wood. On bamboo, Bayer 29493 gave ctive kills for longer periods than any the other three toxicants. Previous oratory data for tests with Sevin on ply-od panels showed it to give essentially nplete kills for 24 and 32 weeks at ages of 100 and 200 mg./sq. ft., respec-ely.

The poor results obtained on clay with ecticides that persisted for 10 to 14 eks on other surfaces follow the same nd exhibited in previous laboratory iel tests with malathion, DDT, and diel-n. However, formulations of malathion t showed little residual action after eral days in the laboratory were found be adequate for 2 to 3 months when ted under field conditions (Schoof *et al.*, 61). Bayer 29493 also produced similar ervals of effectiveness. From the lack correlation between field and laboratory ts it is apparent that factors that in-ence the effectiveness of treatments on y differ markedly under laboratory sus field evaluation.

Based on the over-all comparison of face versus toxicant, residues on cement l clay were the least effective, while se on thatch, followed by plywood and itewashed plywood, were the most able.

Past experience has shown that the type surface may exert a profound effect on the persistence of a residual appli-on. The current data likewise show t any one surface may show a negative positive effect upon a residue depending the toxicant involved. Because of this or, the possibility exists that insecticidal nds may prove satisfactory under cir-nstances where a single toxicant may . On this premise, insecticidal formu-ons could be prescribed in accordance h the surfaces found in a project area.

SUMMARY. At Savannah, Georgia, suspension residues of Hercules 5727 and 7522H, Union Carbide 10854, Sevin, and Bayer 39007 were evaluated against *Anopheles quadrimaculatus* on surfaces of clay, thatch, bamboo, cement (plaster), plywood, whitewashed plywood, galvanized metal, and brick. All surfaces were maintained under a shed out-of-doors. Based on 1-hour exposure of adult females at biweekly intervals, Hercules 5727 and 7522H at 25 and 50 mg./sq. ft. and Union Carbide 10854 at 50 and 100 mg./sq. ft. offered little promise as residual agents; Hercules 7522H was the least effective; it failed on nine surfaces at 4 weeks. Both Sevin and Bayer 39007 showed promising results in giving 90 percent kills for 12 to 14 weeks on thatch, whitewashed plywood, plywood, and metal when applied at 200 mg./sq. ft. Bayer 39007 was superior to Sevin on clay but inferior to it on cement. Residues of all compounds were least effective on cement followed by clay. Deposits on thatch gave the maximum periods of effectiveness.

ACKNOWLEDGMENT. These studies were accomplished as part of a contractual agreement between the Communicable Disease Center and the Agency for International Development.

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STUDIES ON *CULISETA INORNATA* AS A POSSIBLE VECTO OF ENCEPHALITIS VIRUSES IN CALIFORNIA [1]

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INTRODUCTION. In the western United States, *Culex tarsalis* is the primary vector of both western equine (WEE) and St. Louis (SLE) encephalitis viruses (Reeves, 1953). It has been postulated that this mosquito may also serve to carry these viruses through the winter and re-establish infection in vertebrate hosts on which they feed in the following spring. Data bearing on this postulate were given by Bellamy *et al.* (1958, 1962) and Reeves *et al.* (1958). In Kern County, California, where both viruses are endemic, isolations of SLE virus from *C. tarsalis* were essentially limited to specimens collected during summer months, but WEE virus was recovered from specimens collected in all seasons except for the period from mid-November to mid-January when blood-feeding by *C. tarsalis* was greatly reduced. Other mosquitoes feed on blood during this interval and might play an important role in the overwinter maintenance of these viruses. In Kern County, *Culiseta inornata* is the principal mosquito feeding during this winter period.

Culiseta inornata was found naturally infected with WEE virus in the Yakima Valley, Washington (Hammon *et al.*, 1945), and more recently was found infected with Cache Valley virus in Utah (Holden and Hess, 1959). In addition, *C. inornata* can transmit WEE, SLE, and Japanese B viruses under laboratory conditions (Hammon and Reeves, 1943a; Hammon and Reeves, 1943b; Reeves and Hammon, 1946) and has been experimentally infected with California encephal virus (Reeves and Hammon, 1952).

The present studies were to evalu *C. inornata* as a possible vector of cephalitis viruses in Kern County, C fornia, and covered a period from Ma 1959 to June 1962.

