those trees adjacent to infested trees would more likely be infested than those in another part of the plantation. This could result in a contagious rather than a random distribution of tree to tree infestation. A one-sample runs test (Siegel 1956) was applied to the sequence of infested and noninfested trees as they appeared in the rows. There was no information available for relationship across rows. The results showed that the 559 observed runs were not significantly different from the mean expected number of runs of 542. The two-tailed probability for rejecting the hypothesis of randomness did not approach significance at the 5% level. It appears that the proportion of newly attacked trees in this plantation are infested at random, and this is reflected in the over-all distribution of infested trees.

The data presented here suggest that much of the apparent immunity reported by early workers can be accounted for by insect behaviour, which at best, is poorly understood.

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Insectary Production of *Stethorus* Species[1]

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ABSTRACT

A program of mass production and periodic release of imported *Stethorus* sp., a predator of tetranychid mites, is being conducted by the University of California Department of Biological Control. Large numbers of the Pacific spider mite (*Tetranychus pacificus* McG.) are reared on linted oranges, under controlled conditions. The mite-infested fruit is used to mass-produce the *Stethorus* sp. in specially designed rearing units. The *Stethorus* adults are collected from the units and released on mite-infested plants.

Members of the genus *Stethorus* are widely distributed predators of tetranychid mites and are often effective in controlling outbreaks of these mites. Other predators, such as predaceous mites, thrips, and green lacewings, are instrumental in maintaining mite populations at low densities, but generally they are not effective in controlling high-density populations of mites.

Although native species of *Stethorus* are usually present in areas infested with tetranychid mites, it may be desirable to introduce new *Stethorus* species into an environment. Such characteristics as cannibalism, searching capacity, weather tolerance, mite species preferred, and mite-density requirements might favor one species in one microhabitat and another species in another microhabitat. Therefore, introduction of a new *Stethorus* may improve or extend an existing biological balance.

The mass production of *Stethorus* species requires a tremendous supply of host mites. Fleschner (1950) found that *Stethorus picipes* Casey required at least 135 mites to complete development, and that the larvae were capable of consuming up to 486 mites each. If we estimate that 300 mites are required for oviposition and larvae development, then approximately 6,000,000 mites would have to be produced to rear 20,000 *Stethorus* adults, which is the present monthly production at the University of California's Riverside insectary.

Several species of mites have been used for rearing *Stethorus* species. Finney (1953) used the six-spotted mite, *Eotetranychus sexmaculatus* (Riley), and Fleschner (unpublished data) used the spider mite, *Tetranychus cinnabarinus* Bois. The Pacific spider mite, *Tetranychus pacificus* McG., has proved to be superior for mass-production purposes at the Riverside insectary. It produces tremendous populations on Valencia or navel oranges, and it is not sensitive to trace pesticide residues on the fruit. Purifying the air with activated charcoal is not necessary with this mite, as is the case with the insectary rearing of certain other species of tetranychid mites.

METHODS AND MATERIALS.—Washed, waxed, and graded oranges are obtained from a packing house and placed in cold storage (45° F.) until needed. Fruit which has been treated with ethylene gas to improve its color is not satisfactory because it frequently decays too soon under insectary conditions. The fruit is prepared for mite infestation by first being placed on 15¾×28-inch hardware-cloth trays which hold 55 size 138 fruit (fig. 1). Briefly, the cold fruit is held in a steam cabinet for "sweating" (fig. 2). Then, the tray of moist fruit is placed in a linting box and flocking lint is applied to the fruit (fig. 3). About 25 ml. of flocking is distributed over the curved bottom of the linting box, a horizontal perforated tube 1 inch above the curved bottom is connected to air pressure, and the flocking is blown up through the hardware-cloth tray and onto the fruit.

Two colors of flocking lint are used to distinguish the DDT-treated from the untreated fruit. All trays of fruit used to sustain the mite culture are linted with a mixture of 10% DDT (standard 50% DDT WP is used), 10% diluent, and 80% green flocking by weight. This prevents various mite predators, particularly predaceous mites,

[1] Paper No. 1211, University of California Citrus Experiment Station, Riverside, California. Accepted for publication May 2, 1960.

