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STUDIES ON THE STERILE-INSECT TECHNIQUE AS A MEANS OF CONTROLLING THE STABLE FLY, *STOMOXYS CALCITRANS* (L.), IN A BEEF CATTLE FEEDLOT IN BROOKINGS, SOUTH DAKOTA

BY

MICHAEL AGUILAR CATANGUI

A thesis submitted in partial fulfillment
of the requirements for the degree
Master of Science
Major in Entomology
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1987

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STUDIES ON THE STERILE-INSECT TECHNIQUE
AS A MEANS OF CONTROLLING THE STABLE
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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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Thesis Advisor

Date

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Head, Plant Science Dept.

Date

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TABLE OF CONTENTS

LIST OF FIGURES

Page

LIST OF TABLES

INTRODUCTION

The stable fly, Stomoxys calcitrans (L.), is usually the only biting, blood-sucking fly breeding in appreciable numbers in and around confined animal facilities (Axtell, 1986). In Nebraska, this pest is known to cause up to 3.5 kilograms production loss per animal per season (Berry et al., 1983). An average number of 50 flies per animal can reduce the weight gains of calves on a growing ration by 0.9 kilogram per day (Campbell et al., 1977). At present, the level of 5 stable flies per front leg of confined beef cattle is used as an indicator of the economic threshold when control measures must be instituted (Hall and Smith, 1986). Since the immature stages of the stable fly develop in decaying organic matter, the most practical control measure has been sanitation or the proper storage and management of grains, silage, haylage, and manure which can support breeding in the feedlot. Campbell and McNeal (1979) in Nebraska, found chemical control to be of value when residual sprays were applied to fly resting areas such as the feedbunks, farm buildings, or vegetation surrounding the feedlots. Feedlot operators in South Dakota generally do not have the equipment necessary to carry out this means of control. Effective biological control strategies against the stable fly in feedlots have yet to be developed since releases of pteromalid pupal-parasites have met limited success (Petersen and Meyer, 1983).

The sterile-insect technique as a method of controlling or eradicating harmful insects gained popularity after the successful eradication of the primary screw-worm fly in the United States by the U.S. Department of Agriculture (Knipling, 1960; Bushland, 1975). It involved the mass-production of the fly, sterilization by gamma radiation, and release of the sterilized flies over infested areas. The sustained release of a constant number of sterile flies which greatly outnumbered the wild population, completely nullified normal reproduction in the field. This resulted in total elimination of the screw-worm fly over large geographical areas. Several workers subsequently employed the sterile-insect technique against other insect pests such as the melon fly (Steiner et al., 1965) and the stable fly (Patterson et al., 1981).

Compared to chemical control, the sterile-insect technique is highly selective and does not cause insect resistance. Sterilization is also the only proven pest control method that can actually eradicate an insect species over large areas. The main requirements for the technique to work according to Knipling (1968) are: (1) The availability of a method for producing sterility without serious adverse effects on the mating behavior and competitiveness of the insect, (2) A practical method for rearing the insects in large numbers, and (3) Quantitative information on the natural population density. Williams et al. (1981), demonstrated that the stable fly can be easily mass-produced and sterilized by 2 kR gamma radiation without affecting its competitiveness and

longevity in the field. Previous studies in Florida (LaBrecque et al., 1972) and on St. Croix, U.S. Virgin Islands (Patterson et al., 1981) indicated that the sterile-insect technique shows promise against the stable fly.

This research was initiated to investigate the feasibility of controlling a stable fly population in a beef cattle feedlot in Brookings, South Dakota using the sterile-insect technique. The objectives of the studies were: (1) To determine the population dynamics of the stable fly in the feedlot during the summer season of 1986, (2) To design a practical method of mass-producing the flies in the laboratory, (3) To determine whether actual releases of sterilized flies will have an effect on the reproductive potential of the wild population in the feedlot, and (4) To evaluate a chemosterilant, bisazir, as a possible substitute for gamma radiation in producing sterility in flies to be released in the field. All experiments in this study were carried out at the SDSU Cattle and Sheep Nutrition Research feedlot and at the Physiology Laboratories on SDSU campus from May, 1986 to July, 1987.

REVIEW OF LITERATURE

The Sterile-Insect Technique

The first successful insect eradication program using the sterile-insect technique was carried out by the U.S. Department of Agriculture on Curacao Island in the Netherland Antilles in 1954 (Knipling, 1955). The primary screw-worm fly, Cochliomyia hominivorax whose larvae develop in the living flesh of wounded calves, lambs, kids, and pigs, was the insect eradicated. Screw-worm flies were mass-produced at Orlando, Florida, sterilized in the pupal stage with 7.5 kR gamma radiation, transported to Curacao by regular commercial airlines, then released in the field as young adults using crop-dusting aircrafts. The low natural population density of the screw-worm fly (less than a hundred per square-kilometer) in a particular region and its habit of only mating once are characteristics that made this insect an ideal candidate for eradication. Eradication was accomplished within 6 months by releasing 309 sterile flies per square-kilometer per week over the 66 square kilometer island (Baumhover et al., 1955). After this successful eradication campaign, the screw-worm fly was systematically eradicated in Florida in 1958, Texas and New Mexico in 1964, and Arizona and California in 1966 (Bushland, 1975). The release rate was at 386 sterile flies per square-kilometer per week, slightly higher than the 309 flies per square-kilometer per week rate at Curacao. In these eradication campaigns, the U.S.

Department of Agriculture constructed several fly factories the largest of which was capable of producing 100 million flies per week.

Immediately following the initial successes of the screw-worm eradication program, other insects were investigated by the U.S. Department of Agriculture as possible candidates for eradication or control by using the sterile-insect technique. In 1963 the melon fly, Dacus cucurbitae was eradicated from the 13 square-kilometer island of Rota, Mariana Islands (Steiner et al., 1965). In this program about 3 million flies per week were produced in Hawaii then transported to the island by plane. The flies were sterilized by exposure to the 10 kR gamma radiation in their pupal stage. In another study, Rhode et al. (1971) greatly reduced but did not eradicate the Mediterranean fruit fly, Ceratitis capitata, in a 48 square-kilometer coffee and citrus plantation in Nicaragua. The latter project was unsuccessful mainly due to the high density of the native fly population at the time of release, and the lack of sufficient isolation to prevent continual reinfestation. These reasons are also believed to have contributed to the failure of Steiner et al. (1965) to eradicate the Oriental fruit fly, Dacus dorsalis on an island in Hawaii.

The application of the sterile-insect technique to the stable fly, Stomoxys calcitrans, was initiated by LaBrecque et al. (1972) in Florida. The authors reported up to 64% induced sterility in the wild female stable fly population at a farm containing 200

dairy cattle when releases of 8,000 to 14,000 sterile flies were made every 3 to 4 days over a period of 6 months. The stable flies were sterilized by dipping the pupae in a 5% aqueous solution of metepa or methiotepa for 60 minutes. The wild population on the dairy farm was unfortunately not eradicated due to immigration of flies from nearby sources of re-infestation (LaBrecque et al., 1981).

The feasibility of eradicating the stable fly from an insular area was investigated on St. Croix (218 km^2) in the U.S. Virgin Islands from 1974 to 1977. The first 18 months of the project were devoted to field population studies during the wet and dry seasons on the island (LaBrecque et al., 1981), The authors estimated that about 475,000 adult flies sterilized with 2 kR gamma radiation had to be released per day to outnumber the wild population 2 to 1. However, their colony was capable of producing only 300,000 flies per day (Williams et al., 1981). Due to this, the St. Croix study team resorted to integration of the sterile-insect technique with the use of a 1% methoxychlor insecticide spray, sanitation, release of a pupal parasite (Spalangia endius), and the use of attractant-toxicant traps (Patterson et al., 1981). It was not possible to release flies in urban areas since stable flies are vicious biters. Patterson et al. (1981) reported a 99.9% elimination of the wild stable fly population on the island during the last 6 months of their study. Fertile flies however were collected throughout the period due to the presence of isolated breeding sites and fly migration from other islands.

Field Population Studies

The 2 methods popularly used in sampling populations of wild adult stable flies in the field are through actual fly counts made on the animals and with the use of traps constructed of translucent Alsynite@ fiberglass panels which are coated with insect trapping adhesive. Williams (1973) was the first to discover that the translucent white color or the Alsynite@ fiberglass material was highly attractive to adult stable flies in the field. Williams' traps were normally composed of two 35 x 45 cm Alsynite@ panels coated with Tack Trap@ insect trapping adhesive and fitted together at their midpoints to form intersecting planes with the flat surfaces perpendicualr to the ground. These planes were then positioned in slots in 4 x 4 cm wooden stakes 138 cm from the ground. Meifert et al. (1978) modified the Williams' trap with an attractant-toxicant system by treating the panels with permethrin, a synthetic pyrethroid insecticide. Broce (1986) and Greene (1986) in recent population studies in Kansas changed the original crossed-panel design of the trap into a cyclindrical one. The new trap consisted of a 31 x 91 cm Alsynite@ panel rolled into a cylinder, mounted on a stake then covered with a sleeve coated with a sticky material. According to Greene (1986), the cylindrical design had 1.7 times greater surface area than a 20 x 41 cm crossed-panel Williams' trap. Regarding actual counts of stable flies on animals, some workers count the flies on the entire body of each animal (LaBrecque et al., 1981) while others count only the flies on the

half side (Campbell et al., 1977) or those only on the front legs (Berry et al., 1983). Leg counts are generally preferred since Berry et al., (1983) concluded that the number of flies on the entire body of an animal was equal to the sum of the flies counted on the legs below the chest floor.

In a 4-year study on stable fly populations in Western Kansas feedlots, Greene (1986) observed that the peak activity each year was from June to September with considerable differences between years. The peak stable fly populations were much lower during the dry years of 1983 and 1984 than during the wet years of 1982 and 1985. Higher stable fly populations were observed in feedlots with cattle receiving high roughage diets (growing ration) than in feedlots receiving high grain diet (finishing ration). Also, there were more flies in feedlots with fine silt loam soil than in feedlots with coarse sandy soil.

Meyer and Petersen (1983) studied the breeding sites of stable flies in Eastern Nebraska feedlots and dairies. In small feedlots (100-800 head), they found that fence lines, drainage ditches, and haylage were the preferred breeding sites of the fly. Spilled feed was a consistent breeding site at a large feedlot (ca. 10,000 head) while stored manure and soiled beddings were the main breeding sites on the dairy lots. Scholl et al. (1981) found the open sillage storage system to be an overwintering site of the stable flies in Eastern Nebraska. Williams et al. (1980) reported decomposing silage and green chop (cut sorghum) as the preferred breeding media in Northwestern Florida.

Mass Colonization of Stable Flies

Bailey et al. (1975) studied the biology of the stable fly for purposes of mass rearing. To find a suitable artificial medium for the development of the larvae in the laboratory, these authors tested various combinations of such materials as wheat bran, cotton-seed meal, sawdust, wood shavings, shredded paper, and water. They finally found the 3:1:5 combination of wheat bran, bagasse (crushed sugarcane pulp), and water to be the best artificial medium based on the number and weight of the pupae harvested. The swelling action of bagasse when combined with water formed a fluffy well aerated medium favorable for stable fly larvae development. Bridges and Spates (1983) found that a mixture of bagasse, meat-bone meal, and whole wheat flour (2:1:1) was comparable to a medium composed of bagasse, fish protein, whole wheat flour, sodium bicarbonate, and water (5:1:1:0.1:3.6) in producing optimum numbers of pupae.

In connection with the releases of sterile stable flies at St. Croix, U.S. Virgin Islands, Williams et al. (1981) used a medium composed of 1 part bagasse, 3 parts wheat bran, and 6 parts water. Thirty milliliters of Tedion organosulfur insecticide was added to prevent mites from infesting the medium. The larval medium was placed in 61 x 45 x 10 cm PVC trays then seeded with 60,000 eggs per tray. The average number of pupae harvested was 21,400 per tray or a 36% pupation rate. Their established colony at St. Croix was capable of producing 200,000 to 300,000 adults per day. Two technicians were hired to take care of the colony and the estimated

cost of maintenance was $300 per week in 1976, exlcuding the cost of housing and the initial equipment such as racks and larval trays.

Insect Sterilization

Gamma radiation and chemosterilants are the 2 main agents that can induce sterility among insects. During the screw-worm eradication program, sterilization was accomplished by exposing the pupae to 7.5 kR gamma radiation (Baumhover et al., 1955). The melon flies released at Rota, Mariana Islands, were sterilized with 10 kR gamma radiation (Steiner et al., 1965). A lower dose of 2 kR was used by Williams et al. (1981) to sterilize the stable flies released at St. Croix. Their primary concern was to sterilize the insects without affecting their competitiveness in the field.

The use of chemicals as a substitute for radiation to sterilize insects had been investigated by several workers. Among the earlier chemicals found to be insect sterilants were the aziridine compounds aspholate, metepa, tepa, hempa, tretamine, and thiotepa (LaBrecque, 1968). LaBrecque et al. (1972) immersed stable fly pupae in 5% aqueous solution of metepa or methiotepa for 60 minutes to sterilize the emerging adults. The sterility produced in the flies was about 90%.

Lofgren et al. (1973) reported that aziridine compounds also sterilized the mosquito Anopheles albimanus when pupae were immersed in various concentrations of the compounds. Most promising of the aziridine compounds tested was ENT 61585 or bisazir. Seawright et al.

