but the data should provide information relative to dosage formulation and exposure times. Control programs involving mass release of sterile males, if practical, may benefit from the rearing and sterilizing techniques presented.

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APPENDIX

Figure 1. Environmental chamber constructed of black plastic sheeting with overhead lighting source.

Figure 2. Aluminum test cages within environment chamber.

Figure 3. Petri dish containing manure covered with filter paper. Eggs were easily removed for counting, sampling, and incubation.

Figure 4. Petri dish with fly eggs on moistened blotting paper ready for incubation.