References Cited

Anderson, J. F. and Ringo, S. L. 1969. *Entomophthora aquatica* sp. n. infecting larvae and pupae of floodwater mosquitoes. J. Invertebr. Pathol. 13(3):386–393.

Andrade, Z. A., Paula, L. A., Sherlock, I. A. and Cheever, A. W. 1967. Nasal granuloma caused by *Entomophthora coronata*. Amer. J. Trop. Med. Hyg. 16(1):31–33.

Bras, G., Gordon, C. O., Emmons, C. W., Prendegast, K. M. and Sugar, M. 1965. A case of phycomycosis observed in Jamaica; infection with *Entomophthora coronata*. Amer. J. Trop. Med. Hyg. 14(1):141–145.

Clark, T. B., Kellen, W. R., Fuduko, T. and Lindegren, J. E. 1968. Field and laboratory studies on the pathogenicity of the fungus *Beauveria bassiana* to three genera of mosquitoes. J. Invertebr. Pathol. 11(1):1–7.

Emmons, C. W. and Bridges, C. H. 1961. *E. coronata*, the ethiologic agent of a phycomycosis of horses. Mycologia 53:307–312.

Gridley, M. F. 1953. A stain for fungi in tissue sections. Amer. J. Clin. Pathol. 23:303–307.

Humason, G. L. 1967. Animal Tissue Techniques, 2nd edition. W. H. Freeman and Company, San Francisco, 569 pp.

Lowe, R. E., Rumbaugh, R. G. and Patterson, R. S. 1968. *Entomophthora coronata* as a pathogen of mosquitoes. J. Invertebr. Pathol. 11(3):506–507.

Prasertphon, S. and Tanada, Y. 1968. The formation and circulation, in *Galleria*, of hyphal bodies of entomophthoraceous fungi. J. Invertebr. Pathol. 11(2):260–280.

Yendol, W. G. and Paschke, J. D. 1965. Pathology of an *Entomophthora* infection in the eastern subterranean termite, *Reticulitermes flavipes* (Kollar). J. Invertebr. Pathol. 7(4):414–422.

Yendol, W. G., Miller, E. M. and Behnke, C. N. 1968. Toxic substances from entomophthoraceous fungi. J. Invertebr. Pathol. 10(2):313–319.

EFFECTS OF AIR CURRENTS UPON LIFE SPAN (LONGEVITY) OF ADULT *AEDES AEGYPTI* (L.) IN THE LABORATORY[1]

E. M. McCRAY, JR.,[2] T. L. McCRAY[3] AND H. F. SCHOOF[2]

INTRODUCTION. In much of the laboratory research on mosquito biology the length of adult life or the daily mortality in the laboratory is used as an indicator of the effects of various factors such as chemicals, diets, pathogens and ionizing radiation. When life span is used to demonstrate the deleterious or beneficial effects of some factor being investigated, the specimens are usually maintained in some regulated, standard environment.

While studying the effect of temperature stress on *Aedes aegypti*, the adult life span was one of the criteria used for evaluation. Throughout the preliminary studies, marked variations in daily mortalities were noted among the replicates and appeared to be related to the location of the individual containers on the shelves in the room. These observations prompted the investigation of the possible effects of air movement upon the life span of adult *Ae. aegypti* in the laboratory.

MATERIALS AND METHODS. The standard holding conditions for adult specimens consisted of confinement of 50 virgin female *Ae. aegypti* in a pint ice cream carton covered with nylon tulle, kept in a dark room 10 ft. x 13 ft. and maintained at 80° F (±1°) and 80 percent (±2 per-

[1] These studies were supported in part by the U. S. Army Biological Laboratories, Frederick, Maryland.

[2] From the Biology Section, Technical Development Laboratories, Malaria Program, Center for Disease Control, Public Health Service, Health Services and Mental Health Administration, U. S. Department of Health, Education, and Welfare, Savannah, Georgia 31402.

[3] Present address: Stored Product Insects Research and Development Laboratory, Agricultural Research Service, U. S. Dept. of Agriculture, Savannah, Georgia 31403.

cent) R.H. Fresh cotton pads with 10 percent sucrose were provided daily. Because many hundreds of such containers were inspected daily and dead specimens removed, each container required ready access. This was accomplished by placing the containers upon tiers of eight shelves, mounted as free standing units (4 by 1½ by 6½ ft. high) on rollers. The locations of seven such units were carefully selected in relation to the gentle stream of conditioned air which entered the room from a single baffled duct. Smoke was used to study air turbulence and eddies, so that no specimens were placed in what appeared to be a drafty location.

TABLE 1.—Percent mortalities of virgin female *Aedes aegypti* at weekly intervals.

Age group	Replicates A	B	C	D	Mean mortality
9 days old	2	0	0	4	1
16 days old	13	12	29	13	17
23 days old	45	31	64	25	41
30 days old	96	79	86	76	84
37 days old	100	98	98	96	98
Half life (days)	25	26	19	27	..