(1973) found that 100% of the emerging mosquitoes were sterilized when pupae were dipped in 1% aqueous bisazir for 30 to 60 minutes. It was also determined in their study that less than 0.05 ng/mg bisazir residue was present on the adults 3 days after eclosion. The authors expressed doubt that any environmental hazard could be caused by releasing treated mosquitoes. El-Gazzar and Dame (1983) found that the males of Culex quinquefasciatus mosquitoes sterilized with bisazir were 96% competitive under laboratory conditions whereas males sterilized by irradiation were only 26% competitive.

Langley and Carlson (1986) were able to sterilize the tsetse fly Glossina morsitans morsitans by exposure to bisazir vapors. The most promising system designed by the authors exposed the flies at a distance of 1 to 5 cm from a silicone rubber membrane impregnated with bisazir inside a plastic chamber with air temperature ranging from 30 to 35°C. Estimated exposure times that would produce 50% sterility (ED_{50}) were 5.6 to 8.6 minutes for males and 0.4 to 0.5 minute for females. In a separate study, Langley et al. (1982) applied bisazir on pheromone-baited traps in the field. A 50% sterility was induced on the flies following contacts of more than 2 minutes with decoy-traps dosed with 1.2 mg/cm of bisazir. Other insects known to be sterilized by bisazir include the tobacco budworm (Crystal and Haught, 1982) and the cotton boll weevil (Haynes and Wright, 1979).

MATERIALS AND METHODS

This research on the stable fly consisted of 4 parts namely: (1) Field population Studies, (2) Mass Colonization, (3) Field Releases of Sterile Flies, and (4) Evaluation of Bisazir as a Chemosterilant Against the Stable Fly. The field studies were carried out at the SDSU Cattle and Sheep Nutrition Research feedlot while laboratory studies were conducted in the basement of the Physiology Laboratories on the campus of South Dakota State University, Brookings, South Dakota from May, 1986 to July, 1987.

Field Pupolation Studies

A wild stable fly population in the feedlot was studied through actual fly counts on confined beef cattle and through the use of sticky traps. Stable flies biting or alighting within 1 minute interval on the posterior aspect of the forelegs of cattle were observed with the naked eye and counted by using a hand counter. Timing was done with an alarm wrist chronograph (Casio, Japan). There were 52 pens in the feedlot each containing 8 animals. The pens were arranged in 6 parallel rows with 1 head row. Each parallel row contained 8 pens and the head row was composed of 4 pens. Fly counts were made on 20 animals chosen at random to cover all the rows. Fly counts were done each week from June 12 to September 4, 1986 at 9:00 a.m., 12:00 noon, and 3:00 p.m. on each counting date. The average number of stable flies biting or alighting on each animal was estimated.

In addition to actual fly counts, 2 sticky traps (Williams, 1973) were positioned along the periphery of the feedlot. Each trap consisted of two 20.3 x 40.6 cm translucent fiberglass panels (Alysnite@ series 600, 1.2 mm thick) fitted together at their mid-points to form 4 planes with the flat surfaces perpendicular to the ground. These panels were mounted on a 5.1 x 5.1 x 89 cm wooden stake which was previously driven to the ground with a post driver. The panels were covered with four 20 x 47 cm clear plastic sleeves (Flex-O-Glass Inc., Chicago, Illinois), held in place with staples on their open ends, then coated with an insect trapping adhesive (The Tanglefoot Co., Grand Rapids, Michigan). These sleeves were replaced with new ones and the stable flies trapped on the old sleeves counted in the laboratory. The average number of flies per trap per week was noted. The relationship between leg count and trap count was tested by simple linear correlation analysis.

To determine the influence of weather parameters on the population of the stable fly in the feedlot, a CR21 Micrologger@ (Campbell Scientific Inc., Logan, Utah) was installed in one of the empty pens. This instrument recorded various weather parameters on an hourly basis. The relationship between fly counts and air temperature, solar radiation, and precipitation were tested by simple linear correlation analyses.

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Mass Colonization

The colony was started from wild stable flies collected from the Cattle and Sheep Nutrition Research feedlot during the summer of 1986. It was maintained in the basement of the Physiology Laboratories. The temperature and relative humidity of the room where the flies were reared were 25 to 30°C and 58 to 78% respectively. Illumination from 4 overhead flourescent bulbs was continuous for 24 hours. The rearing precedure in this study was modified from the one currently used at the USDA, ARS Laboratories in Lincoln, Nebraska (Easton, 1986).

Adult Stage

The adult stable flies were maintained inside screened aluminum cages measuring 35 x 35 x 35 cm (Figure 1). A 50 x 25.4 cm (length x diameter) orthopedic stockinette (Abco Dealers Inc., Milwaukee, Wisconsin) was attached in front of the cage for easier access into it. The flies were fed with bovine blood obtained from a local locker (Artz Locker, Brookings, South Dakota). Sodium citrate at the rate of 3 grams per liter of blood was added as an anticoagulant. Two 27.5 x 7 x 1 cm obstetrical pads (Diana Mfg. Co., Green Bay, Wisconsin) soaked with citrated bovine blood were provided on top of each cage and changed daily (Figure 2). To prevent the pads from drying too quickly, the upper surface of each pad was moistened with tap water then covered with a clear polyethylene plastic wrap.

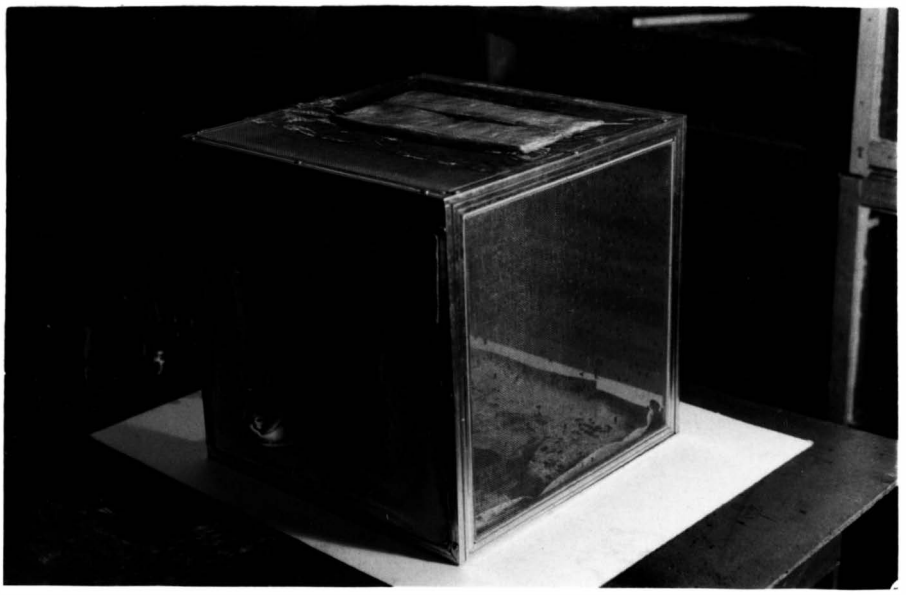


FIGURE 1 A screened aluminum cage measuring 35 x 35 x 35 cm used for maintaining adult stable flies in the laboratory.

FIGURE 2 A close-up of the roof of the cage showing stable flies feeding on citrated bovine blood absorbed on 2 obstetrical pads.

Gravid female flies were allowed to lay eggs on a 14 x 35 cm black cotton cloth suspended in tap water like a wick (Figure 3). The cloth was held with a plastic petri dish cover with a 4 x 0.5 cm slit in the middle. The jar which was filled with tap water measured 8 x 11 cm (diameter x height). The black cloth was left inside the cage for 24 hours and the eggs collected daily by lifting the petri dish cover with the cloth out of the cage, then returned as soon as the eggs were collected. The number of eggs laid by a known initial number of female flies was recorded daily for at least 8 days. The black cloth was placed inside the cage on the day when eggs were first observed on the obstetrical pads. Female stable flies generally preferred the black cloth over the pads as an ovipositional site.

Egg Stage

Eggs laid on the black cloth were flushed into a 26 x 20 x 14 cm plastic container using tap water supplied from a faucet, then poured into a plastic funnel equipped with a strainer. The top and bottom diameters of the funnel were 14 and 5 cm respectively. A 10 x 10 cm cloth attached onto the bottom of the funnel by silicone glue was used to strain the eggs.

Eggs were quantified volumetrically by transferring the strained eggs by a camel's hair brush into a modified 5 ml plastic disposable syringe. The needle assembly of the syringe was cut off so the eggs were held by a 2 x 2 cm cloth fastened onto the end of

FIGURE 3 A black cotton cloth suspended in tap water used as an oviposition site for caged gravid female stable flies.

the syringe with a rubberband. The syringe was calibrated by first knowing the number of eggs occupying a certain volume, i.e., by counting the number of eggs in a conveniently small tube measuring 0.3 x 0.5 cm (diameter x height). Based on 3 replicates, approximately 11,000 eggs occupied 1 cm volume. The syringe which had a diameter of 1.1 cm^3 was calibrated as follows:

$$1\ cm^3 = (3.1416)(1.1/2)^2(L)$$

$$L = 1.1\ cm$$

Each 1 cm length of the syringe corresponded to 10,000 eggs, i.e., 11,000 eggs divided by 1.1 cm. The number of eggs in the syringe was estimated by using a plastic ruler.

The hatching rate of the eggs were determined on the second day of egg-laying by taking 3 samples from each cage. At least 100 eggs per sample were transferred into a petri dish lined with a moistened black filter paper (Eaton-Dikeman, Mt. Holly Springs, Pennsylvania) (Figure 4). The filter paper was kept moist by misting with a hand sprayer to prevent the eggs from dessication. The number of eggs hatching was counted under a dissecting microscope after 4 days of incubation at 25 to 30°C. The contrast provided by the black filter paper greatly facilitated examination and counting of the white eggs.

Larval Stage

A known number of eggs was washed from the syringe onto the surface of the larval medium by using a wash bottle. Only freshly

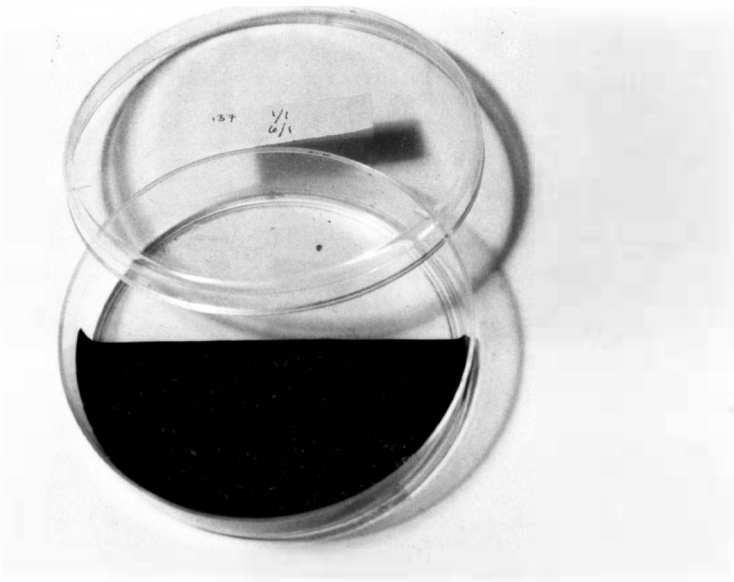


FIGURE 4 A petri dish lined with a moistened black filter paper used to check the hatchability of stable fly eggs.

harvested eggs were used. The eggs were left uncovered by the medium since prior observations indicated that buried eggs did not hatch. The larval container used was a 29.2 x 34.3 cm dishpan (Rubbermaid Inc., Ohio) (Figure 5). The trays were placed inside a 74 x 52 cm cloth bags then fastened with rubberbands to prevent contamination by eggs of other insects especially houseflies. The larvae hatching from the eggs were allowed to develop undisturbed until pupation.

The larval medium was composed of 2 liters zonolite, 2 liters wheat bran, 0.2 liter fishmeal and 2.5 liters water (Figure 6). The dry ingredients were mixed before and after addition of water by hand. The depth of the medium was approximately 6 cm.

Pupal Stage

Four days were allowed to elapse after the initial appearance of pupae along the sides of the tray to allow the majority of the larvae to pupae before they were harvested. The pupae were harvested by scooping them from the sides of the tray by hand. They were cleaned by flotation in tap water, straining the pupae and larval medium afloat, drying by an electric fan, then finally blowing-off the remaining larval medium with forced air coming from a hairdryer (The Pro 1250, K-Mart Corp., Troy, Michigan). Most of the zonolite and some wheat bran floated with the pupae and did not pass through the strainer but could be easily blown off after air-drying. The waste larval medium was placed inside plastic bags and disposed of carefully since it contained some pupae and larvae.

FIGURE 5 A 29.2 x 34.3 x 13.3 cm dishpan containing the artificial medium where the larvae were allowed to develop.

FIGURE 6 Mature stable fly larvae developing in the larval medium composed of zonolite, wheat bran, fishmeal and water (2:2:0.2:2.5).

The cleaned puape were weighed and quantified by first ascertaining the average weight of each pupa. The average weight of each pupa was determined by weighing three 1 gram samples of pupae using a platform balance then counting the number of pupae contained in the 1 gram samples. The number of pupae harvested from each tray was determined by dividing the total weight of the pupae by the average weight of each pupa in that container. The differences in the mean number of pupae developing from known numbers of eggs initially placed in the container were analyzed using the t-test.