MATERIALS AND METHODS. Field stuc were concentrated in three study ar 20 to 30 miles west of Bakersfield, C fornia, where WEE and SLE viruses r ularly occur at endemic levels. These ar ranged in size from 9 to 18 square mi Cotton, rice, and alfalfa were the princi irrigated crops in the general area, wh also included desert tracts and areas "wasteland" that were flooded in the to attract migratory waterfowl and mained flooded until February or Mar Additional observations were made in irrigated, wooded recreational area ab five miles east of Bakersfield, and oc sionally in other areas of Kern County.

From March 1959 through Decemi 1960, a wide variety of aquatic situati were repeatedly inspected for mosqu larvae and pupae. Many of the larval s vey observations were part of an int sive *C. tarsalis* surveillance and cont program conducted during the sumn of both years and were influenced mosquito control activities.

Three mosquito light traps were op ated in one of the study areas to obt adult *C. inornata* for virus tests and to p vide data on their seasonal occurrer Traps were run from one to seven nig per week from September 1960 throu October 1961. Eight additional traps ted with cloth collecting bags in place cyanide jars were operated periodic to collect supplementary females for vi tests. All traps were operated from least one-half hour before sunset until ter sunrise.

[1] This investigation was supported, in part, by a research grant, E-3028, from the National Institute of Allergy and Infectious Diseases, U. S. Public Health Service.

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Adult collections were made with aspirators from a variety of shelters, including culverts, under bridges, farmyard sheds, animal shelters, privies, and standard reduced collecting units. Collections were most intensive during the period October 1960 through June 1961, although similar data from previous and later years were utilized. All shelter collections were utilized for virus isolation tests, host preference studies, and to provide biological data on seasonal population activities and changes.

Adult mosquitoes were brought to the laboratory, anesthetized with chloroform, identified, and in the case of females, classified to the following categories: blood-engorged, gravid, empty, and "unclassified." Specimens with any visible blood in the abdomen were considered blood-engorged; those without blood but with obvious developing eggs were considered gravid; and those with neither blood nor eggs were called empty. Specimens with abdomens broken off and those which could not be classified with certainty were placed in an "unclassified" category. Females were pooled into groups of 50 specimens or less, flame-sealed in soft-glass tubes and stored at temperatures below -40 C. until tested for virus. Dead and dry specimens were not used for virus tests. Abdomens of blood-engorged specimens were removed, individually placed in small gelatin capsules, and stored in rubber stoppered glass tubes in the freezer until precipitin tested. The remaining parts of dissected females (head and thorax) were included in virus test pools. All frozen specimens were packed with dry ice in insulated cartons and shipped to the virus laboratory at Berkeley, California for subsequent testing.

The procedures for virus testing and isolation have been described fully (Scrimenti and Reeves, 1962). Each pool was tested in 5 adult mice, and 2 hamster kidney and 2 embryonated egg tissue culture tubes.

Precipitin tests of the blood-filled abdomens were modified from the usual procedures and are described fully (Tempelis and Lofy, 1962, Tempelis and Reeves, 1962a and b). In summary, each abdomen was triturated in 1.0 ml of saline and centrifuged. The supernatent fluid was precipitin screen tested against two antisera, one reacting to the serum proteins of a wide range of mammalian species and the other to a wide range of avian species. The screening antisera were reactive at antigen dilutions about 1:10,000 in the micro-capillary test. Samples that reacted with either antiserum were tested further against more species specific antisera for final identification.

RESULTS. Larvae of *C. inornata* were found in 218 of 14,665 inspections. They were collected in all months except August and September, and all stages of larvae and pupae were present from November through May. Larvae were found in a wide variety of aquatic situations, including duck club ponds, ditches, canals, irrigation and tail water impoundments, seepages, and rainpools. Larvae were most abundant in duck club ponds from December through February and apparently were not adversely affected by thin sheets of ice that occasionally formed on the ponds. Larvae of *C. inornata* were found more frequently with *C. tarsalis* than with larvae of any other species.

Data on light trap collections and temperature averages are presented in Figure 1. Weekly average-per-trap-night collections are plotted in three-week moving averages. The maximum number of *C. inornata* taken in one night in any trap was 25 males and 109 females (December 20/21); whereas, none were captured during the very warm months of July and August.

