Attractants for Synanthropic Flies. 2. Response Patterns of House Flies[1] to Attractive Baits on Poultry Ranches[2]

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ABSTRACT

A proteinaceous attractant, prepared by freeze-drying fermented whole egg solids, was found to attract *Musca domestica* L. and other synanthropic Diptera. In field experiments, a mixture of 2 g of the attractant and 2 g of dichlorvos sugar-bait increased fly collection two-fold on manure substrate in trays set on the ground and three-fold in suspended bait units over the sugar-toxicant bait alone. This increase was due primarily to the increased response of nulliparous and parous females in which vitellogenesis was about to occur. Attractancy of the bait declined sharply after 48 hours of field exposure.

The location of bait stations significantly affected the number, sex ratio, and female age structure of the flies collected. Stations collecting the greatest number of flies were generally situated at the ends of the cage rows in sunlight-shade border areas. Fly collections from areas of greatest fly activity were characterized by a high proportion of both nulliparous and parous protein-searching females, and the sex ratio in these high-activity zones approached 1. East-west and north-south preferences of certain female age groups were manifested in the collections.

Studies on use of poison baits for fly control were reported as early as 1914 by Morrill, and use of such baits was recommended by Howard and Hutchinson (1917). The early poison baits included such food attractants as beer, fish, and bananas. The efficacy of dry baits was reported by Gahan et al. (1954) and Mayeux (1954), and commercial emphasis centered on dry sugar-toxicant formulations which lack volatile attractants.

Weismann (1960) studied the sensory functions of house fly antennae. He concluded that their sense of smell is not highly developed, and questioned the potential of odoriferous baits for fly control. Mourier (1964) found that the searching behavior of a fly leads it to nonattractive sugar-baits and that the "herd instinct" of flies will cause more to follow. Thus, factors other than volatile attractiveness influence fly response to baits.

Attractants for house flies were studied by Brown et al. 1961), Beroza and Green (1963), and Frishman and Matthysse (1966). These attractants generally fell within the categories of putrefaction products, fermentation products, and simple carbohydrates. Since the house fly requires both carbohydrates and protein at various stages of its adult lifespan (Derbeneva-Ukhova 1935, Goodman et al. 1968), a bait should include an admixture of substances which will be attractive both to adult flies in search of sugar and to those searching for protein.

A volatile attractant, prepared by freeze-drying fermented whole egg solids, has been developed for the control of *Hippelates* eye gnats[4] at the University of California, Riverside (Mulla et al. 1973) and it is attractive to most synanthropic Diptera. This attractant is now known as Lursect™[5]. The attractant is easily mixed with commercial poison sugar-baits to provide an attractive bait mixture consisting of proteinaceous attractant, sugar arrestant, and a knockdown toxicant (dichlorvos). Such an admixture facilitates application of measurable quantities into "bait units" (Mason et al. 1971), which can be placed at selected "bait stations" (Keller et al. 1956). In addition, such a bait mixture can be placed in strategic situations on the ground in fly-infested areas.

The importance of integrating bait location with fly behavior has been emphasized by Davison (1962) and Keiding (1965). Observations on the ethology of various Diptera on poultry ranches by Anderson and Poorbaugh (1964) demonstrated aggregation of fly populations that varied from species to species. Presumably this dispersion of fly populations would influence effectiveness of poison-bait stations using low-order attractants.

This study was initiated to determine the efficacy of an attractant used with a poison sugar-bait against house flies in various locations on poultry ranches. In addition, the effect of the location of bait stations was studied in regard to the number, sex ratio, and age structure of flies collected.

Materials and Methods

This study was conducted on 2 caged layer poultry-houses in southern California. One ranch was near the community of Perris and henceforth is referred to as Ranch PA. This ranch housed approximately 30,000 layers in 12 houses interconnected by

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[4] Diptera: Chloropidae.