Field Releases of Sterile Flies

Field releases of sterile stable flies were done twice per week from July 25 to September 22, 1986. On the average, 25,725 sterile male and female flies were released per week. A total of 231,527 flies were released throughout the 9 week period.

To sterilize the flies, about 44,000 pupae per week were sent to USDA Radiation and Metabolism Research Laboratories at Fargo, North Dakota by bus. The pupae were placed inside quart size Ziploc® storage bags (The Dow Chemical Co., Indianapolis, Indiana). Not more than 15,000 pupae were placed inside each bag to facilitate handling and irradiation (Fgiure 7). To prevent the emergence of adult flies while in transport, the bags containing the pupae were placed inside a cold chest at approximately 10 to 15°C temperature. In Fargo, the pupae were irradiated with 2 kR of gamma radiation coming from a cobalt 60 source. The irradiated pupae were returned to SDSU via the earliest southbound bus.

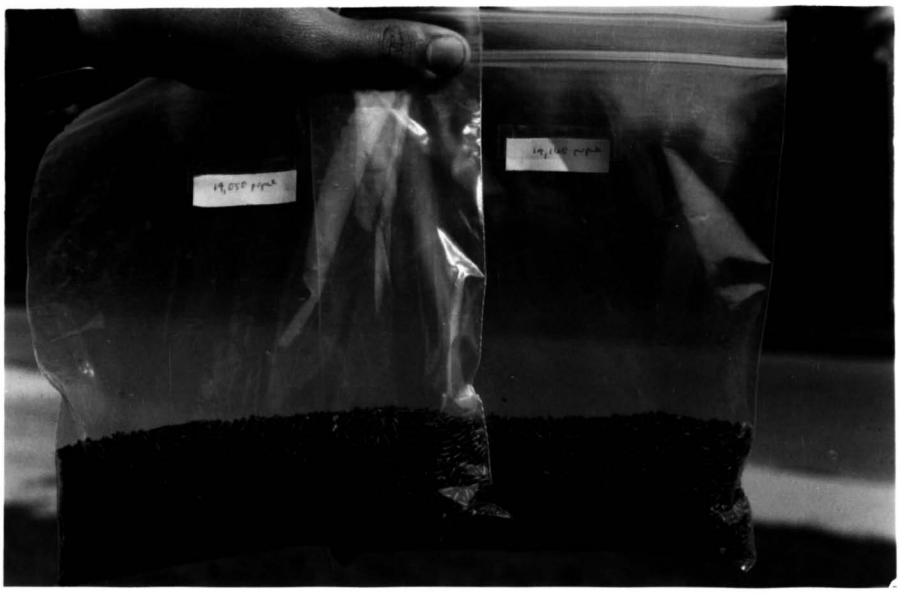


FIGURE 7 Stable fly pupae inside plastic storage bags (ca. 14,000 pupae per bag) ready to be sent for irradiation.

The irradiated pupae were allowed to eclose inside 35 x 35 x 35 cm metal cages described previously. Approximately 10,000 adults were contained per cage. The flies were fed with citrated bovine blood in the laboratory and then marked with a fire orange fluorescent pigment (Day-Glo Corp., Cleveland, Ohio) by dusting the adults in a cage lined with newsprint using a powder insufflator (DeVilbiss, Somerset, Pennsylvania) then transported to and released into the feedlot. Marking was essential to distinguish the sterile from wild or fertile flies at sampling time. The number of flies released was estimated by determining the percent emergence of adults from the pupae placed in the cage for eclosion. Three pupal samples were taken from each cage and the number of empty pupal cases was counted.

To assess the effect of the released sterile flies on the reproductive potential of the wild population, sampling of adult stable flies was done once a week immediately after the first release. At least 200 female flies were captured from the cattle pens using a standard aerial insect net. Sampling was done in the late afternoons when stable flies congregate on the wooden fences bordering the feedlot. The captured flies were placed inside a 15 x 15 x 20 cm wooden cage, transported back to the laboratory then sorted. Sorting was accomplished by first immobilizing the flies by placement inside a freezer for 10 minutes. To separate the marked from wild flies, a desk lamp fitted with two F15T8 BLB Black Light Bulbs® (General Electric, USA) was used. Marked flies

glowed when examined under this ultraviolet light source and thus could be easily counted and separated. The ratio of the sterile flies to the wild ones was recorded.

At least 200 wild female stable flies were placed inside a 35 x 35 x 35 cm metal cage, fed with citrated bovine blood, then set for egg-laying as described previously under the mass colonization part. The percent hatchability of the eggs was determined by transferring a known number of eggs into a petri dish lined with moistened black Filter paper as described earlier (Figure 4). Ten replicates with each replicate containing at least 100 eggs were made. The number of eggs hatching was determined under a dissecting microscope after 7 days of incubation at 25 to 30°C. The relationship between the number of sterile flies released in the field and the percent hatchability of the eggs laid by wild female flies was tested by simple linear regression analysis.

Evaluation of Bisazir as a Chemosterilant Against the Stable Fly

The procedure of sending the pupae to Fargo, North Dakota for irradiation was proven to be incovenient and limited the maximum number of flies that could be released per week. Also, losses due to overexposure to cold temperature were estimated at 40%. A thioaziridine chemosterilant called bisazir[1] (Figure 8) was therefore evaluated as a possible substitute for gamma radiation as a

[1] P, P-bis (1-aziridinyl)-N-methylphosphinothioic amide

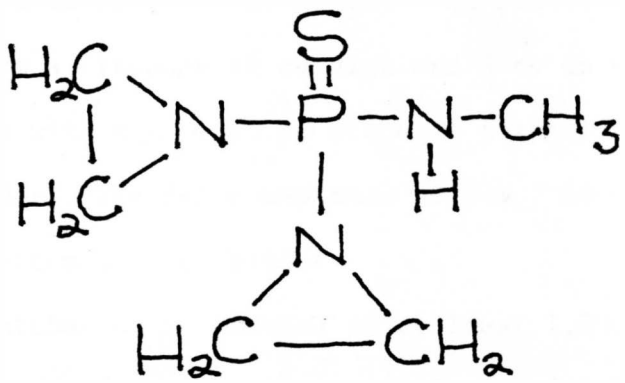


FIGURE 8 The structural formula of bisazir or P, P-bis (1-aziridinyl)-N-methyl phosphinothioic amide.

means of sterilizing the stable fly. Bisazir was evaluated in the laboratory from December, 1986 to February, 1987.

Small plastic cages were constructed to carry out single-pair matings of stable flies (Figure 9). These experiments were necessary to accurately test the degree of sterility of the treated male and female stable flies. Each cage was cylindrical and made mostly of clear plastic (Flex-O-Glass Inc., Chicago, Illinois). A rectangular 20 x 12 cm piece of plastic was rolled and stapled on its ends to form a cylinder 12 cm high and 5 cm in diameter. The top was covered with a 10 x 10 cm piece of plastic netting stapled onto the cylinder. The floor was made of 7 x 7 cm clear plastic taped at the bottom of the cylinder.

Four batches or replicates of at least 1,000 pupae were dipped in 50 ml of 1% aqueous bisazir solution for 90 minutes in a 100 x 20 mm plastic perti dish (Figure 11). The pupae were air-dried then allowed to eclose inside a quart size Ziploc bag (Figure 12) to prevent them from mating. The adults emerging from the treated pupae were immobilized in cold temperature then sexed. Twenty-five males and 25 females from each batch of dipped pupae were transferred individually into the plastic cages. Each fly was then paired with a normal virgin male or female. A total of 100 treated female-normal male single pairs and 100 treated male-normal female single pairs were set up. Twenty-five normal female-normal male single pairs were included as controls.

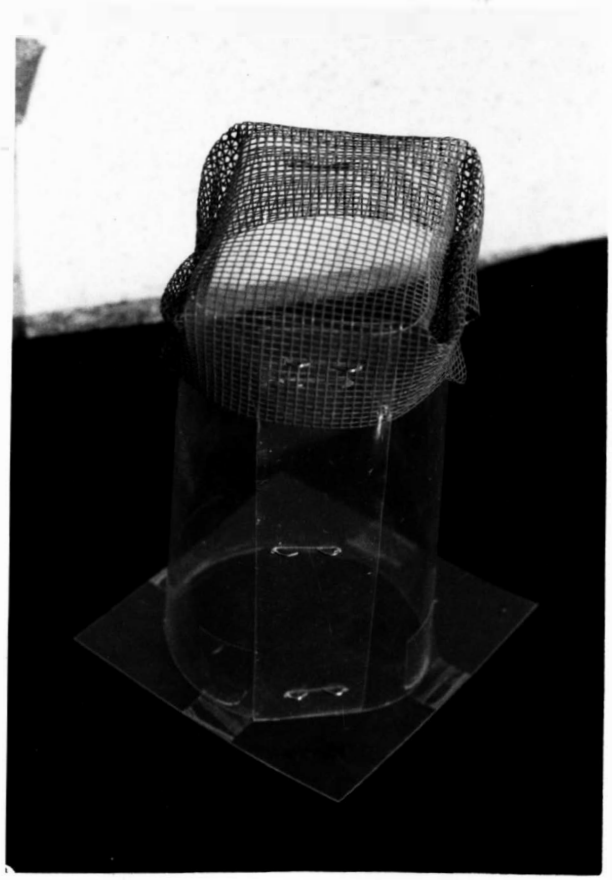


FIGURE 9 A 5 x 12 cm (diameter x height) plastic cage designed to allow single-pair matings of stable flies.

FIGURE 11 Stable fly pupae immersed in 50 ml of 1% aqueous bisazir solution in a 100 x 20 mm plastic petri dish.

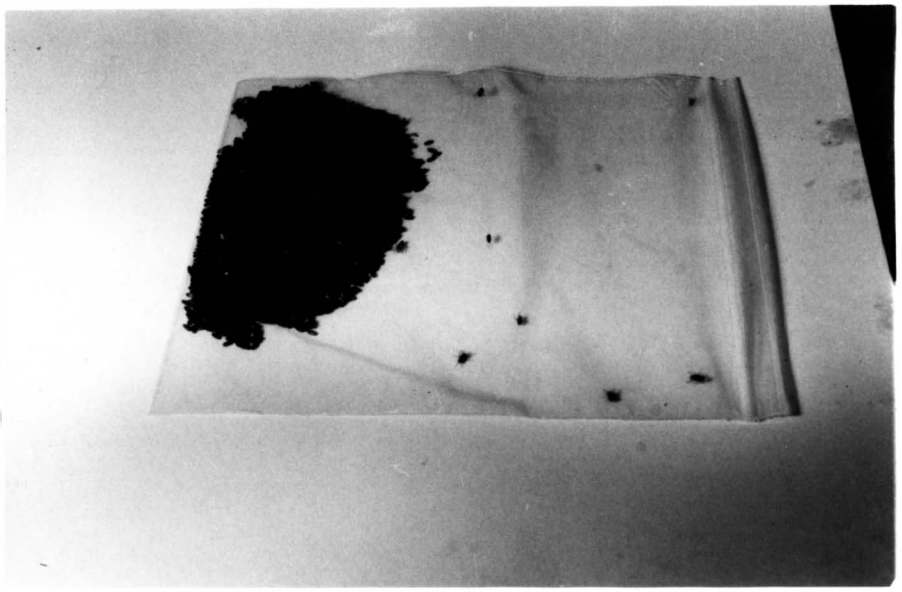


FIGURE 12 Adult stable flies emerging from bisazir-treated pupae inside a plastic bag.

Each pair was provided with a 2 x 2 cm cotton pad soaked in citrated bovine blood as a food source and oviposition site (Figure 10). The surface of the pad was moistened with tap water then covered with a 10 x 10 cm piece of polyethylene plastic wrap to prevent the blood from dessicating too quickly. The blood-soaked pads were changed and examined for eggs daily. The eggs laid by each female were transferred to a petri dish lined with a moistened black filter paper (Figure 4). After 7 days of incubation at 25 to 30°C, the percent hatchability was determined under a dissecting microscope. The longevity of each fly inside the cage and the total number of eggs laid by each female fly during its lifetime were also noted. The differences in longevity between the treated and normal flies were analyzed using the t-test.