Monthly patterns of blood-feeding and ovarian development for 1,877 females collected from shelters over a ten-year period are summarized in Figure 2.

No virus was isolated from 4,301 females collected in light traps and shelters during the most intensive sampling period from November 1960 through June 1961 (Table 1) or from an additional 134 specimens collected in December 1959, and 465 in the period July 1961 through June

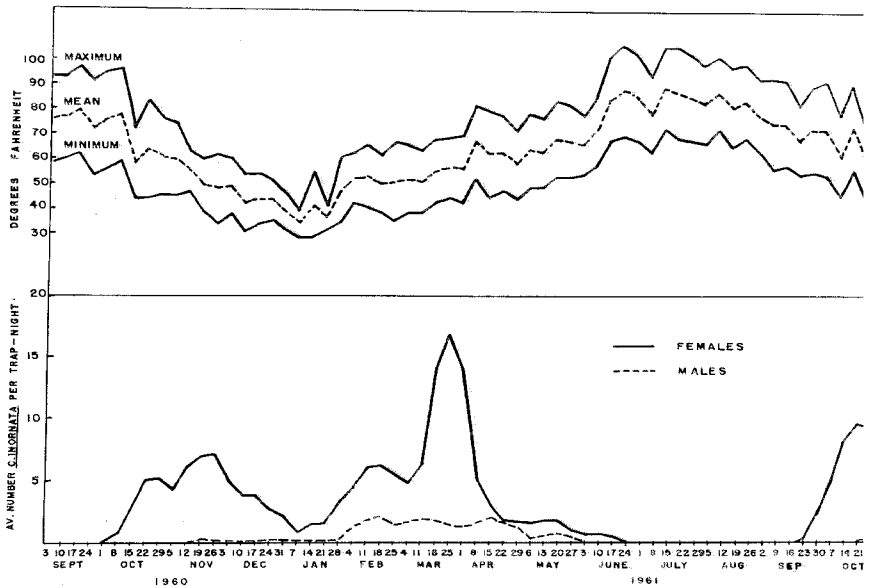

FIG. 1.—Weekly temperatures (U. S. Weather Bureau, Buttonwillow, California) and three-we moving averages of number of *Culiseta inornata* collected from three light traps, Jerry Slough, K County, California, 1960–1961

1962. Of the females tested, over 50 per cent were blood-engorged or gravid.

Precipitin tests of blood from 126 engorged specimens are summarized in Table 2. There was a definite preference for mammalian hosts, particularly cattle. The two avian-positive samples had fed chicken. Of 30 blood-engorged specime collected in chicken houses, 28 had f on mammals, one on chicken, and o gave no reaction.

DISCUSSION. In Kern County, Californ

TABLE 1.—*Culiseta inornata* tested for virus with negative findings in Kern County, California, 1960–1961

Month	Metabolic state of females				To
	Empty	Blood engorged	Gravid	Unclassified	
October	0	3	0	0	
November	60	13	87	7	16
December	339	22	314	23	69
January	237	14	484	30	76
February	446	20	691	47	1,20
March	425	21	723	70	1,23
April	71	15	70	2	15
May	10	20	8	1	3
June	7	13	8	6	2
Total	1,595	141	2,385	180	4,30

. *inornata* is essentially a fall, winter, id spring mosquito. A similar seasonal currence was reported in the southern nited States (King *et al.*, 1960; Mcregor and Eads, 1943; Rozeboom, 1942). decrease in the number of adults colcted in light traps in January coincides ith the year's coldest temperatures (Figre 1). Although the apparent bimodal inter population distribution may reflect ı actual decrease in the population, it ıore probably reflects a failure of *C. inorata* to respond to traps under unfavorıle conditions. Data on shelter populations would further support this interpretation (Table 3).