[5] Registered trade name applied for by McGlaughlin, Gormley, King Co., Minneapolis, Minn.

screened ceilings and enclosed aisles which ran perpendicular to the cage rows. Poultry droppings were removed semimonthly, and naled fly spray was applied when considered necessary. The other ranch was in the town of Mentone and henceforth is referred to as Ranch MA. This ranch housed 15,000 layers in 4 separate buildings. Poultry droppings were allowed to cone and were partially removed semiannually. This practice of manure management was part of a biological control program in which parasites were released semimonthly, and use of fly sprays was avoided.

Four experiments were conducted: 2 to determine efficacy of the attractant, and 2 to study the effect of bait placement on fly collection.

Experiment I: Sugar-Toxicant Bait With and Without Attractant.

In the 1st experiment, dischlorvos sugar-bait with attractant was compared to dichlorvos sugar-bait alone. For the 1st tests, the baits were placed on a poultry-manure substrate in bake pans (10×10×2 in.). Pairs of these bait units, one with attractant and one without, were placed under the cage rows of Ranch PA. After 24 h, the bait units were collected and the flies were removed, identified, sexed, and counted. Table 1 shows bait dosages and number of replications.

In 5 tests, dichlorvos sugar-bait with and without attractant was compared in matched pairs of bait units suspended from the ends of the cage rows (Fig. 1). The bait unit was a small 276-ml cup. For 24-h tests were run on Ranch PA, and one 24-h test was run at Ranch MA. In addition to recording species, sex, and numbers collected, physiological age of female house flies was determined by examination of a representative sample of adults drawn from the collection of each bait station. To determine physiological age, the females were dissected in saline solution in spot plates, ovaries were removed and transferred to a glass slide, and the stage of cogenesis was noted according to the criteria of Adams and Mulla (1967) developed for eye gnats. Parity was determined by presence or absence of fat bodies, condition of ovariole pedicels and tracheoles, and bunched condition of ovarioles (Anderson 1964).

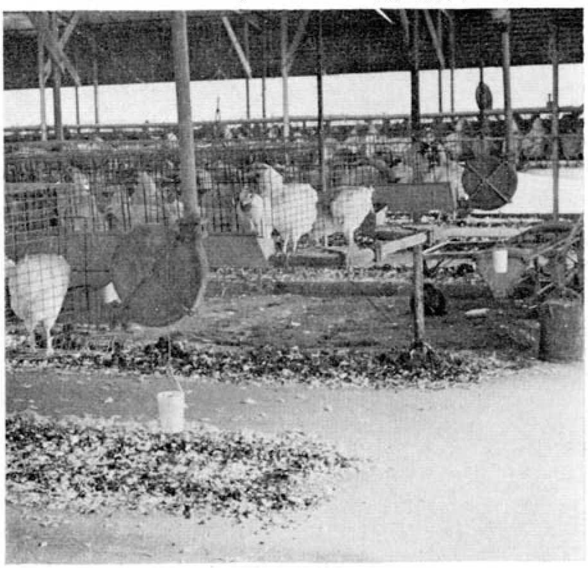


FIG. 1.—A pair of suspended cup units used for evaluating attractiveness of baits.

Experiment II: Longevity of the Attractant on Exposure.

The 2nd experiment on attractant efficacy measured effectiveness of the attractant over time. Exposure periods of 24, 48, and 72 h were compared in an experiment using 18 bait units, one unit representing each of the 3 time periods at 6 selected loci on Ranch MA. The bait unit used was the larger 1.5-liter can unit (Willson and Mulla 1973a). Each replicated set of 3 units was suspended from the ends of 3 adjoining cage rows. To compensate for a possible spatial effect on a particular unit within a set of 3 units, 3 baitings were run in which the position of each time-designated unit was rotated. In each baiting all 18 bait units were started simultaneously, and thereafter the units designated for specific exposure periods were capped 24, 48, and 72 h after initial placement.

Experiment III: Effect of Directional Exposure on Fly Collection.