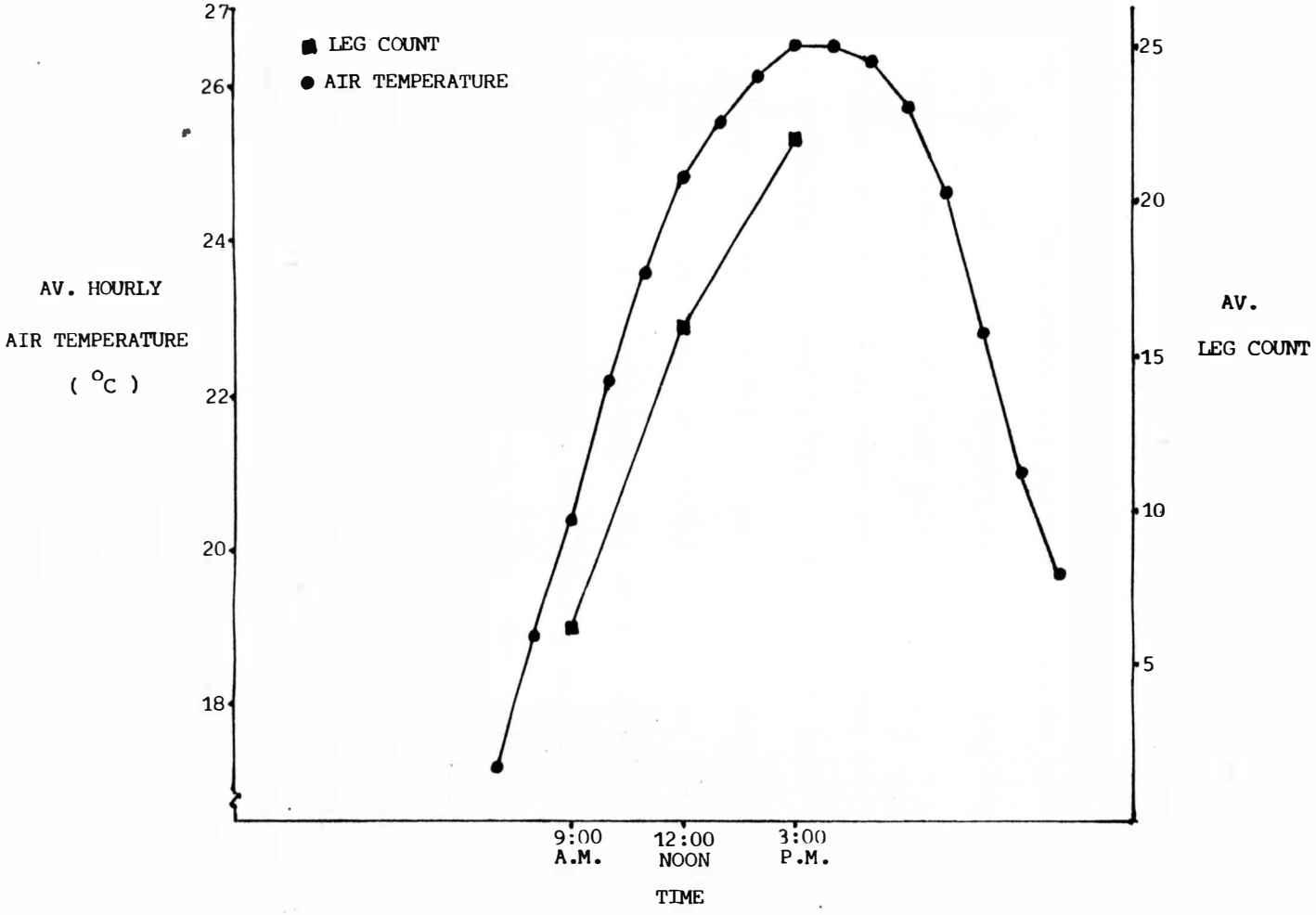
RESULTS AND DISCUSSION

Field Population Studies

In Figure 13, the average number of stable flies biting or alighting on the posterior aspect of the forelegs of confined beef cattle at 3 different periods of the day is shown. The relationship between the fly counts and the average hourly air temperature changes from June 12 to September 4, 1986 is included. The number of stable flies on the forelegs of cattle was highest at 3:00 p.m., corresponding to the highest air temperature recorded for the day. An average of 22 flies per animal was observed at 3:00 p.m. when

FIGURE 10 A close-up of the cylindrical plastic cages showing the 2 x 2 cm blood-soaked cotton pads provided for each cage as a source of food and oviposition site.

hourly air temperature (°C) changes.



the average air temperature reached 26.5°C. Analysis by simple linear correlation indicated an association between the number of stable flies and the prevailing air temperatures from 9:00 a.m. to 3:00 p.m. (Table 1). Evidence is also provided that the increase in numbers of stable flies from 9:00 a.m. to 3:00 p.m. was due to the increasing temperature. The association between fly counts and air temperature after 3:00 p.m. was not investigated considering the fact that air temperatures declined after this time.

The relationship between fly counts on the forelegs of cattle and solar radiation is shown in Figure 14. A peak solar radiation value of approximately 2,700 kilojoules per square-meter was recorded at 1:00 p.m. then declined thereafter, whereas the peak fly count was observed 2 hours later. The effect of solar radiation on the numbers of flies biting or alighting was probably indirect, i.e., air temperature rose which in turn resulted in increased numbers of stable flies observed until 3:00 p.m. There was no linear correlation between the numbers of stable flies and solar radiation from 9:00 a.m. to 3:00 p.m. (Figure 14, Table 2).

The population of stable flies in the SDSU Cattle and Sheep Nutrition Research feedlot can be roughly estimated by multiplying the number of cattle by twice the average fly numbers at 3:00 p.m. (416 x 2 x 22 = 36,608 flies). This estimate is based on the observation of Berry et al. (1983) that the number of flies on the forelegs is about equal to the number of flies on the hindlegs of each animal. Through counting the numbers of stable flies on the

Table 1 Relationship between the average number of stable flies on the forelegs of confined beef cattle and the average air temperature (°C) from June 12 to September 4, 1986 at the SDSU Cattle and Sheep Nutrition Research feedlot.

Time	X_1: Leg counts	X_2: Air temperature(°C)
9:00 a.m.	5	20.4
12:00 noon	16	24.8
3:00 p.m.	22	26.5
	$Sx_1^2 = 148.7$	$Sx_1x_2 = 53.8$ $Sx_2^2 = 19.6$
	$\bar{x}_1 = 14.3$	$r = 0.997$ $\bar{x}_2 = 23.9$

The tabular value of r=0.997 (p=0.05; d_f=1). There is a significant linear correlation between leg counts and air temperature.

FIGURE 14 The average number of stable flies biting or alighting on the forelegs of confined beef cattle at 3 different times of the day from June 12 to September 4, 1986 at the SDSU Cattle and Sheep Nutrition Research feedlot in relation to the average solar radiation (KJ/m^2) changes.

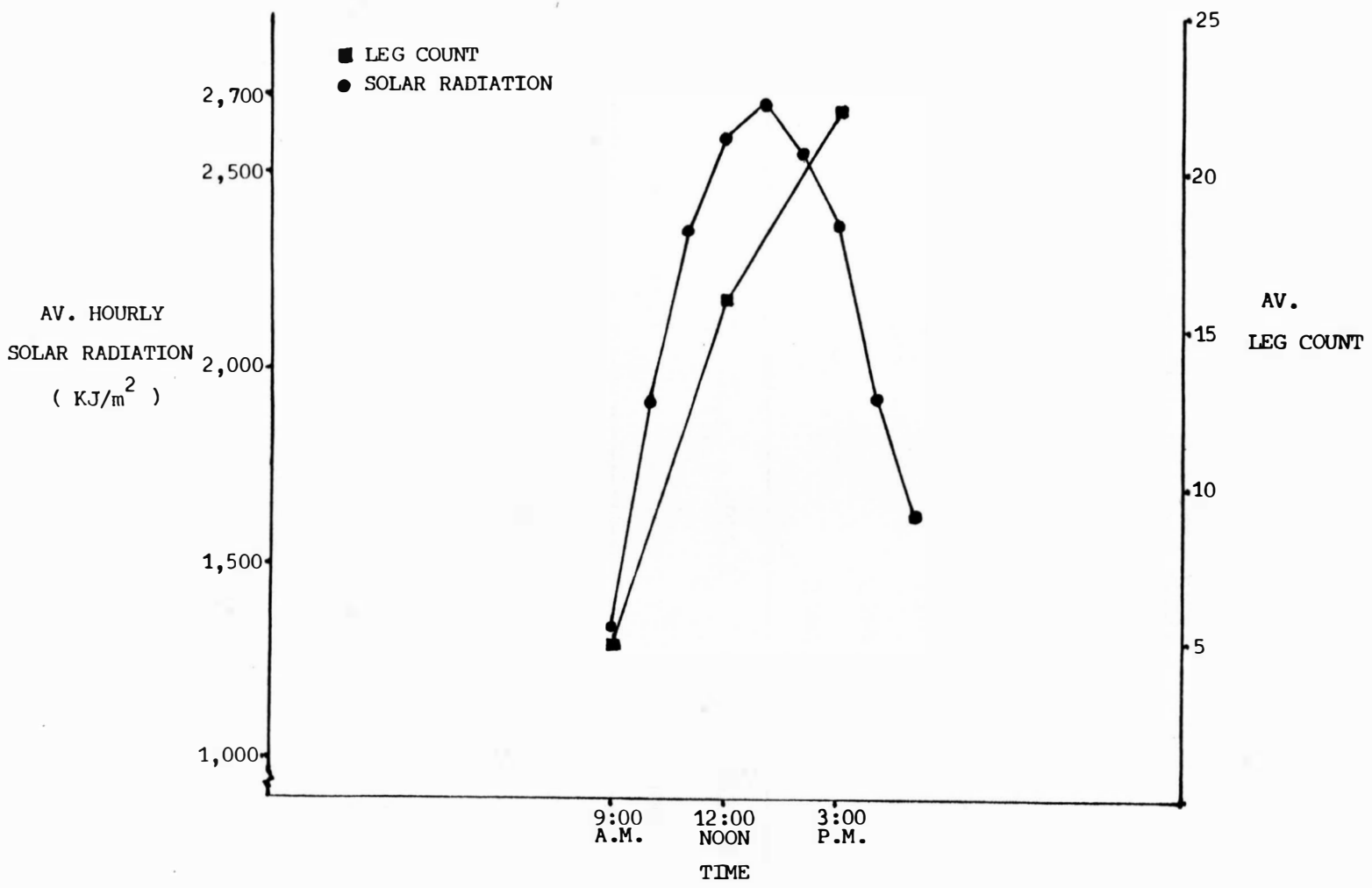


Table 2 Relationship between the average number of stable flies on the forelegs of confined beef cattle and the average solar radiation (KJ/m^2) at 3 different times of the day from June 12 to September 4, 1986 at the SDSU Cattle and Sheep Nutrition Research feedlot.

Time	X_1: Leg counts	X_2: Solar radiation(KJ/m^2)
9:00 a.m.	5	1341.9
12:00 noon	16	2594.1
3:00 p.m.	22	2364.3
	$Sx_1^2 = 148.7$	$Sx_1x_2 = 9925.3$ $Sx_2^2 = 888625.8$
	$\bar{x}_1 = 14.3$	$r = 0.864$ $\bar{x}_2 = 2100.1$

The tabular value of r=0.997 (p=0.05); d_f=1). There is no linear correlation between leg counts and solar radiation.

forelegs and hindlegs of cattle simultaneously, Berry et al. (1983) noted that the foreleg counts must be multiplied by 2.81 to estimate the number of flies on all the legs. Since almost all of the stable flies observed to feed or alight below the chest floor of a standing animal, these authors assumed that leg counts represented total body counts. According to LaBrecque et al. (1972), a single fly on a cow may actually represent 56 flies in the total fly population in an area.

The weekly variations in the average number of stable flies biting or alighting on the forelegs of cattle at 3:00 p.m. is presented in Figure 15. The 3:00 p.m. counts were utilized for comparing weekly counts since the highest counts within a day was observed at this time. The highest count of 37 flies per animal occurred in July and the lowest counts were observed in early June and late August. However, there was no clear pattern observed as the counts fluctuated widely from June 12 to September 4, 1986. There was no linear correlation between weekly variations in the average leg counts and the weekly air temperature readings at 3:00 p.m. (Figure 16, Table 3).

Figure 17 shows the weekly variations in the average number of stable flies captured per sticky trap. There was no predictable pattern observed although the highest count was in July. There was also no linear correlation between weekly average trap counts and weekly average air temperatures (Figure 18, Table 4). Air temperature

FIGURE 15 Weekly variation in the number of stable flies biting or alighting on the forelegs of cattle at 3:00 p.m. from June 12 to September 4, 1986 at the SDSU Cattle and Sheep Nutrition Research feedlot.