Studies in Texas (Wilkins and Breland, 1949; Buxton and Breland, 1952) suggested that at least a part of the *C. inornata* population in that area might pass the summer in the egg stage. In spite of intensive surveillance, neither eggs nor larvae were found in August or September of 1960 in Kern County. Moreover, some of the habitats in which larvae of *C. inornata* were first found in October contained water but no larvae and had been repeatedly treated with insecticide several

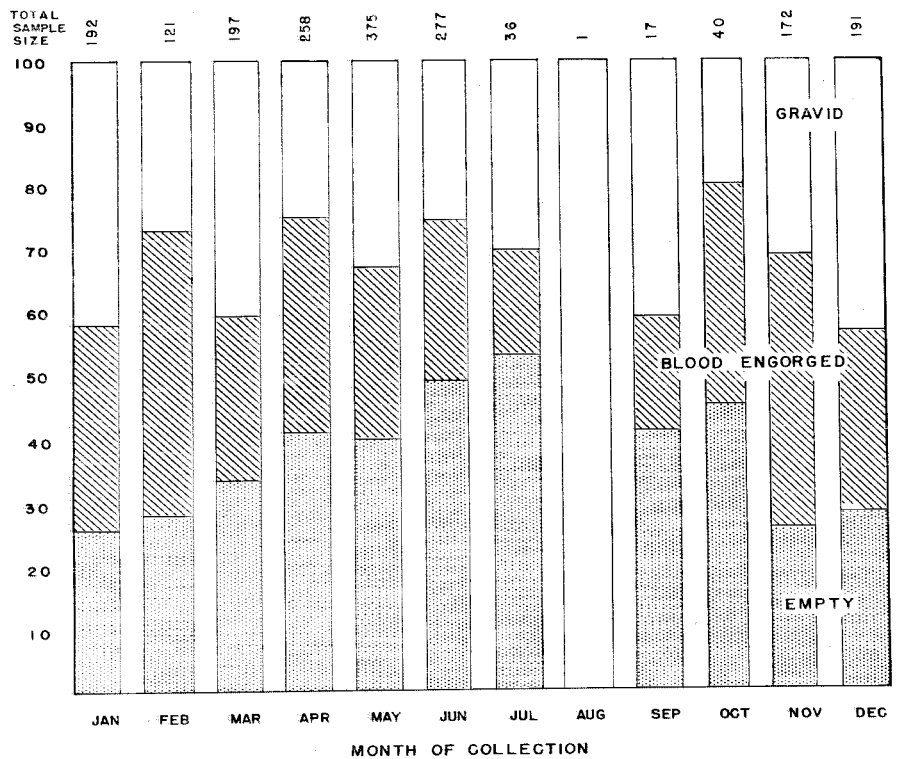

FIG. 2.—Seasonal pattern of feeding and egg production of female *Culiseta inornata*, Kern County, alifornia, 1952–1961 inclusive

TABLE 2.—Precipitin test identification of blood samples from engorged *Culiseta inornata* collected in Kern County, 1960–1962

Host animal	Number positive	Percent positive
Cow *	96	76.2
Horse	19	15.0
Jackrabbit	4	3.2
Dog	1	0.8
Mammal **	3	2.4
Chicken	2	1.6
Negative	1	0.8
Total	126	100.0

* This antiserum cross-reacted broadly with sheep but very few sheep occurred in the area.
** Samples reacted with high titer screening antiserum but not with 10 more specific mammalian antisera.

TABLE 3.—Numbers of male and female *Culis inornata* in shelter collections, by month, 1952 to 1961, Kern County, California

Month	Number of males	Number females
January	21	192
February	50	121
March	103	197
April	158	258
May	178	375
June	75	277
July	12	36
August	0	1
September	1	17
October	2	40
November	7	172
December	24	191
Total	631	1,877

weeks before the larvae were found. If the egg provided a means for passing the summer in Kern County, males should have been more common in fall shelter collections as they were later in the winter. The ratio of males to females in shelter collections in September, October, and November was considerably lower than in later periods when adult emergence from pupae was known to be occurring on a large scale, beginning in December (Table 3). Adult females were collected in every month, although in greatly reduced numbers during the summer. Consequently, it seems likely that the survival of this mosquito through summer periods in Kern County depends upon adult females persisting in small numbers. Owens (1942) reported that female *C. inornata* in a laboratory colony had an average life span of 97 days and a maximum of 145 days. If long life is characteristic of females under field conditions, their chances for survival through summer periods of minimum reproduction would be enhanced.