The 3rd experiment was designed to measure the effect of directional exposure on the numbers, sex, and age of flies collected. The larger 1.5-liter bait units, baited with 4 g of attractant and sugar-toxicant bait mixture (50:50 by wt), were suspended from the ends of the cage rows nearest the 4 corners of Ranch PA. This experiment included 3 24-h collections.

Experiment IV: Effect of Bait Placement Along the Cage Rows.

The 4th experiment was designed to determine the effect of bait station loci on numbers, sex, and age structure of flies collected. At Ranch PA, 5 bait stations were established along the central cage rows in the E half of the ranch and 5 in the W half, and each station consisted of 2 bait-pan units having manure substrates. Three collections, 24 h in duration, were obtained 2 weeks apart during the months of April and May. Age-structure analysis was limited to flies collected in the stations at the E half of the ranch. At Ranch MA, two 24-h collections were made to determine fly activity along the cage rows from the E to the W ends. As stated earlier, poultry droppings at Ranch MA were dry and coned in contrast to biweekly removal of droppings on Ranch PA; thus, a moist substrate had to be provided for the attractant sugar-bait mix. A bait unit was designed that consisted of a 500-ml plastic cup containing a moist vermiculite bait substrate and a hood to shield the bait from fresh droppings. Five stations were designated along a cage row from E to W. Each station consisted of 3 bait units situated along 3 parallel cage rows.

Statistical Treatments

Statistical separation of means resulting from matched fair trials was made with Student's t-test for paired variates. Data from the exposure-period experiment was analyzed by a random plot analysis of variance. The chi-square test for independence was used to detect significant variation among the numbers, sex, and age-group proportions in collections obtained from various bait stations.

Results

Experiment I. Sugar-Toxicant Bait With and Without Attractant

The number of flies collected by dichlorvos sugar-bait with the attractant was significantly greater than that collected by sugar bait alone in every comparison test conducted. When the baits were placed in pan units having manure substrate, addition of the attractant increased fly collection 2-fold. In suspended bait units, however, the mean ratio of the number of flies collected by sugar-toxicant-attractant mixture to sugar-toxicant bait alone was 3:1, and the presence of attractant increased the percentage of females in the catch by about 10%. As a result, the ratio of females collected in suspended units with attractant to those without was approximately 4:1 (Table 1).

In the physiological age analysis of females collected in units with and without attractant, the average number of females collected by each bait was multiplied by the corresponding age-group proportions to obtain the age structure characterizing the sample collected. Fig. 2 illustrates the results. Nulliparous age groups 1+2 and 7+8 and parous age groups 7+8 did not appear to be affected by the presence of the attractant. Nulliparous (N) and parous (P) groups 3+4, 5+6 and 9+10 showed increased response to the attractant. The peak response of nulliparous females was confined to stages 3+4 in contrast to the parous peak in stages 3 through 6.

Experiment II. Longevity of the Attractant on Exposure

The number of flies collected for the 24-, 48-, and 72-h exposure periods per site in the 3 baitings were averaged to give a mean count per period per site (Table 2). Of the total 72-h collection, approximately 75% was obtained in 24 h and 93% in 48 h, respectively. The proportion of females in the counts declined with increased baiting period.

Experiment III. Effect of Direction Exposure on Fly Collection

Fig. 3 shows the age structure of female house flies collected from the 4 corners of Ranch PA. Most of the flies were collected at the SE corner and were characterized by an average sex ratio of 48.5% ♀; the fewest were collected at the opposing NW corner and were characterized by a sex ratio of 41.5% ♀. A chi-square test for independence between proportion of age groups per station did not demonstrate significant difference between the age structure of flies collected per station. The relative proportion of nulliparous females in age group N 3+4 appears to correlate with the sample size. As a result, the stations collecting the most flies are characterized by a high proportion of both nulliparous and parous protein-searching females. This relationship is similar to that found for the sex ratio which approaches one in high activity zones. The proportion of nulliparous females in the S corner units was higher than that of the N units. In isolatel incidences, the age group, P 5+6, would occur in relatively high proportions in one SW corner.