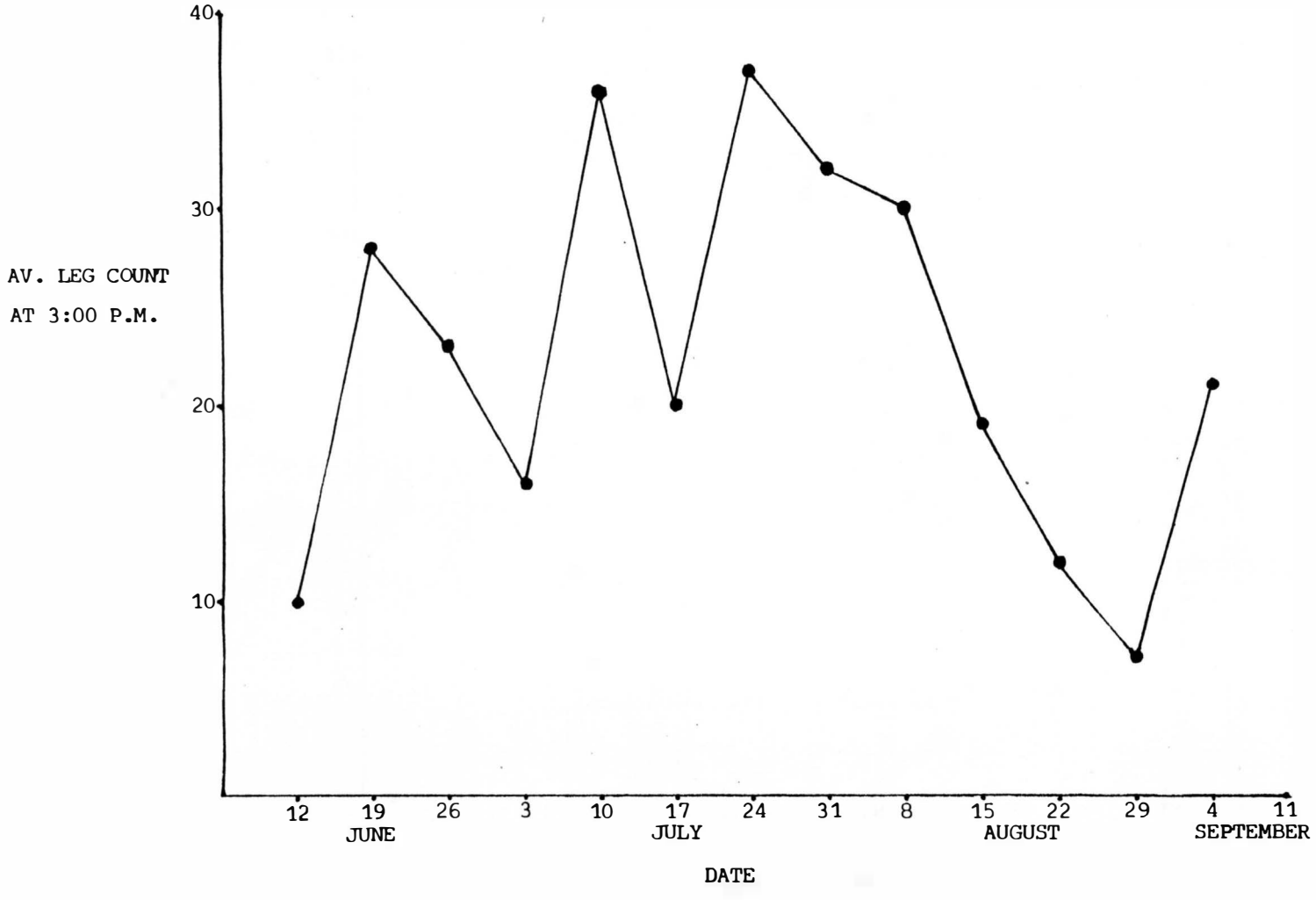


FIGURE 16 The degree of association between the weekly average number of stable flies biting or alighting on the forelegs of confined beef cattle and the weekly air temperature (°C) at 3:00 p.m. from June 12 to September 4, 1986 at the SDSU Cattle and Sheep Nutrition Research feedlot.

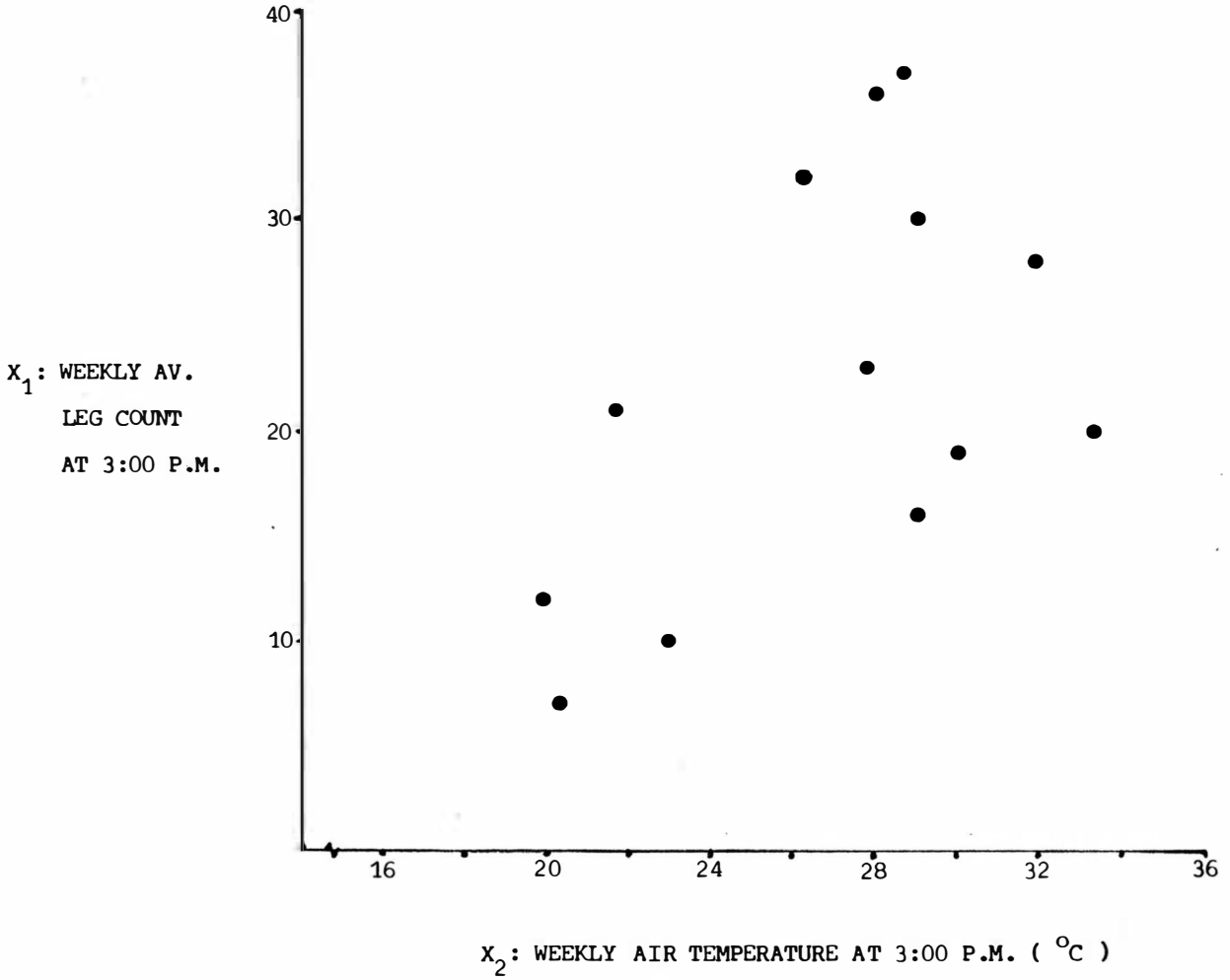


Table 3 Relationship between the weekly average number of stable flies on the forelegs of confined beef cattle and the weekly air temperature (°C) at 3:00 p.m. from June 12 to September 4, 1986 at the SDSU Cattle and Sheep Nutrition Research feedlot.

Week	X_1: Leg count at 3:00 p.m.	X_2: Air temperature at 3:00 p.m.(°C)
1	10	25.8
2	28	34.0
3	23	27.8
4	16	29.2
5	36	28.1
6	20	33.4
7	37	28.8
8	32	26.3
9	30	29.1
10	19	31.0
11	12	19.9
12	7	20.3
13	21	21.7
	$Sx_1^2 = 1139.1$ $Sx_1x_2 = 246.5$	$Sx_2^2 = 244.2$
	$\bar{x}_1 = 22.4$ $r = 0.467$	$\bar{x}_2 = 27.3$

The tabular value of r=0.553 (p=0.05; d_f=11). There is no linear correlation between weekly average leg count and air temperature at 3:00 p.m.

FIGURE 17 Weekly variation of average number of stable flies captured per sticky trap from June 12 to August 29, 1986 at the SDSU Cattle and Sheep Nutrition Research feedlot.

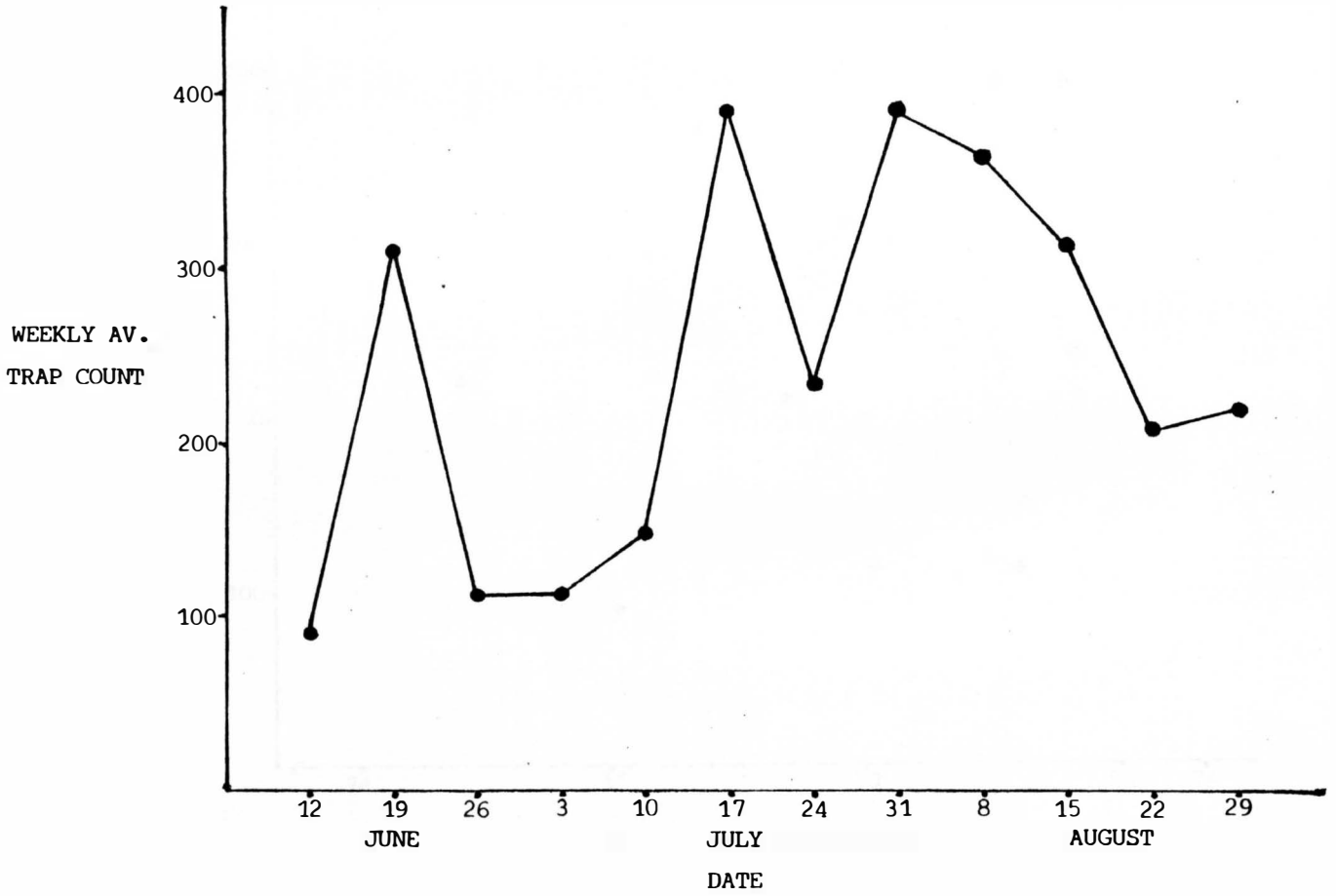


FIGURE 18 The degree of association between the weekly average number of stable flies captured per sticky trap and the weekly average air temperature (°C) from June 12 to August 29, 1986 at the SDSU Cattle and Sheep Nutrition Research feedlot.

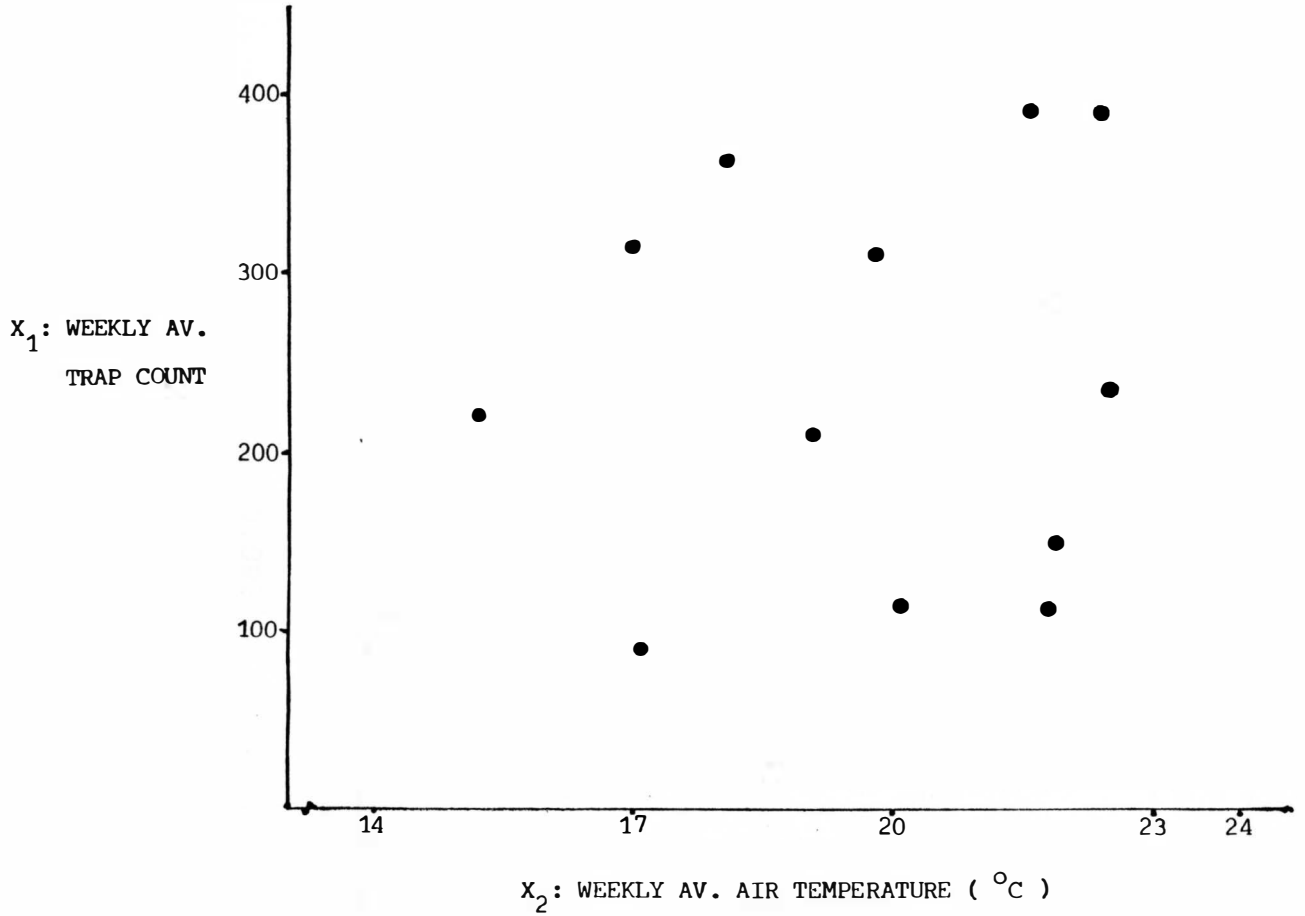


Table 4 Relationship between the weekly average number of stable flies captured per sticky trap and the weekly average air temperature (°C) from June 12 to August 29, 1986 at the SDSU Cattle and Sheep Nutrition Research feedlot.