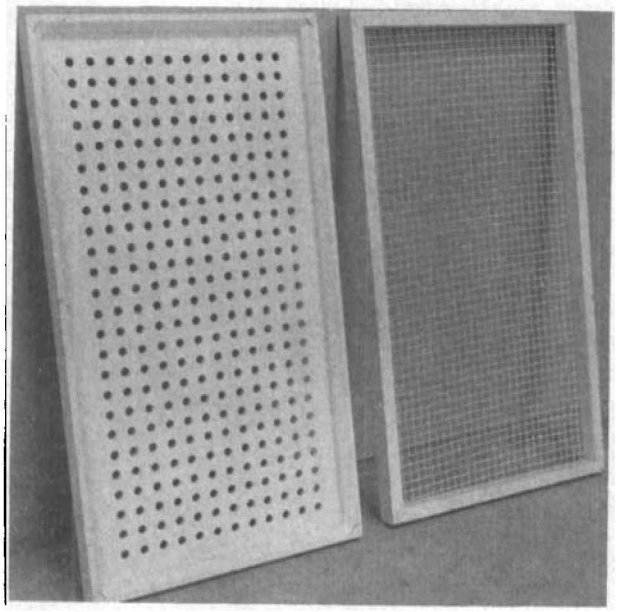

FIG. 1.—Left: Perforated masonite tray for rearing of *Stethorus* on mite-infested oranges.

Right: Hardware-cloth tray for linting and infesting oranges.

from contaminating the mite culture. Since *Stethorus* adults are sensitive to DDT, the trays of fruit used in the *Stethorus* oviposition units are linted with white DDT-free flocking. The flocking lint is composed of viscose rayon fibers "Verlon F21" and it provides a light, fuzzy covering over the fruit which encourages the female mites to commence feeding and ovipositing.

Routine for Operating Mite Culture.—To provide the *Stethorus* culture with a predictable and constant supply of mite-infested fruit, it was necessary to establish a standardized routine for operating the mite culture. The culture is kept in a dark room where the temperature is maintained at 78° to 80° F. and the relative humidity at 45% to 55%. All of the mite culture operations are done on Monday, Wednesday, and Friday. The culture is divided into four groups, each consisting of one or more units. Each unit is a tray of fruit heavily infested with mites which usually has on top of it a fresh tray of fruit ready to be infested (fig. 4). The operations conducted on each group are staggered so that no two groups have the same operations on the same day. A typical operation sequence for one group would be to place trays of DDT-linted fruit for infesting on the units Monday, remove the trays of spent fruit Friday, and add trays of untreated fruit the following Monday for infesting.

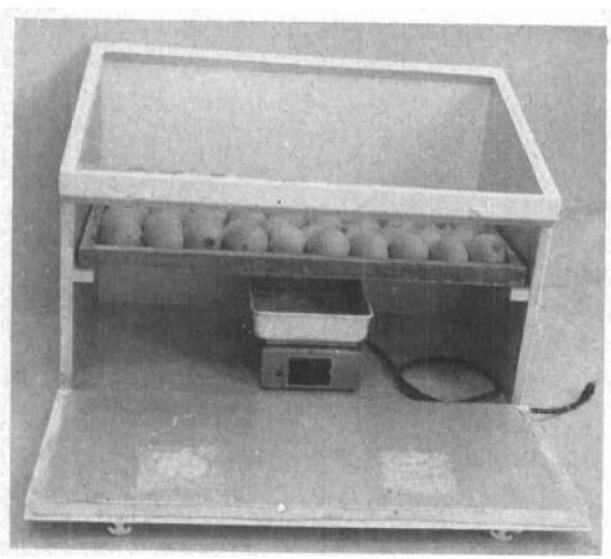

FIG. 2.—Steam cabinet containing hot plate, water pan, and a tray of fruit.