Similar results, concerning the effect of directional exposure on fly collection, were extracted from the suspended-bait comparison tests of Experiment I. In the age analysis of females baited by the attractant-sugar-bait mixture at the E and W ends of Ranch PA, a low but significant (<0.10) level of directional

Table 1. — Numbers and sex ratios of house flies collected by units baited with dichlorvos sugar-bait alone and sugar-bait plus attractants.

Collection period	No. replicates	Mean no. house flies/unit: Sugar-bait + attractant	Mean no. house flies/unit: Sugar-bait	% females: Sugar-bait + attractant	% females: Sugar-bait
		Pan units with manure substrate along cage rows *Bait-dosage/unit = 2.5 g sugar-bait and 5.0 g mixture (50:50 by wt.)*			
Oct. 6, 7	9	16.9	2.4*[a]	75.0	40.9
Oct. 12, 13	9	23.9	12.0	71.6	75.9
Oct. 27, 28	9	92.3	49.8***	73.0	78.3
		Cup units suspended at end of cage rows *Bait-dosage = 2.0 g sugar-bait and 4 g mixture (50:50 by wt.)*			
June 30, July 1	8	57.6	17.4*	44.7	31.6
July 8, 9	8	147.9	45.6***	43.2	34.0
July 15, 16	8	110.4	54.0**	43.6	36.1
July 28, 29	8	108.4	31.9***	46.9	34.9
Sept. 28, 29	6	94.7	21.3***	65.5	50.8

[a] Significant level of difference between means: * = $P<0.10$; ** = $P<0.05$; *** = $P<0.01$.

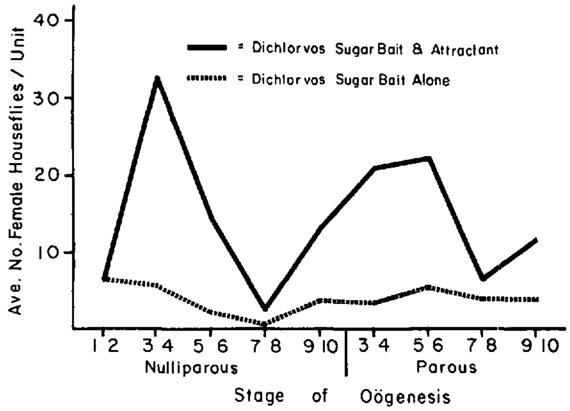


FIG. 2.—Numbers of females per stage of oogenesis collected in suspended units having dichlorvos sugar-bait with and without the attractant Lursect™.

preference by specific age groups was indicated. The difference in age structure was primarily caused by predominance of nulliparous and parous flies in oogenic stages 5 and 6 at the W end (Fig. 3). At the time of this experiment, the number and sex ratio in the collections did not differ from one end to the other.

Experiment IV. Effect of Bait Placement Along the Cage Rows

The baitings conducted along the central cage rows provided a cross-section view of fly behavior as it related to the peripheral and interior zones of the poultry houses. The number of flies collected per pan unit in the cage row experiment at Ranch PA was greatest at the E and W ends of the rows, least at the center, and increased slightly near the center aisle (Fig. 4). The proportion of females per station in this experiment was inversely proportional to the sample size. A test for independence of both the sample size and the female proportions per station from the end to center aisles of the E and W sections showed no significant differences between the 2 sections. Within the 5 stations of the E half of the ranch, the proportions of females per physiological age were determined for each station, and significant ($P<0.05$) independence in the proportion of age groups along the cage row was indicated.