Week	X_1: Trap count	X_2: Average air temperature(°C)
1	88	17.1
2	309	19.8
3	113	21.8
4	114	20.1
5	148	21.9
6	392	22.4
7	236	22.5
8	389	21.6
9	364	18.1
10	314	17.0
11	208	19.1
12	220	15.2
	$Sx_1^2 = 135912.3$	$Sx_1x_2 = 275.7$ $Sx_2^2 = 65.4$
	$\bar{x}_1 = 241.2$	$r = 0.092$ $\bar{x}_2 = 19.7$

The tabular value of r=0.576 (p=0.05; d_f=10). There is no linear correlation between weekly trap count and weekly average air temperature.

therefore had an effect on the hourly leg counts but not on the weekly leg and trap counts.

Figures 19 and 20, and Tables 5 and 6, indicate that rainfall had no influence either on the leg or trap counts of stable flies. In contrast, Greene (1986) observed an increased fly population immediately following rainfall of 1.91 cm or more in feedlots with fine silt loam soil in Western Kansas. He attributed this to a response of the stable flies to availability of breeding sites and the increased chance of eggs resulting in pupae before the breeding areas dry out.

Figure 21 and Table 7 show that there was no correlation between leg counts and trap counts. This lack of correlation between leg and trap counts was also observed by Berry et al. (1983). The authors favored the use of leg counts over trap counts in estimating production losses because leg counts have the advantage of indicating the degree of irritation of fly bites to the host animals. Berry et al. (1983) furthermore believed that leg counts may be less influenced by environmental factors than are trap counts.

Mass Colonization

Tables 8 and 9 summarize the most important information needed to mass-produce the stable fly for field releases of sterile insects. Table 8 shows that egg production was highest on the second day of egg-laying then declined thereafter. The female flies were 6 days old on the first day of egg-laying. The daily mortality rate

FIGURE 19 The degree of association between the weekly average number of stable flies biting or alighting on the forelegs of cattle at 3:00 p.m. and the total weekly precipitation (cm) from June 12 to August 29, 1986 at the SDSU Cattle and Sheep Nutrition Research feedlot.

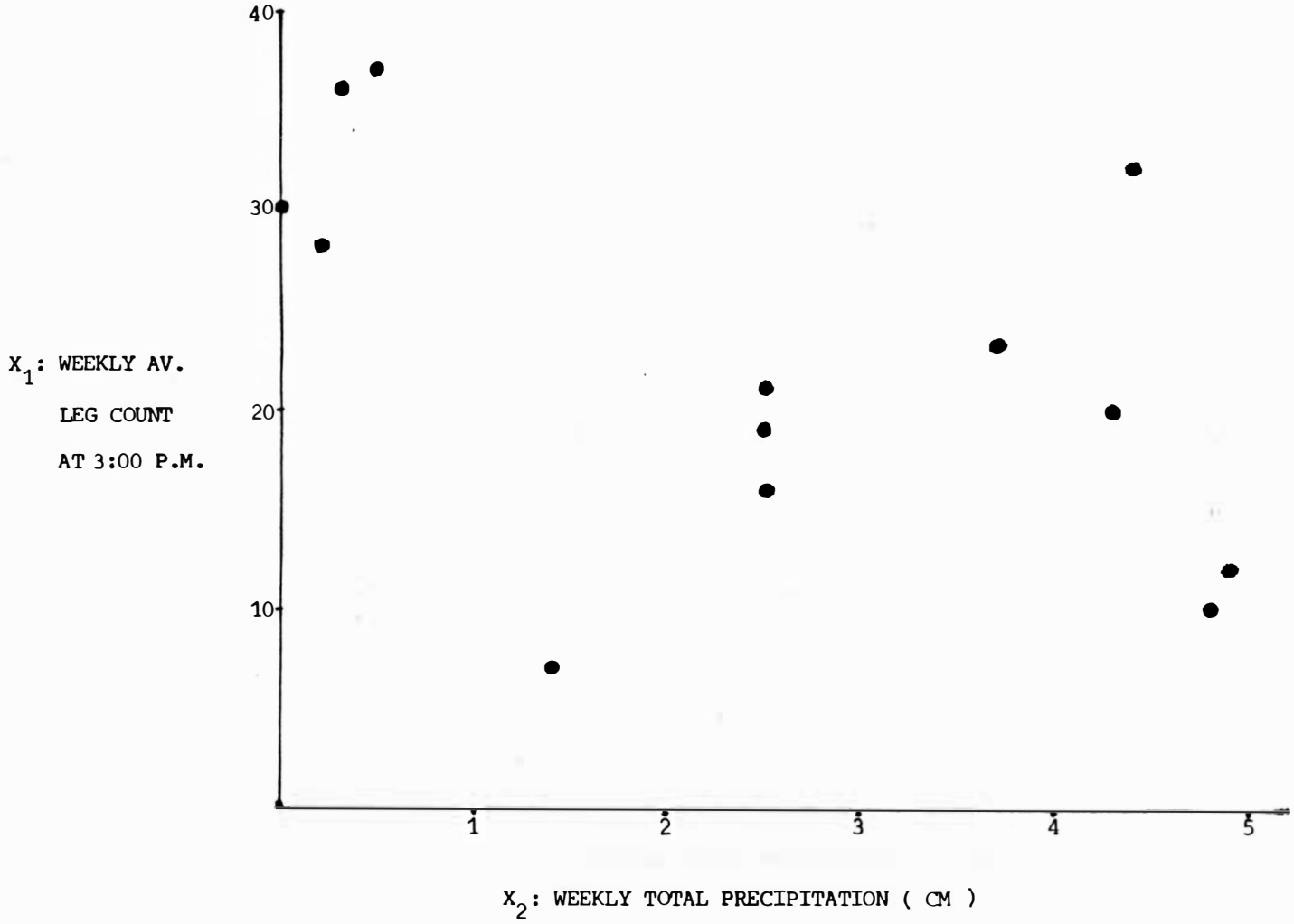


FIGURE 20 The degree of association between the weekly average number of stable flies captured per sticky trap and the weekly total precipitation(cm) from June 12 to August 29, 1986 at the SDSU Cattle and Sheep Nutrition Research feedlot.

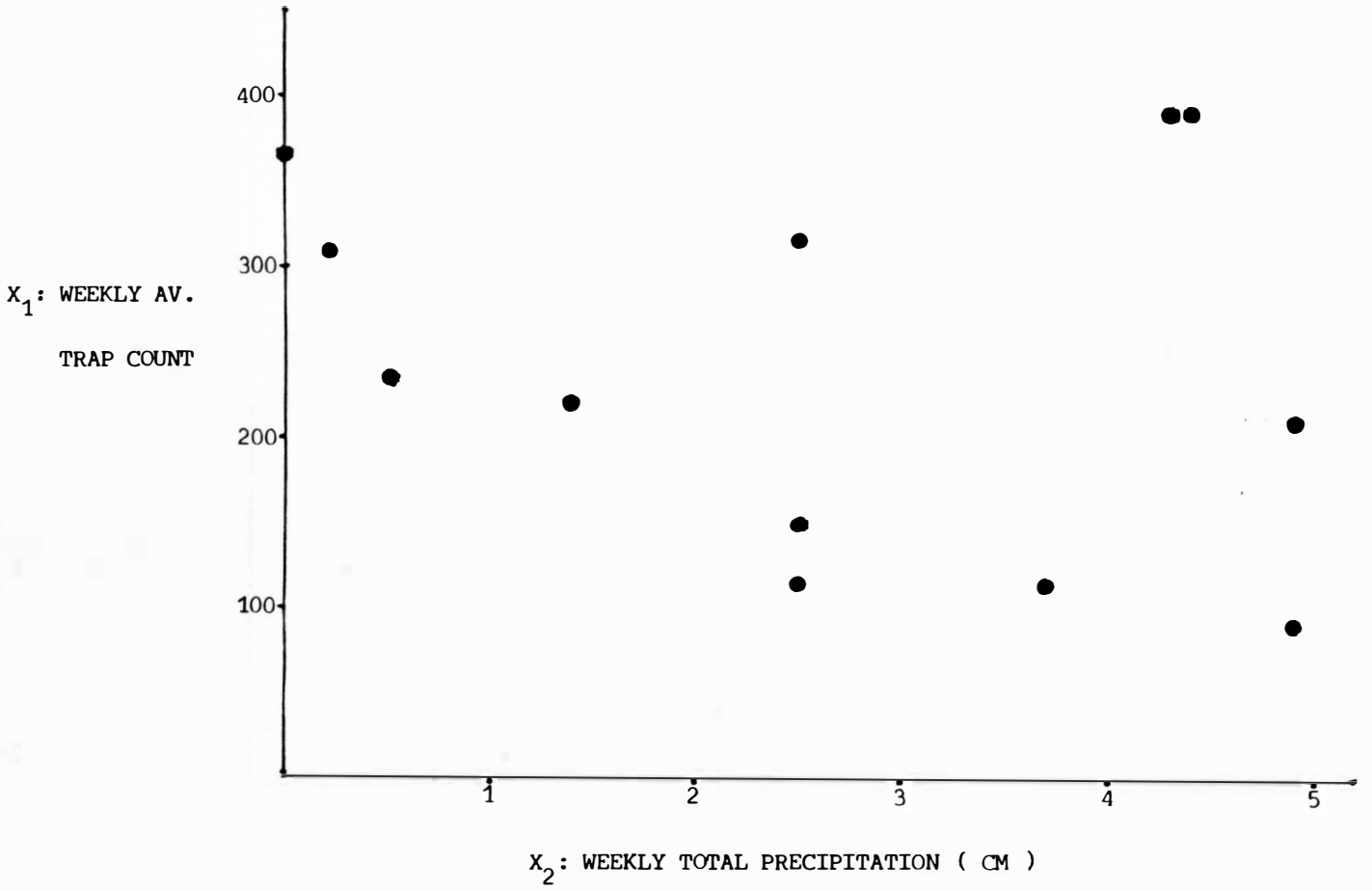


Table 5 Relationship between the weekly average number of stable flies on the forelegs of confined beef cattle at 3:00 p.m. and the weekly total precipitation (cm) from June 12 to September 4, 1986 at the SDSU Cattle and Sheep Nutrition Research feedlot.

Week	X_1: Leg count at 3:00 p.m.	X_2: Total precipitation (cm)
1	10	4.85
2	28	0.15
3	23	3.71
4	16	2.46
5	36	0.25
6	20	4.27
7	37	0.46
8	32	4.44
9	30	0.00
10	19	2.46
11	12	4.88
12	7	1.37
13	21	2.51
	$Sx_1^2 = 1139.1$	$Sx_1x_2 = -113.6$ $Sx_2^2 = 41.8$
	$\bar{x}_1 = 22.4$	$r = -0.520$ $\bar{x}_2 = 2.4$

The tabular value of r=0.553 (p=0.05; d_f=11). There is no linear correlation between weekly leg count at 3:00 p.m. and weekly total precipitation.

Table 6 Relationship between the weekly average number of stable flies captured per sticky trap and the weekly total precipitation (cm) from June 12 to August 29, 1986 at the SDSU Cattle and Sheep Nutrition Research feedlot.

Week	X_1: Trap counts	X_2: Total precipitation(cm)
1	88	4.85
2	309	0.15
3	113	3.71
4	114	2.46
5	148	0.25
6	392	4.27
7	236	0.46
8	389	4.44
9	364	0.00
10	314	2.46
11	208	4.88
12	220	1.37
	$Sx_1^2 = 135912.3$	$Sx_1x_2 = -260.4$ $Sx_2^2 = 41.8$
	$\bar{x}_1 = 241.2$	$r = -0.109$ $\bar{x}_2 = 2.4$

The tabular value of $r=0.576$ ($p=0.05$; $d_f=10$). There is no linear correlation between weekly trap count and weekly total precipitation.

