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THE EFFECT OF PHOTOPERIOD ON TRANSMISSION EFFICIENCY OF JAPANESE ENCEPHALITIS VIRUS BY *CULEX TRITAENIORHYNCHUS SUMMOROSUS* DYAR [1]

MERRILL D. CATES AND W. C. HUANG [2]

Medical Ecology Department
U. S. Naval Medical Reesarch Unit No. 2
Taipei, Taiwan

INTRODUCTION. *Culex tritaeniorhynchus summorosus* Dyar (CTS) has been implicated as the vector of Japanese encephalitis virus (JEV) by Wang *et al.* (1962) and Hurlbut (1964). Furthermore, Hurlbut (1964) reported that CTS is involved in a "pig-mosquito cycle" which precedes and coincides with late summer encephalitis epidemics in Taiwan. To the present time, these outbreaks in both porcine and human populations have been attributed only to a rapid increase in CTS populations (Hurlbut, 1964).

Hurlbut (1964) and Hayashi *et al.* (1966) emphasize the necessity of more investigations into the ecology of vector species of JEV. Such a pursuance might explain what factors favor CTS population explosions and what factors may favor JEV transmission. In this respect, Eldridge (1963) has shown that long photoperiods induce feeding by *Culex tritaeniorhynchus* Giles mosquitoes but that short photoperiods depress feeding activity. This paper deals with the effect of daylength on JEV transmission by CTS.

MATERIALS AND METHODS. Newborn white Swiss mice were infected by an intracerebral injection of 0.02 ml mouse brain suspension containing JEV-Nakayama virus. Each mouse was incubated for 2 days and then offered to about 5-day-old adult female CTS mosquitoes for 4 hours. The viremia was determined in these mice before and after they were offered to mosquitoes. Bioassay of mouse blood was performed by logarithmically diluting the blood with PBS and 0.5 percent bovine albumin and injecting 0.03 ml. of one dilution, i.c., into each of six litter-mates 21–23 days old. All mice offered to mosquitoes had 10^{-3} to 10^{-4} log dilution virus titer.

Engorged females were divided into

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three treatment groups containing about 50 mosquitoes each while unengorged mosquitoes were discarded. One group was treated with 0 hours continuous darkness (CD), one with 12 hours half daylight (HDL), and one with 24 hours continuous light (CL) per day for 12 days. At the end of the incubation period salivary secretions and whole mosquitoes were assayed for JEV according to the method of Hurlbut and Nibley (1964).

To test the persistance of JEV in mosquitoes held under varied light treatments, salivary secretions from 15 mosquitoes from each treatment were assayed on the 12th, 30th, 45th, and 55th day after feeding on infected mice. Salivary secretions were collected according to the method of Hurlbut and Nibley (1964) and were diluted logarithmically from 10^{-0} through 10^{-5} log dilutions.

RESULTS. More mosquitoes held under CL were positive according to whole-mosquito and salivary-secretion assay than mosquitoes from HDL and CD treatments. According to salivary secretion titrations, 75 percent of all CL-treated mosquitoes were JEV-positives whereas only 42 percent of HDL-treated and 54 percent CD-treated mosquitoes were JEV-positive. The results of whole mosquito assays revealed that 88 percent CL-, 79 percent HDL-, and 74 percent of CD-treated mosquitoes were positive. Although the data do not present a clear picture of virus harbored by CD- and HDL-treated mosquitoes, it does indicate that CL-treatment was more favorable for virus development within vector mosquitoes.

Salivary secretion titrations from mosquitoes treated for 12 days revealed that CL-treated mosquitoes secreted significantly more virus than CD-treated mosquitoes and that HDL-treated mosquito virus titers were not significantly different from other treatments (Table 1). As in assays for percent positive mosquitoes, CL-treatments favor development of JEV in the host mosquito.

Figure 1 shows the development of JEV titer in salivary secretions of mosquitoes held under various treatments over extended time periods. The salivary secretions from CL-treated mosquitoes reach their peak virus titer readily but the titer decreased rapidly and the mosquitoes died shortly after the 45th day. The virus titer of HD-treated mosquitoes generally fell between the CL- and CD-treated ones but these also died after the 45th day. CD-treated mosquitoes reached the highest level of JEV titer of all but this level was not reached until the 30th day. This group of mosquitoes died after the 55th day.

DISCUSSION. CL-treatments clearly favor virus development until the 12th day but higher virus titers are reached by mosquitoes treated under shorter photoperiods. For experimental transmission in laboratories the best results would be obtained by holding infected mosquitoes under continuous light. Peaks are reached more readily in this manner and transmission successes could be great enough, according to percent-positive salivary secretion, to warrant use of this treatment. To insure greatest success in recovery, when holding field-collected mosquitoes prior to JEV isolation attempts, continuous light should also be used.

In nature the advantage of increased daylength is not too obvious. In Taiwan the difference in daylength from June to September, when *C. tritaeniorhynchus summorosus* is active, is only about one hour. The summer solstice occurs in late June, well before the actual JEV epidemic. The solstice period is very important, however, since it is during mid-summer that the amplifying host population is infected

TABLE 1.—Mean LD_{50} log dilutions of JEV in salivary secretions of CTS mosquitoes held at indicated photoperiods for 12 days.