Fig. 5 illustrates the spatial pattern of the age structure of females collected along the cage row of Ranch PA. Fly activity is clearly shown to be greatest at the E end where the exposure to light coming through the open end of the house was maximum. The age structure of the flies collected at this station was dominated by the protein-searching age groups, N 3+4, P 3+4, and P 5+6. The preovipositional groups N 9+10 and P 9+10 increase in proportion to other groups toward the interior. The low number of females in age group N 5+6 is unusual and should, in theory, be greater than age group N 7+8, which was documented by Smith (1968) as an uncommon group. However, these dissections were done on flies collected in the E half of the house, and the results of the corner collections and the end row collections (Fig. 4) shows that group N 5+6 had a prefence for the W end.

The numbers and proportion of males collected per station are also greatest at the end of the row and correspond to the abundance of age groups N 5+6 and N 7+8, which are preferred by males (Adams and Hintz 1969).

The results of the cage row experiment at Ranch MA are similar to that of Ranch PA. Number of flies collected per unit was greatest at the E end, dropped sharply, and gradually to the W end. The proportion of females was lower at the end stations than the neighboring stations, but an overall correlation of sex ratio and number of flies collected was not present as in the experiment at Ranch PA. This discrepancy between the results of Ranch MA and Ranch PA could be due to the fact that the difference in surface area studied was nearly 10-fold. Although the sample size showed distinct differences in stations along the cage rows, significant independence of the proportion of age groups per station was not demonstrated ($P>0.10$) by a chi-square test.

Fig. 5 illustrates the spatial patterns of age structure derived from the cage-row collections of Ranch MA. This house was small compared with that of Ranch PA, and the dominance of preovipositional age groups in the interior is not so clearly defined. The protein-searching age groups dominated the ends of the house, but the directional preferences of nulliparous and parous age groups were in opposite direction from each other. The N 3+4 group was abundant in the E end but not the W, whereas the age groups P 3+4 and P 5+6 were abundant at both ends. The relationship between age groups N 5–8 (which included mating females) and high male activity did not appear so obvious in this experiment as that of Ranch PA.

A valid comparison can be made between the results of the female age data from the corner collections (Experiment III) and cage-row collections (Experiment IV) on Ranch PA. A test for independence of age-group proportions between the cage-row and corner collections showed a highly significant difference ($P<0.01$). In addition, the average number of flies collected per corner unit (388 flies/unit) was significantly greater than that collected

Table 2.—Effect of exposure period on the number and sex of house flies collected in suspended units baited with dichlorvos sugar-bait plus attractant.

Collection period	Mean no. house flies/unit	% females
24 h	164.1±48.3 a[a]	49.9
48 h	200.4±24.4 b	47.5
72 h	214.6±53.9 b	45.5

[a] Means bearing different letters significantly different from one another at $P<0.05$.

along the cage rows (23 flies/unit). And, the proportion of females in corner collections (46%) was less than that of the cage-row collections (73%).

Discussion

Efficacy of the Attractant

Addition of the proteinaceous attractant to dichlorvos sugar-bait clearly improved the fly-collecting capability of the latter. The increase in flies collected was expressed in both sexes, and an explanation of this phenomenon is provided by analysis of physiological age structure of the females collected. The most prominent age group of females responding to the attractant was nulliparous Group 3+4. This group corresponds to the "potential resting phase" which was found to be a stage at which the female house fly requires protein before ovarian development can continue (Goodman et al. 1968). Adams (1970) noted a decrease in area of the corpus allatum during N 3+4 and P 3 to 5 which implied a decrease in the production of juvenile hormone during those particular stages, and as a result, a pause in ovarian development. Therefore, the prime response of female house flies is during a stage of oogenic development in which protein requirement must be satisfied before normal development can proceed. As a result, the volatile attractant, derived from proteinaceous material, lures a segment of the fly population that would have ignored the poisoned sugar-bait lacking the volatile attractant.

The increased response of stages N 9+10 and P 9+10 is possibly due to the attractant acting as an ovipositional lure. Larsen et al. (1966) found that house flies in the preovipositional period respond to odors eminating from suitable ovipositional media. A brief field test using CSMA fly media with and without the attractant has demonstrated that the attractant can stimulate additional oviposition.