FIGURE 21 The degree of association between the weekly average number of stable flies captured per sticky trap and the weekly average number of stable flies biting or alighting on the forelegs of cattle at 3:00 p.m. June 12 to August 29, 1986 at the SDSU Cattle and Sheep Nutrition Research feedlot.

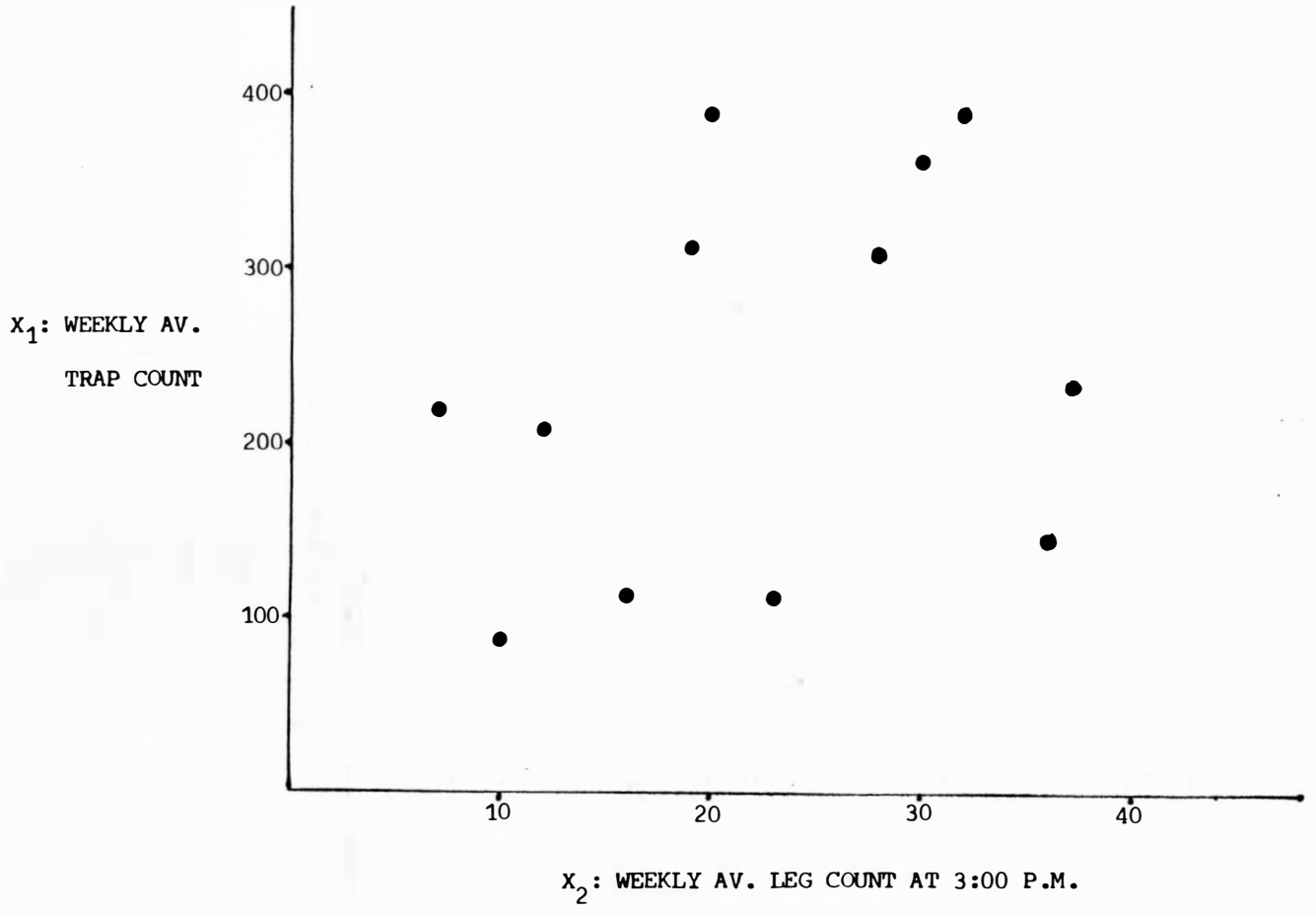


Table 7 Relationship between the weekly average number of stable flies captured per sticky trap and the weekly average number of stable flies on the forelegs of confined beef cattle at 3:00 p.m. from June 12 to August 29, 1986 at the SDSU Cattle and Sheep Nutrition Research feedlot.

Week	X_1: Trap count		X_2: Leg Count
1	88		10
2	309		28
3	113		23
4	114		16
5	148		36
6	392		20
7	236		37
8	389		32
9	364		30
10	314		19
11	208		12
12	220		7
	$Sx_1^2 = 135912.3$	$Sx_1x_2 = 4087.5$	$Sx_2^2 = 1137.0$
	$\bar{x}_1 = 241.2$	$r = 0.328$	$\bar{x}_2 = 22.5$

The tabular value of $r=0.576$ ($p=0.05$; $d_f=10$). There is no linear correlation between trap count and leg count.

Table 8 Total number of eggs produced by fertile female stable flies in 4 separate cages.*

Cage	Initial number of females per cage	Total number of eggs laid			
		Day 1	Day 2	Day 4	Day 8
A	2,137	59,000	59,000	30,000	21,000
B	1,362	14,000	24,000	25,000	11,000
C	1,244	30,000	41,000	39,000	18,000
D	1,527	57,000	55,000	47,000	30,000
TOTAL	6,270	160,000	179,000	141,000	80,000
Average number of eggs laid per initial female in the cage		26	28	22	13

* The female flies were 6 days old on Day 1.

was not determined hence the egg production data were based on the initial number of female flies in the cage. The number of females was estimated by dividing the initial number of flies by 2, assuming a 1:1 male to female ratio. The initial number of flies was determined from the percent eclosion of pupae placed inside the cage. One thousand female flies can be expected to produce 13,000 eggs on their eighth day of egg-laying. Whether the decrease in egg production after the second day was due to a decrease in egg-laying capacity or fly mortality, or both, was not ascertained.

Table 9 indicates that at least 5,000 pupae can be harvested per tray if 15,000 eggs were initially introduced on the larval medium or a 36% pupation rate. The optimum number of eggs introduced into the tray was 15,000. Twenty thousand eggs resulted to a significant reduction in percent pupation as well as a reduction in the size or weight of the pupae produced. This was due to overcrowding of the developing larvae in the tray. Increasing the initial numbers of eggs from 10,000 to 15,000 resulted in a significant increase in the number of pupae harvested without a decrease in percent pupation and pupal weight. Although the competitiveness of smaller flies was not investigated, larger pupae were considered more desirable for field releases.

Table 10 presents additional data pertinent to mass-rearing of the stable fly. The eggs hatched within 2 days when incubated at 25 to 30°C in a petri dish lined with a black moistened filter paper. The adult female flies were observed to lay eggs 6 days after

Table 9 Production of stable fly pupae from plastic dishpans initially seeded with known numbers of eggs laid in the laboratory.*

No. of eggs introduced	No. of pupae harvested ($\bar{x}$±SD)	% pupation ($\bar{x}$±SD)	Duration from egg to harvest of pupae (days)	Av. weight of each pupa (mg)
10,000	3,655[a]±361	37[a]±1.4	11	10
15,000	5,337[b]±1,062	36[a]±5.7	11	10
20,000	5,357[b]±924	27[b]±4.3	12	8

* Figures are averages of 8 replicates. Means within a column followed by the same letter are not significantly different ($p=0.05$; $d_f=14$; t-test).

Table 10 Pertinent data for mass-rearing the stable fly under laboratory conditions.*

1.	% hatch of eggs:	87.4 ± 3.8
2.	% adult emergence from pupae:	87.2 ± 5.4
3.	Duration of egg stage:	2 days
4.	Duration from adult emergence to egg-laying:	6 days
5.	Duration from egg stage to gravid adult stage:	25 days

* Figures are averages of 3 replicates taken from 4 cages wherever applicable.

eclosion. Twenty-five days were required for female stable flies to develop from egg stage to gravid adult stage.

The maximum number of pupae that the established laboratory colony was capable of producing was about 20,000 per day. At maximum production, 4 trays each with at least 5,000 pupae were harvested and 4 trays each seeded with approximately 15,000 eggs were prepared daily. To sustain the colony, 4 additional trays were prepared every 6 days. At any given time, 8 cages each containing about 4,000 adult stable flies and 60 larval trays were maintained in the laboratory.

Field Releases of Sterile Flies

Figure 22 and Table 11 represent a simple linear regression analysis in which the number of sterile stable flies released per week was the independent variable and the average percent hatch of the eggs laid by wild females was the dependent variable. The analysis shows that the decline in the percent hatch of the eggs was due to the release of sterile flies in the feedlot. From the regression formula $Y = 100.5 - 0.000417X$, it was estimated that 241,007 sterile flies had to be released per week to result in a 0% hatch of the eggs laid by the wild females or 100% induced sterility. Assuming that the released sterile flies live for at least 7 days, 34,430 flies must be released per day. However, this is only a theoretical estimation and must be tested by actual releases in the

FIGURE 22 The degree of association between the number of sterile stable flies released and the average percent hatch of eggs laid by wild females per week from July 30 to September 25, 1986 at the SDSU Cattle and Sheep Nutrition Research feedlot.

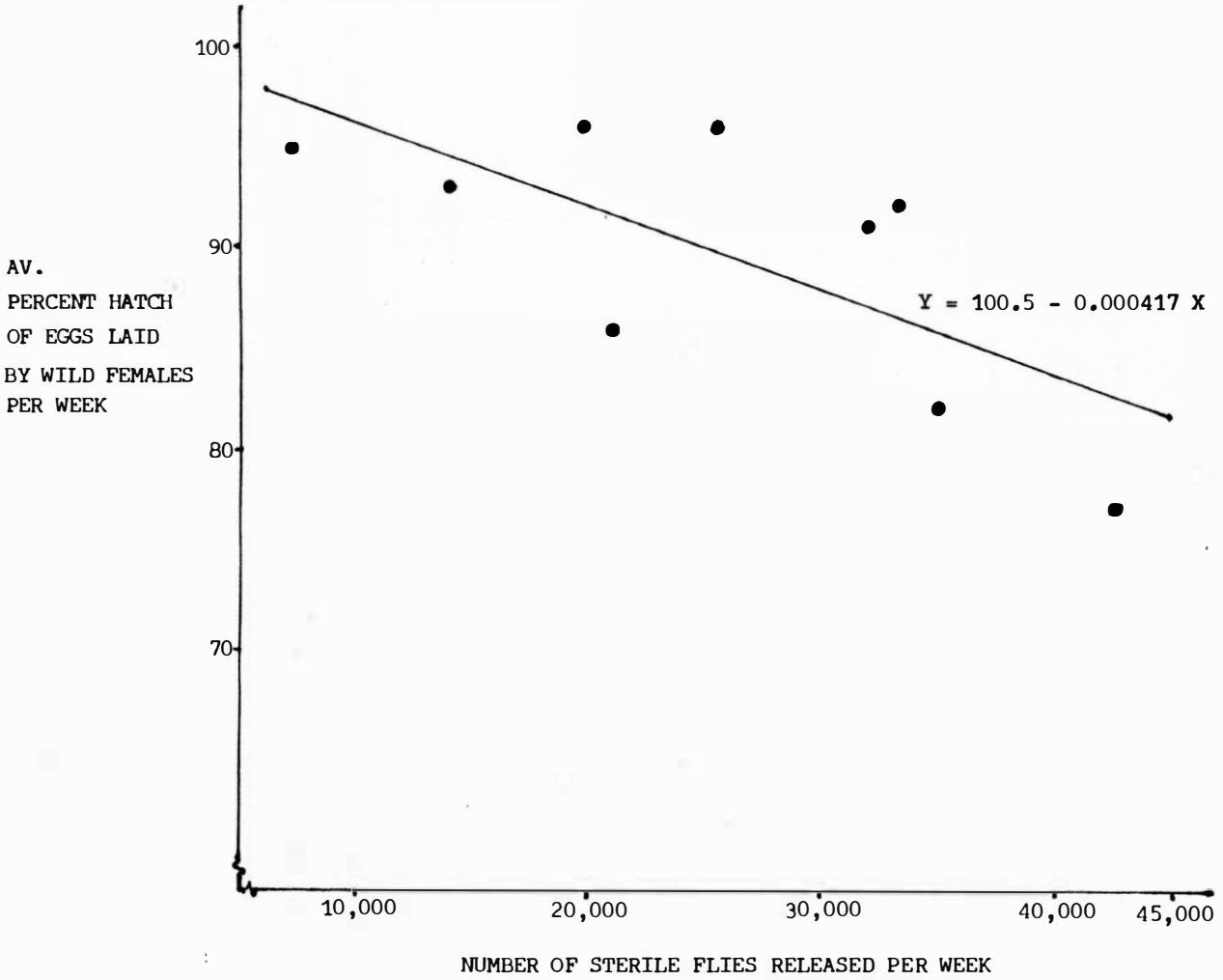


Table 11 The relationship between the number of sterile stable flies released and the average percent egg hatch from wild female stable flies caught per week from July 30 to September 25, 1986 at the SDSU Cattle and Sheep Nutrition Research feedlot.