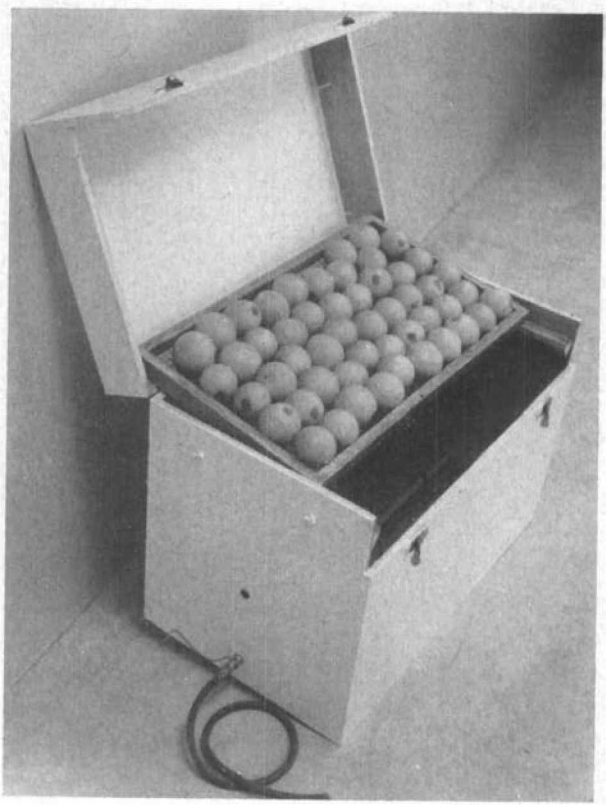

FIG. 3.—Linting box, showing curved bottom, perforated air tube, and a tray of fruit ready for linting.

Each unit remains as an individual mite culture. This reduces the chance of contamination and also allows for flexibility. The following procedure represents the handling of one unit. First, a tray of DDT-treated fruit is placed on top of the tray of heavily mite-infested fruit; the mites move upward by negative geotropism onto the new fruit. Four or five days later the tray of old fruit is removed and replaced by the newly infested tray of fruit. The new culture of mites is allowed to incubate for 2 or 3 days. Then, a tray of DDT-free linted fruit is placed on top of the incubating culture. The DDT-free fruit is infested for 4 or 5 days; then it is replaced by a tray of DDT fruit, which completes one cycle. One cycle can take 11 to 14 days, depending upon the position of weekends in the cycle. The life cycle for the mite at this temperature is about 10 days. A standard flashlight is useful to determine

FIG. 4.—A rack containing six mite units divided into two groups: one group is incubating, the other is infesting.

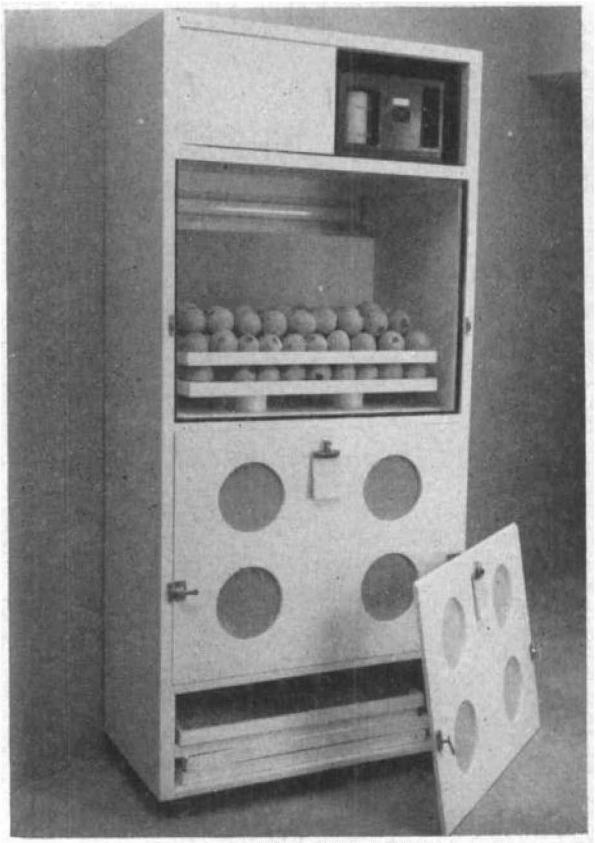

FIG. 5.—*Stethorus* rearing unit. The upper cage contains developing predator larvae, and the closed lower cage contains an oviposition unit.

the progress of the mite cultures. When the light is held at the proper angle, the mites and their webbing show clearly.