The added response of adult males to bait units with Lursect is difficult to explain. Laboratory observations in a turntable olfactometer demonstrated that females respond more to the attractant than males (Willson, unpublished data). Murvosh et al. (1964) and Rogoff et al. (1964) reported that male house flies are attracted to dead virgin females, and they related this behavior to the presence of a sex pheromone. Adams and Hintz (1969) reported that females with ovaries in stages 3 and 4 rarely mated, but thereafter mating steadily increased and males preferred females with ovaries in stages 6–10.

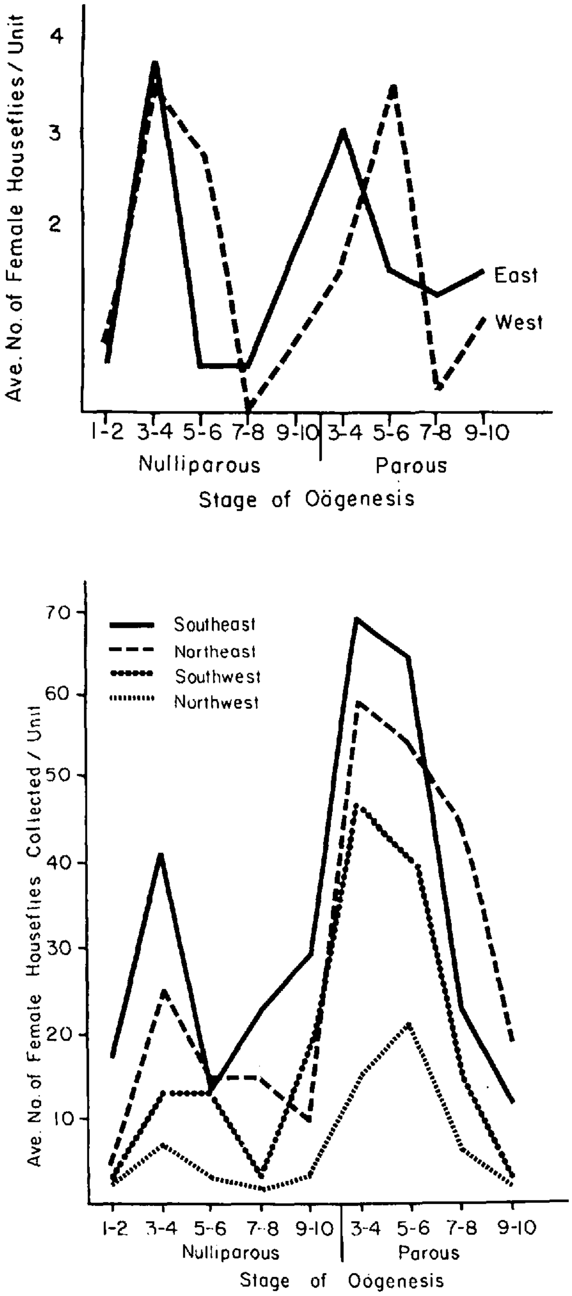


FIG. 3.—Influence of bait-station placement on the number of females collected per stage of oogenesis in bait units suspended at the end of cage row nearest the corners and along the east and west sides of Ranch PA.

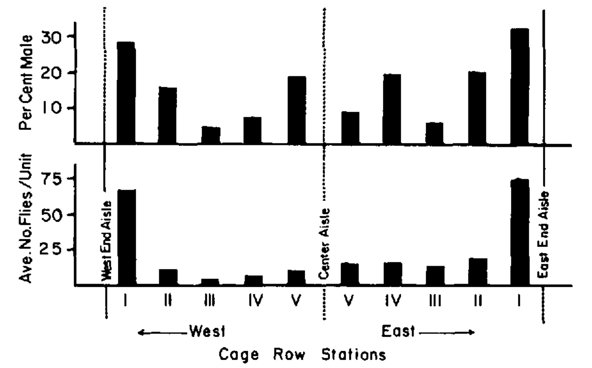


FIG. 4.—Number and sex of house flies collected along central cage rows of Ranch PA.