Week	Number of sterile flies released (X)	Percent hatch of eggs laid by wild females (Y)
1	7,559	94.8
2	19,737	96.1
3	31,916	91.4
4	14,258	92.6
5	33,379	91.8
6	25,551	96.3
7	42,528	77.1
8	35,244	81.7
9	21,355	86.3

$Sx^2 = 986343331.6$ $Sxy = -411786.4$ $Sy^2 = 360.5$

$\bar{x} = 25725.2$ $t_b = 2.527$ $\bar{y} = 89.8$

* The tabular value of $t=2.365$ ($p=0.05$; $d_f=7$). The number of sterile flies released is linearly associated with the decline in the % hatch of eggs laid by wild female flies.

* The regression formula is: $Y = 100.5 - 0.000417X$.

feedlot. The maximum production capability of our laboratory colony was 20,000 flies per day.

According to Knipling (1968), if enough sterile male insects were released so that the initial sterile to fertile male ratio is 2:1, theoretically, an eradication of the wild population would result during the fourth generation of the insect. Table 12 indicates that the 2:1 sterile to fertile ratio was never attained in this study on the stable fly during the summer of 1986. The sterile stable flies were always outnumbered by fertile wild ones. Nevertheless, some matings between the released sterile males and the wild females occurred resulting in a reduction in the percent hatch of eggs laid by the wild female flies.

Based on actual numbers of stable flies biting or alighting on the forelegs of cattle at 3:00 p.m. as discussed earlier, it was estimated that the actual population of stable flies in the feedlot at any given time from June 12 to September 4, 1986 was approximately 37,000. Therefore, 74,000 sterile flies would have to be maintained in the feedlot to produce the 2:1 sterile to fertile ratio. Assuming that the released flies live for 7 days, 10,571 flies should have been released daily. This number is less than the 34,430 daily releases estimated from the regression formula. The estimate from leg counts however may be less accurate since it does not take into consideration the number of stable flies not on the animals.

The theoretical estimates in sterile-insect studies are never realized in the field so that in actual practice, preliminary

Table 12 The sterile to wild stable fly ratios following weekly releases of sterile flies from July 30 to September 25, 1986 at the SDSU Cattle and Sheep Nutrition Research feedlot.

Week	Number of sterile flies released per week	Sterile:Wild ratio
1	7,559	0:199
2	19,737	30:202 (1:7)
3	31,916	23:388 (1:17)
4	14,258	10:459 (1:46)
5	33,379	39:186 (1:5)
6	25,551	20:410 (1:21)
7	42,528	67:210 (1:3)
8	35,244	39:155 (1:4)
9	21,355	51:220 (1:4)

experiments have to be undertaken. For example, in connection with the eradication of the screw-worm fly in the Island of Curacao in 1954 (Bushland, 1975), USDA scientists started initially with a release rate of 77 flies per square-kilometer per week. This release rate resulted in only 15% sterile matings or 85% of the egg masses still hatched. However, when the release rate was increased from 77 flies to 309 flies per square-kilometer per week, the percent hatch of eggs laid was reduced to 30%. This caused a marked decline on the wild screw-worm fly population and the percentage of non-hatching egg masses increased. Eradication was thus accomplished within 6 months. In a Florida experiment in which the release rate of sterile flies was increased to 386 flies per square-kilometer per week, the wild population almost disappeared after 6 months but the last wild fly was not found until 5 months later.

Patterson et al. (1981), in their attempt to eradicate the stable fly population at St. Croix in the U.S. Virgin Islands, released about 100,000 sterile flies per day (5 days a week) over the 218 square-kilometer island. Over 99% induced sterility was observed during the last 6 months of their 18-month study. However, eradication was not accomplished due to isolated breeding sites and fly migration. Releases of sterile flies were not possible in towns, villages and employment districts, nor in areas inaccessible by land vehicles. The stable fly, unlike the screw-worm fly is a vicious biter and because of this, Patterson et al. (1981) were

reluctant to increase the number of sterile flies released and resorted to partial insecticide use.

The fact that stable flies are capable of migrating up to 117 kilometers (Patterson et al., 1981) is an important consideration when attempting to eradicate this insect from a certain area. The SDSU Cattle and Sheep Nutrition Research feedlot is surrounded by other fly breeding places like the Dairy Research feedlot which is only about 400 meters away. The complete elimination of the stable flies in the feedlot may not be possible but the sterile-insect technique can still be used as a component of an integrated pest management scheme.

Evaluation of Bisazir as a Chemosterilant Against the Stable Fly

The use of gamma or x-rays to sterilize insects may not be possible in some areas where there is no proximity of an irradiation source. Under these circumstances, an alternative is the use of chemosterilants which may be more convenient and economical. Chemosterilants are considered more versatile than radiation because they can be applied like insecticides. For example, resting places of insects in the field might be treated with chemosterilants to produce sterility directly in the field. In this study, a thioaziridine chemical called bisazir (Figure 8) was evaluated as a possible substitute for gamma radiation in sterilizing stable flies for field releases.

A normal virgin female stable fly mated with a sterile male is expected to lay eggs that will not hatch. The eggs will not hatch because of the failure of the sterile male to fertilize the eggs normally. A sterile female mated with a normal male is not expected to lay any eggs due to some abnormalities or even destruction of its ovaries caused by the sterilizing agent. Sterility is considered permanent if the insect remain sterile throughout its lifetime.

Table 13 indicated that the method of dipping the stable fly pupae in 1% aqueous bisazir solution for 90 minutes produced complete and permanent sterility in male adults emerging from the treated pupae. Table 14 shows that the females were also completely and permanently sterilized. The fact that the flies in the control laid eggs that eventually hatched indicates that mating occurred satisfactorily inside the small cage designed by the author. The dimensions of the cage also permitted normal egg-laying by the female stable fly.

Table 15 presents the effect of the treatment on the longevity of the male and female stable flies. The longevity of male stable flies was not affected but a significant reduction in the longevity of the females was observed. In connection with the releases of sterile flies in the field, the males are considered more important than the females since the latter mate only once (Hall and Smith, 1986). The effects of bisazir on the sexual aggressiveness of the male flies was not investigated.

Table 13 The sterility observed in male bisazir-treated stable flies when paired singly with a normal female mate.*

Replicate	No. of treated male-normal female pairs	No. of eggs laid by the normal female ($\bar{x}$±SD)	% hatch of eggs ($\bar{x}$±SD)	% sterility of the treated males ($\bar{x}$)
1	25	370±250	0	100
2	25	425±238	0	100
3	25	361±212	0	100
4	25	349±151	0	100
Control[a/]	25	396±214	92±4	0

* Each fly was initially virgin when placed inside the cage.

a/ Normal male-normal female pairs.

Table 14 The sterility observed in female bisazir-treated stable flies when paired singly with a normal male mate*.

Replicate	No. of treated female-normal male pairs	No. of eggs laid by the treated female ($\bar{x}$±SD)	% hatch of eggs ($\bar{x}$±SD)	% sterility of the treated females ($\bar{x}$)
1	25	0	-	100
2	25	0	-	100
3	25	0	-	100
4	25	0	-	100
Control[a/]	25	396±214	92±4	0

* Each fly was initially virgin when placed inside the cage.

a/ Normal male-normal female pairs.

Table 15 The longevity (in days) of bisazir-sterilized and normal stable flies.

Condition/ Sex	Sterile $\bar{x}$ (range)	Normal $\bar{x}$ (range)	Observed t
Male	14 (5-30)	13 (2-30)	1.19^{ns}
Female	14 (6-28)	21 (4-47)	6.46^{*}

ns = not significant

* = significant at 5% level; d_f=98

When a pupa is dipped in a chemosterilant solution, the chemical does not actually penetrate the puparium. The fly collects the sterilizing dose on its mouthparts and tarsi from the residue on the exterior of the pupal case during eclosion (LaBracque, 1968). Bisazir, like other aziridine chemosterilants, is an alkylating agent (Langley and Carlson, 1986). Alkylating agents produce breakages in the chromosomes of sperm cells in male insects and inhibit oogenesis in females (LaChance et al., 1968). Since cytological examinations were not carried out in this study, it cannot be verified whether or not bisazir produced these abnormalities in the stable fly. In mammals and birds, alkylating agents are known to cause mutagenesis, teratogenesis, carcinogenesis, and sexual sterilization especially in males (Hayes, 1968). Caution must be exercised when handling or working with bisazir.

SUMMARY AND CONCLUSION

To investigate the feasibility of the sterile-insect technique in controlling the stable fly in a beef cattle feedlot in Brookings, South Dakota, four studies were undertaken namely: (1) Field Population Studies, (2) Mass Colonization, (3) Field Releases of Sterile Flies, and (4) Evaluation of Bisazir as a Chemosterilant Against the Stable Fly.

A wild stable fly population at the SDSU Cattle and Sheep Nutrition Research feedlot was estimated through actual counts of

flies biting or alighting on the forelegs of confined beef cattle and through the use of sticky traps. The number of adult stable flies on the forelegs of cattle was highest at 3:00 p.m. during the summer season corresponding to the highest air temperature recorded for the day. An increase in the numbers of flies on the forelegs from 9:00 a.m. to 3:00 p.m. was linearly correlated with increasing air temperature. Weekly average leg counts taken at 3:00 p.m. and weekly average trap counts indicated that the stable fly population reached a peak during July and was lowest in early June and late August, although there were wide variations from week to week. There was no correlation between weekly average numbers of flies on the forelegs of cattle and the weekly average numbers of flies captured per sticky trap. Air temperature and rainfall had no influence on either the leg counts or the numbers of flies on the traps. The average stable fly population in the feedlot from June 12 to September 4, 1986 was estimated to be approximately 37,000 insects.

The stable fly was successfully mass-produced in the basement of the SDSU Physiology Laboratories under relatively constant temperature and humidity by feeding the adult flies with citrated bovine blood and allowing the larvae to develop in a medium composed of zonolite, wheat bran, fishmeal, and water (2:2:0.2:2.5). At least 5,000 pupae were harvested from 6.7 litres of artificial media contained in 29.2 x 34.3 x 13.3 cm dishpans or trays that were initially seeded with 15,000 eggs. The maximum

number of pupae that the established colony was capable of producing was about 140,000 per week. At maximum productivity, 4 trays with at least 5,000 pupae per tray were harvested per day and 4 trays each seeded with approximately 15,000 eggs were prepared daily. To sustain the colony it was necessary to prepare 4 additional trays every 6 days. At any given time, eight 35 x 35 x 35 cm screened aluminum cages each containing 4,000 adult stable flies and 60 larval trays each containing either eggs, larvae or pupae were maintained in the laboratory.

The release in the feedlot of stable flies sterilized with 2 kR gamma radiation at an average rate of 25,725 flies per week from July 30 to September 25, 1986 resulted in a decline in the average percent hatch of eggs laid in the laboratory by wild caught female flies. Through simple linear regression analysis, it was estimated that 241,000 sterile flies per week had to be released to result in 0% hatch of the eggs laid by wild females in the feedlot. The laboratory colony was capable of producing only about 140,000 flies per week. However, the release rate could have been lowered if releases were initiated early in June when the fly population was lowest. A complete elimination of the stable fly population in the feedlot may not be possible because of its close proximity to other sources of infestation, however the sterile-insect technique can always be utilized in combination with other components of an integrated pest management program.

Through single-mating experiments in the laboratory using small, cylindrical plastic cages designed by the author, it was shown that both male and female stable flies emerging from pupae dipped in a 1% aqueous solution of bisazir for 90 minutes were completely and permanently sterile. The treated males were not able to fertilize the eggs of normal females at any point in their adult life while the treated females did not produce any eggs. Further experiments on the sexual competitiveness of the treated males are necessary before bisazir can be recommended as a substitute for gamma radiation in sterilizing stable flies for field releases. Caution also must be exercised when handling bisazir because of its known detrimental effects on the chromosomes of mammalian cells.

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APPENDIX

APPENDIX I. Hourly Average Air Temperature (°C) and Solar Radiation (KJ/m^2) from June 12 to September 4, 1986 at the SDSU Cattle and Sheep Nutrition Research feedlot.

Time	Av. Air Temperature (°C)	Av. Solar Radiation(KJ/m^2)
4:00 a.m.	16.4	0.0
5:00	16.1	2.1
6:00	16.1	81.3
7:00	17.2	405.4
8:00	18.8	833.7
9:00	20.4	1341.9
10:00	22.2	1919.5
11:00	23.6	2352.1
12:00	24.8	2594.1
1:00 p.m.	25.5	2681.0
2:00	26.1	2550.5
3:00	26.5	2364.3
4:00	26.5	1934.1
5:00	26.3	1632.7
6:00	25.7	908.3
7:00	24.6	412.8
8:00	22.8	96.5
9:00	21.0	2.5
10:00	19.7	0.0

APPENDIX II. Average number of stable flies biting or alighting on the forelegs of confined beef cattle from June 12 to September 4, 1986 at the SDSU Cattle and Sheep Nutrition Research feedlot.

Date		Time		
		9:00 a.m.	12:00 noon	3:00 p.m.
June	12	1	14	10
	19	13	24	28
	26	8	22	23
July	3	3	13	16
	10	11	18	36
	17	11	18	20
	24	7	22	37
	31	1	23	32
August	8	6	26	30
	15	4	9	19
	22	1	8	12
	29	1	5	7
September	4	1	3	21