The Rearing Unit.—The *Stethorus* rearing unit (fig. 5) is a multipurpose cabinet. The top part with sliding doors is for equipment storage, the two large cages are for rearing, and the bottom part is for tray storage. The cabinet is on casters, which allow rapid movement from room to room or to the fumigator. The cabinet is 74 inches high, 34 inches wide, and 19 inches deep. The two rearing cages are each 31 inches wide and 23 inches high. The back of the cages is covered with organdy cloth and the front is closed with a 32×24-inch plywood door.

The trays used to hold fruit in the rearing units are the same size as the trays used for infesting the fruit with mites. However, the tray bottom is made of perforated masonite (fig. 1) instead of hardware cloth. The masonite gives better support to the fruit in the emergence units. These trays are also easier and cheaper to make and last longer than the hardware-cloth trays.

Operation of Stethorus Culture.—The operation of the *Stethorus* culture is also standardized; however, considerably more flexibility must be allowed to utilize the fruit to maximum advantage. All *Stethorus* rearing is done at about 83° F. which is usually the highest temperature that the fruit can withstand without excessive drying and breakdown. The trays of infested DDT-free fruit are allowed to incubate for several days after removal from the mite culture, then the fruit is used in *Stethorus* oviposition units. A *Stethorus* oviposition unit (fig. 5) uses one tray of well-infested fruit with an abundance of mite eggs and usually 500 well-fed adult *Stethorus*. The *Stethorus* feed on the mites and oviposit for 4 days. Then the *Stethorus* adults are removed with a vacuum aspirator. After the *Stethorus* eggs hatch, additional trays of mite-infested fruit are added to the original tray when the *Stethorus* larvae begin to search actively for mites (fig. 5). The feeding of the young larvae is extremely critical; the tiny larvae must have an abundant supply of mite eggs, larvae, and nymphs. The first-instar larvae are very weak and slow and usually they cannot overpower an adult mite. After the larvae reach the third instar, they can move rapidly from fruit to fruit seeking mites. Old fruit, previously treated with DDT, may, in some cases, be used at this time. Some species of *Stethorus* larvae have considerable resistance to DDT, and the emerging adults are not seriously affected by the old DDT residue. Mites should be readily available to the larvae prior to pupation or considerable mortality will result in the first few hours after emergence.

The adult female *Stethorus* can lay about 6 eggs per day and since the sex ratio is about 1:1, an oviposition unit using 500 *Stethorus* for 4 days theoretically could produce 6,000 eggs, a 12-fold increase. Many adverse factors operate to reduce this ratio. Cannibalism is a constant problem which can be partially reduced by supplying an

overabundance of mites. Other factors, such as mechanical injury during fruit handling, starvation, and emergence mortality, contribute to lowering efficiency. A 7-fold increase is the highest obtained so far.

When emergence begins, a light is located behind the cloth back of the emergence unit. The young adult *Stethorus* fly to the illuminated back of the emergence unit where streaks of honey on an enameled aluminum strip are provided as a supplementary food (fig. 5). A vacuum aspirator is used to collect the *Stethorus* adults into large pyrex test tubes. When the *Stethorus* are to be released in the field, they are collected 500 to a test tube, and a strip of enameled aluminum streaked with honey is slipped into each tube. The open end of the tube is covered with fine-mesh nylon cloth for ventilation. The tubes of *Stethorus* are taken to the field in a small evaporative cooler and released on mite-infested plants.