Nulliparous females in age group 5+6 respond to Lursect and may release sufficient sex pheromone to attract males. Therefore, male response to the attractant probably is due to both the volatile odor of the bait plus excitation by the dead females.

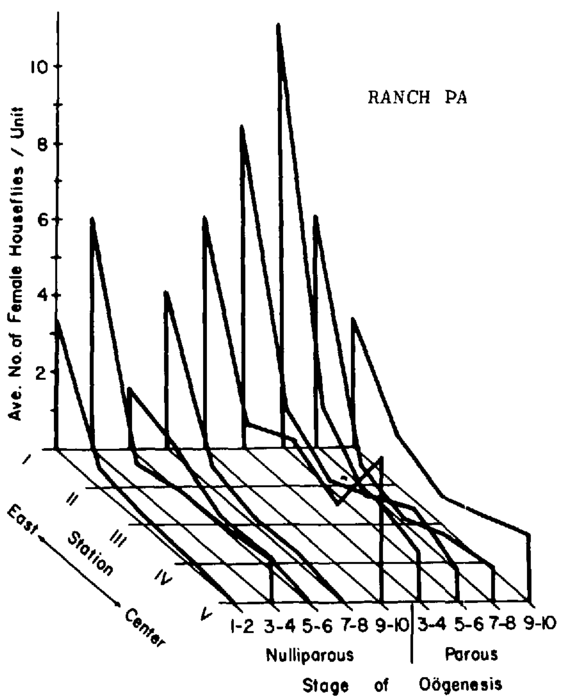
The age structure of flies baited by the attractant sugar-bait mixture would appear to be more representative of a natural population than that baited by sugar-bait alone. Although sugar-bait alone does not attract protein-searching females, it must be recognized that the attractant in Lursect is volatile and may thus attract more protein-searching females than the actual representation of that age group in the field population.

Smith (1968) studied physiological age in relation to the calendar time required for an adult female house fly to pass from one age group to another. He found that the early stages of oogenesis and the preovipositional stage were relatively slow compared with the rather quick development of the follicle-elongation stages which would correspond to Adams' Stages 6 through 8. Suenaga (1969) also studied the calendar age of house flies per physiological age group and noted the seasonal differences in the rate of gonotrophic development. The age charts which he produced showed the early nulliparous stages to be more predominant than the late vitellogenic stages. Therefore, the predominance of early oogenic stages found in the flies baited by the attractant sugar-bait mixture coincide with the observations of Smith and Suenaga.

The data from exposure tests suggest that the primary response occurs within the first 24 h, with a decline in activity during the second 24 h, and almost no activity during the third 24-h period. This decrease in the response may be due to several factors: (1) the attractancy of Lursect declines on exposure (Mulla et al. 1973); (2) the attractancy of the bait and the effectiveness of the toxicant may decline due to dead flies covering the bait; (3) the extreme climatic conditions may adversely affect either fly activity or attractant longevity. However, the attractant has been observed to lure many flies on the 3rd day following an initial 48 h of cool climate, and the number of flies collected has often reached as high as 1000 flies/survey unit. In general, it has been found that the bulk of the flies are attracted during the first 48 h. The 72-h exposure period was adopted for population monitoring, because shorter periods require more frequent field trips, and the longer exposure period results in microbial decay of the collected fly samples.