Photoperiod (hrs. light)	Mean LD_{50} log dilution *
0	1.20 A
12	1.54 AB
24	1.67 B

* Mean followed by the same letter is not significantly different at the 10% significance according to the student's T test.

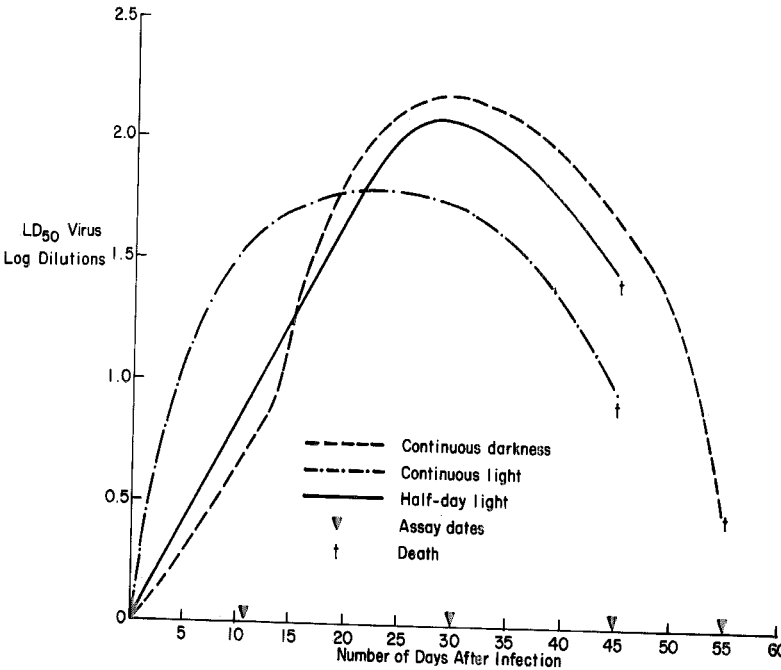

FIG. 1.—Mouse LD_{50} for JEV present in mosquito salivary secretions following indicated post-infection incubation periods and photoperiod treatments.

and vector populations are quite low (Sun, 1964). It is interesting that Japan and Korea experience more severe JEV outbreaks than Taiwan or other more southern Asian areas. This is coincidental with greater daylength at the summer solstice and shorter daylength at the peak of epidemics.

At a low population period a high rate of vector efficiency would favor JEV increase in amplifying hosts. Eldridge (1963) showed that *C. tritaeniorhynchus* feeds more readily during long days. Furthermore, our data indicate higher mosquito infection and rapid rising salivary secretion titer during long days. The two factors combined should favor JEV transmission in late June and July.

This study represents only a limited area of the ecology of *C. tritaeniorhynchus summorosus*. One must consider Shelford's law of tolerance that if one or more factors are near the limits of tolerance for the organism then the tolerance for other factors may be altered. Therefore, deviations in daylength may assume greater import in respect of further information on ecology of JEV vectors. It is imperative that JEV epidemiology and ecology of vectors be pursued further. Based on a more complete knowledge of vector ecology and habits, development of JEV epidemics may be better understood and possibly prevented.

SUMMARY. *Culex tritaeniorhynchus summorosus* Dyar adult females were infected with Japanese encephalitis virus, incubated for 12 days at 0, 12, or 24 hours light per 24-hour day, and salivary secretions and whole mosquitoes were bioassayed to determine the efficiency. Twenty-four hour light-day treatments were more favorable for transmission than 12 and 0 hour days in assays of whole mosquitoes;

and 24-hour light-day treatments yielded significantly higher titers in salivary secretions than 0-hour light day treatments.

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STUDIES WITH THE POWER-DRIVEN BACK-PACK SPRAYER-DUSTER [1]

JOHN L. McDONALD [2] AND L. W. TELLER [3]

Of all the insecticide dispersal equipment, none seems to be as controversial as the motor-driven back-pack. Our experience with these machines shows there are basically two schools of thought concerning this tool; those that like it and those that don't.

The motor-driven back-pack is a tool that has a specific capability and place in insect control, and when anyone using this machine loses sight of this fact, there are unpleasant results. The value of this device varies quite considerably with the different situation and circumstances and this should be kept in mind at all times.

We have successfully used these machines for dispersal of liquids, dusts and granules in various entomological problem areas. Perhaps the greatest advantage we have experienced with this machine is the omipresent opportunity for selective application of insecticide. It can be assumed that wherever a man can go on foot, by boat or wheeled-vehicle, this machine can go also. Much of our work has had to do with controlling mosquitoes utilizing the "source reduction" concept and here the back-pack has proved especially useful. Instead of making blanket aerial applications of insecticide to several acres of suspected mosquito breeding and resting sites, we have typically been able to get to the actual sites and exert prompt selective and efficient control at a fraction of the cost and time otherwise required.

Many times operators of this machine have suggested that these machines cannot disperse the insecticide materials very far. Using a "Day-Glo" dye as a marker mixed with dust and granules, we have consistenly found that granules are distributed in a very even pattern up to 35–60 feet from the nozzle of the machine; and dust as much as 500 feet when assisted by a 2–3 m.p.h. tail wind. Liquids are typically dispersed 40–50 feet from the nozzle in still air.

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[2] NAMRU #2, Box 14, APO San Francisco 96263.

[3] DVCC, Box 43, NAS, Jacksonville, Fla. 32212.