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Some New Mutants and Linkage Groups of the House Fly[1]

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ABSTRACT

A search for new mutants in the house fly, *Musca domestica* L., has been continued and a list of seven new marker strains is included in this paper. They are four eye color mutants, *car* (*carnation*), *cm* (*carmine*), *rb* (*ruby*) and w^{au} (*auburn*); an eye shape mutant, *ro* (*rough*); a wing shape mutant, *ct* (*cut*); and a wing vein mutant, *Lp* (*Loop*).

Linkage tests to classify 17 marker genes found in this laboratory and obtained from other investigators have been performed. Five linkage groups, which are assumed to represent all the autosomes of the house fly, have been demonstrated. No sex-linked mutant has yet been detected.

The discovery of house flies, *Musca domestica* L., resistant to DDT in the late 1940's has led to numerous investigations of insecticide resistance in this and other insects. Genetic investigation of the organism involved is an important and fundamental step toward an understanding of the problem. A number of genetic studies of the resistance problem in the house fly have been reported; however, conclusive results are very few, in part owing to a lack of basic genetic knowledge of this insect. Therefore, a study of the genetics of the house fly with an emphasis on the accumulation of marked chromosomes seemed of great importance.

Since 1953 some useful mutants of the house fly have been reported by Milani and other investigators. More mutants are necessary, however, to further our knowledge of the genetics of this insect and to make possible more refined studies on the inheritance of resistance. We initiated the investigations to find additional mutants, and a list of aberrations and mutants found was previously reported by Sullivan & Hiroyoshi (1960). An additional list of house fly mutants is included in this report. Also, studies of the linkage relationships of these mutants and mutants obtained from other investigators are reported.

MATERIALS AND METHODS.—Examination with a dissecting microscope of adult flies from normal stocks, mutant stocks and experimental crosses has resulted in the finding of a number of morphological aberrations. Mutagenic agents were not used; only spontaneously occurring mutants were studied in this investigation. When an aberration was found, selection and brother-sister inbreeding were used in attempts to establish pure strains. The pure strains thus established were then tested genetically to determine whether the character was monofactorial. The new mutants were then used for linkage tests.

The tests to classify marker genes in linkage groups were performed by the method of F_2-test and/or testcross. These experimental crosses were performed by a single pair mating technique reported by Sullivan (1960). A batch of eggs from a single pair of flies, 100 to 200 eggs, was placed in a one-half-pint milk bottle, half-full of larval medium, which was made up in the following proportions: 1000 gr. CSMA (Chemical Specialties Manufacturers Association) dry house fly medium, 100 ml. dark Karo Syrup, ½ lb. bakers yeast and 2.5 l. water. The top of the bottle was covered with a layer of paper towel and incubated at a temperature of 80° F. for 3 days. Then about 3 tablespoons of fresh medium were added to the cultures in which the larvae were growing and the bottles were returned to the 80° F. room until emergence. A female house fly will continue to lay batches of eggs until she dies, but only two batches of eggs were kept. These egg batches were usually laid when the female was 4 to 5 and 6 to 7 days old. Only eggs from young females were used in this investigation to insure this control on crossing over rates.

Several normal or wild type house fly strains were used as sources of the new mutants and in the linkage tests. As these strains have been maintained in this laboratory for several years since being sent from other institutions and used for research on resistance to certain insecticides, some knowledge of their reactions to insecticides is available. This matter, however, seemed unimportant in this

[1] Contribution No. 1086, Department of Entomology, University of Kansas, Lawrence. This investigation was supported by the Medical Research and Development Division of the Office of the Surgeon General of the Army under Contract No. DA-49-007-MD-738. Accepted for publication May 2, 1960.

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The author thanks Dr. Robert L. Sullivan for his interest and criticism in the experiments and help in revision of the manuscript; Mrs. Elizabeth T. Lichtwardt for her encouragement during the research and for reading the manuscript, and Mrs. Mary Shepard for her technical assistance. Thanks are also given to Dr. Robert R. Sokal and Dr. Charles D. Michener for providing the opportunity to study in their laboratories.