Maximum life span—40 days.

RESULTS AND DISCUSSION. Initially, tests to obtain base line data of the normal life span of adult *Ae. aegypti* produced mortalities which varied markedly from one container to another, as the data from a single test with the Camp Detrick (CD) strain (Table 1) illustrate. In this test, each of the four replicates in each of the five age groups were on a single shelf and, because of the experimental design of the work in which these specimens were involved, were to be removed and used when the specimens were 9, 16, 23, 30 and 37 days of age. It is apparent from these data that there were practically no specimens available for use that were 30 and 37 days old. These data also show that the percent mortalities among the replicates of 16- or 23-day-old specimens varied more than 100 percent. Such a wide variation among the individual replicates of a given age group would completely obscure the effects of some test condition or compound. In these tests the last specimen died on the 40th day, and the half-life (that day on which 50 percent of the specimens were dead) in three of the four groups ranged from 25 to 27 days. In the remaining group the half life was 19 days. These results were like those obtained in all of the early tests and were not acceptable. Similar tests with the Rockefeller (RK) strain showed that none lived longer than 53 days and the mean half-life was 30 days.

If we had not needed large numbers of adults 37 days and older, the data in which most of the specimens died after about 40–50 days may have been accepted, particularly since Christophers (1960) stated that Fielding (1919), MacGregor (1915) and others had reported a life expectancy of 20 to 42 days. However, Putnam and Shannon (1934) reported one female that lived for 17 weeks, and Brues and Sacher (1952) reported 50 percent surviving until the 60th day with one female living slightly longer than 100 days. Since these data were from tests with less than 200 females, one could assume that such data represented an unusual occurrence. In contradiction to this assumption were the daily observations in our preliminary tests which revealed that adults from containers on certain shelves consistently lived longer than the mean longevity, while adults from containers on other shelves consistently lived for periods shorter than the mean longevity. Although it seemed

inconceivable that the scarcely perceptible air movement within the room was responsible for these differences in longevity, no other possible factor was evident.

A limited test was then initiated by hanging a cotton muslin drape around one-half of one shelf. Equal numbers of containers with 50 virgin female adult *Ae. aegypti* (CD strain) of the same age and from the same larval pool were then placed on the shelf and daily mortalities recorded. Those adults on the undraped portion of the shelf died at the same rate as those in earlier tests. Those on the draped half of the shelf lived for 78–87 days with mean half-lives of 40–50 days; quite different from the earlier half-life of 19–27 days. In a second series of tests, specimens that were protected with a muslin drape had a mean mortality of 6 percent at 30 days of age and 22 percent at 37 days of age. This was in marked contrast to the data in Table 1 in which the 30- and 37-day-old specimens had mean mortalities of 84 and 98 percent, respectively.

As a result of these data, all shelf units were modified so that drapes and roller-type window shades covered all sides and individual sections were opened only during servicing. Throughout the 2 years of studies which followed these early tests, specimens frequently lived longer than 100 days and the half-life was consistently 60–65 days. Mortalities of specimens were normally less than 1 percent at 23 days of age and 5 percent at 37 days of age (Table 2). Mortality increased rapidly after 65 days and the total number of specimens living longer than 100 days was very small.

These data illuminate one of the inherent hazards of using adult life span as a criterion for evaluating the effect of some supposedly beneficial or deleterious factor, since even slight variations in air movement can produce markedly different

TABLE 2.—Mean percent mortalities of virgin female *Aedes aegypti* on draped and undraped shelves.

Age group	Mean percent mortality	
	Without drapes	With drapes
9 days old	1	<1
16 days old	17	<1
23 days old	41	<1
30 days old	84	3
37 days old	98	5
Half life (days)	24	63
Maximum life (days)	40	111
Number specimens tested	1,000	4,000

results in longevity. Although the individual differences among replicates may be smoothed out to some extent by arithmetical manipulation, the net result may be such that the effect of the test compound or environmental stress could be altered or even undetectable. This postulate does not mean, however, that adult life span is not a valid indicator; it merely stresses the point that the investigator should be extremely alert to the fact that even slight (and to the worker, insignificant) variations in the laboratory environment can produce such variations in response that the whole validity of the experiment is questionable.

Literature Cited

Brues, A. M. and Sacher, G. A. 1952. In Nickson's Symposium on Radiobiology. Chapman and Hall, London, p. 441.

Christophers, S. R. 1960. *Aedes aegypti* (L.), The Yellow Fever Mosquito. Cambridge University Press.

Fielding, J. W. 1919. Notes on the bionomics of *Stegomyia fasciata* Fabr. Part I. Ann. Trop. Med. Parasit. 13:259–296.

MacGregor, M. E. 1915. Notes on the rearing of *Stegomyia fasciata* in London. J. Trop. Med. (Hyg.) 18:193–196.

Putnam, P. and Shannon, R. A. 1934. The biology of *Stegomyia* under laboratory conditions. Proc. Ent. Soc., Wash. 36:217–242.