The number of female *C. inornata* tested for virus in the present studies was sufficient to yield one or more isolations of virus if infection was as common as in *C. tarsalis*. However, the failure to isolate viruses must be interpreted with the knowledge that there was very little virus activity in other vectors and verte brate hosts in Kern County in the sun mers of 1959 and 1960 but a relativel high level of WEE virus activity in th summer of 1961.

Prior to the present studies, *C. inornat* was intensively tested for virus in Wash ington (Hammon *et al.*, 1941, 1942, 1945 Colorado (Cockburn *et al.*, 1957) an Manitoba (Norris, 1946; J. McLintoc personal communication). Negative stu ies in Oklahoma (Reeves *et al.*, 1947) an Minnesota (Burroughs and Burrough 1954), and earlier studies in Kern Count (Reeves *et al.*, 1958; Reeves and Hammo 1962) were somewhat limited in scop and inconclusive.

Blood-feeding by female *C. inornata* o curred through most of the year in Ker County (Table 1 and Figure 2) an precipitin tests indicated that 98 per cer of these feedings were on mammals (Tab 2). Studies of the feeding habits of *inornata* in Washington (Reeves an Hammon, 1944), Utah (A. D. Hess, pe sonal communication), and Alberta, Ca ada (J. A. Shemanchuck, personal con munication) all showed that it fed main on large domestic mammals. The ho preference of this mosquito may be major obstacle to its serving as a vect of WEE and SLE viruses, since wild an domestic birds seem to provide a mo

tisfactory source of infective blood-meals Reeves, 1953).

Current reinvestigations of *C. inornata* a vector of encephalitis viruses in Kern ounty were based on the observation at this mosquito was abundant and was king blood meals during winter periods hen *C. tarsalis* was relatively inactive and rely infected with viruses (Reeves *et al.*, 958). In addition, *C. inornata* had been und naturally infected with WEE virus Washington and had transmitted WEE nd SLE viruses in the laboratory. The ndings that *C. inornata* was not infected ith virus, in spite of extensive tests, and iat it preferred to feed on mammalian osts, do not support the hypothesis that ie species is an important vector of ncephalitis viruses in Kern County.

SUMMARY. An intensive study of *Culiseta inornata* was made in Kern County, alifornia. Immature stages were found 1 a wide range of aquatic habitats but articularly in fields flooded for duck unting in the fall and winter. Light trap nd shelter collection records indicated a eak population of adult males and feiales in the fall, winter, and spring ionths and the lowest level in the sumier. Blood-feeding and ovarian developient occurred throughout the year, but ere particularly notable in midwinter hen other mosquito species were inctive. Virus was not isolated in tests of ,900 *C. inornata* females, of which over) percent were blood-engorged or gravid. recipitin test identification of blood samles from 126 engorged females indicated marked preference for large domestic iammalian hosts.

ACKNOWLEDGMENT. Dr. R. E. Bellamy ssisted in the collection and identification f specimens and direction of field investiations. Miss M. Loty assisted in precipin test identification of blood-meals.

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BIOLOGY OF *CULISETA MELANURA (COQUILLETT)* IN SOUTHEAST GEORGIA [1]

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INTRODUCTION. Interest in the colonization of *Culiseta melanura* has been stimulated by the implication of this mosquito in the transmission of eastern encephalitis. Isolation of EE virus from wild-captured *C. melanura* was first reported by Chamberlain *et al.*, (1951). Since that study, numerous investigations have been made in connection with encephalitis outbreaks in eastern United States (Chamberlain *et al.*, 1958; Wallis, 1959; Bickley and Byrne, 1960; Hayes, 1960).

That *C. melanura* is important in the transmission of EE is based largely upon circumstantial (rather than experimental) evidence. While there is no unanimou agreement in the conclusions drawn l various workers, the weight of the ev dence suggests that when *C. melanura* involved, the involvement is most like a low-level maintenance cycle which serv to account for endemicity of infectio within an avian population. This mosqui probably is not involved in the type epidemic cycle which accounts for th feed-back of encephalitis virus from avi to human populations.

Experimental evidence is needed to a sess better the role of *C. melanura* transmission of encephalitis. Such data c be obtained most readily by laborato experimentation with living material whi can best be provided through coloniz tion of the suspected vector. Accordingl studies directed toward colonization *C. melanura* were initiated at the Tec nical Development Laboratories, Sava

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