Effect of Bait Unit Placement

The importance of bait location for maximizing fly collection has been demonstrated. If one assumes that a feeding attractant has a limited range in drawing flies to the bait, then one must conclude that the collections obtained at various bait stations are representative of the fly activity of a given area. The distinct patterns of numbers, sex, and age structure obtained in this study suggest that each segment of a house fly population exhibits a unique pattern of spacial activity. Recently emerged and gravid females were fairly evenly dispersed along the cage rows of the poultry house, but protein-



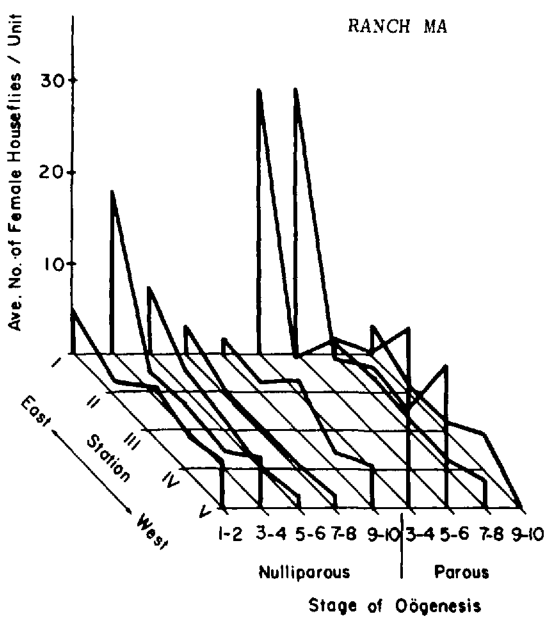


FIG. 5.—Influence of bait station placement on the number of females collected per stage of oogenesis in pan units along cage rows of Ranch PA and in shielded cup units along cage rows of Ranch MA.

searching and mating females were concentrated within distinct zones of activity. Such behavior of female age groups corresponding with concentrations of male activity suggests existence of specific zones of mating activity. These zones of high fly activity were areas where exposure to light was greatest. Such areas could be interpreted as the "border of shade and sunlight," which Davidson (1962) recommended for bait-trapping house flies. However, such a generalization cannot be applied to all situations because fly behavior is regulated by the environmental conditions which vary with climatic changes and differ with the structural design of each ranch situation (Willson and Mulla, unpublished data). As a result, the sex ratio and age structure patterns observed at Ranch MA did not completely coincide with that observed at Ranch PA, and such variations between ranches would be expected.

The implications of these findings upon the use of bait for survey and control are important. Presumably bait stations used for survey should sample a representative segment of the field population. The aggregation of age groups 3+4 and 5+6 combined with the even dispersion of age group 9+10 complicates fly-population sampling. To obtain a representative sample, bait stations would have to be situated to monitor fly populations in both the potential areas of aggregated fly activity and evenly dispersed segments of the population. The aggregated fly activity would inevitably shift with changing environmental conditions. In the case of Ranch PA, bait stations should be placed at the end of the cage rows to monitor aggregated fly activity and along the cage rows to monitor the nonaggregated segments of the fly population. In addition, an adequate number of stations is required to account for the directional preferences of various age groups.

Use of bait for fly control has often been reported as "scattered in areas of fly concentration" (Gahan et al. 1954, Mayeux 1954). But limited attention has been paid to spot treatment of specific areas (Keiding 1965). On the basis of the results described, the effect of a given amount of bait could be maximized considerably by treating only those areas when fly activity is high. In the case of chemosterilant baits (Meifert and LaBreque 1971), which are directed to specific age and sex groups, knowledge of peak areas of fly activity would be very relevant. For example, at Ranch PA, most females aggregate in the peripheral zones during some point of each oogenic cycle. As a result, restriction of bait application to the peripheral ends of the cage rows would limit treatment to less than 10% surface area of the ranch, and the entire female fly population would eventually be exposed to the bait. Male and virgin female activity was confined mainly to the peripheral zones, and treatment of an entire ranch by chemosterilant bait would constitute waste of costly bait.

The examples cited here apply to a situation in which temperatures are generally less than 30°C. High temperatures can change this situation by shifting fly activity in the interior zones (Willson and Mulla, unpublished data). Therefore, fly activity of any given ranch must be evaluated according to both the size and layout of the ranch and the prevailing climatic conditions.

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