TABLE 2.—Larvicides evaluated in screening tests only.

Larvicide	Lowest concentration causing 100% mortality (p.p.m.)	Acute oral toxicity to rats (LD_{50} mg/kg)
Bay 48792	0.1	2.5
Bay 48772	.05	3
Bay 52553	.01	5
Stauffer N-4548	.025	30
Bay 51294	.025	10
Velsicol FCS-303	.025	Unknown

TABLE 2a.—Chemical composition of compounds listed in Table 2.

Bay 48792: (*O*-ethyl *O*-[*p*-(methylsulfinyl) phenyl] ethylphosphonothioate)
Bay 48772: (*O*-methyl *O*-[4-)methylsulfinyl)-*m*-tolyl] methylphosphonothioate)
Bay 52553: (*O*-isopropyl *O*-methyl *O*-*p*-nitrophenyl phosphorothioate)
Stauffer N-4548 (*S*-[[(*p*-chlorophenyl)thio] methyl] *O*-methyl methylphosphonodithioate)
Bay 51294: (*O*-ethyl methylphosphonothioate *O*-ester with 2-chloro-4-hydroxybenzonitrile)
Velsicol FCS-303 (*O*-(4-bromo-2,5-dichlorophenyl) *O*-ethyl phenylphosphonothioate)

9098, Bay 77488, CIBA C-9491, Montecatini L 561, CIBA C-8874, Bay 69047, and Stauffer N-4988 were about 1.5 to 0.5 times as toxic as DDT. The remaining compounds were about one-third to one-seventh as toxic as DDT.

Additional compounds that gave good kills in screening tests but were not tested further because of high mammalian toxicity are listed in Table 2. The chemical composition of each of these compounds is given in Table 2a.

LARVAL DEVELOPMENT OF *AEDES AEGYPTI* (L.) IN USED AUTO TIRES[1]

J. E. KEIRANS

Since the inauguration of the Aedes aegypti Eradication Program in 1964, many new studies on the control of this mosquito have been undertaken, and much new information on its habits has been made available. Tinker (1964) showed that the receptacle most favored as a larval site by *Ae. aegypti* was a used tire. Haverfield and Hoffman (1966) found that the shipment of used tires is probably the most efficient means of dispersing this mosquito and that its larvae are found more often in tires than in any other type of container. The recognition of this fact led to a series of experiments to determine if (1) an auto tire freshly discarded is capable of supporting larval development of *Ae. aegypti*, (2) the length of the larval development span in tires of various ages, and (3) the amount of food available to the larvae in tires of known age.

MATERIALS AND METHODS. Four sites, referred to herein as A, B, C, and D, were selected in and around Savannah, Georgia, each naturally infested with *Ae. aegypti* and each different in its vegetation and proximity to organic food sources for the larvae. All botanical designations are from Small (1933).

Site A. A small ditch beside a frequently used road, the general vegetation being *Phytolacca americana* (pokeweed), *Quercus virginiana* (live-oak), *Quercus ilicifolia* (scrub-oak), *Vitis sp.* (wild

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[1] From the Biology Section, Technical Development Laboratories, National Communicable Disease Center, Health Services and Mental Health Administration, Public Health Service, U. S. Department of Health, Education, and Welfare, Savannah, Georgia 31402.

grape), *Pinus australis* (long-leaf pine), *Ambrosia elatior* (ragweed), and *Alsine* sp. (chickweed).

Site B. A 26- x 28-ft. yard enclosed by a 6.5-ft. brick wall behind an abandoned three-story house. Vegetation—*Melia azedarach* (china-berry), *Quercus virginiana* (live-oak), *Eupatorium capillifolium* (dog-fennel) and *Cenchrus tribuloides* (sand-spur).

Site C. A 19- x 41-ft. used-tire yard surrounded by a 6-ft corrugated fence in a substandard section of Savannah. Vegetation in this area includes *Albizzia julibrissin* (mimosa), *Eupatorium capillifolium* (dog-fennel), *Pharbitis* sp. (morning-glory), *Cenchrus tribuloides* (sand-spur), *Digitaria* sp. (crab-grass), *Amaranthus* sp. (pigweed), *Liquidambar styraciflua* (sweet-gum), and *Platanus occidentalis* (sycamore).

Site D. The northwest corner of a cemetery, 10 yards from a dirt road. Vegetation includes *Celtis* sp. (hackberry), *Liquidambar styraciflua* (sweet-gum), *Mirabilis jalapa* (four-o'clock), *Pueraria thunbergiana* (kudzu vine), *Ambrosia elatior* (ragweed), *Cirsium* sp. (thistle), and *Holcus halepensis* (Johnson-grass).

Used auto tires were obtained locally and were of various sizes. All had been recently removed from autos and stored dry before experimental use. Before being placed in the field, the inside of each tire was vacuumed to remove any previously accumulated food material.

Initially, 10 tires were placed at each site. They were mounted at a 45° angle by tilting them on stakes, and 2 liters of tap water were added to each tire. The water level was maintained at approximately the 2-liter level by additions, if necessary, but excess amounts of rain water were not removed. To each of two tires at all four sites, 25 newly emerged *Ae. aegypti* larvae were added the same day the tires were placed in the field. Likewise, 25 larvae each were added to another two tires at each site after periods of 1, 2, 3 and 4 weeks. This procedure allowed food materials to accumulate in the tires for specific periods of time before the addition of larvae. The time periods required for larval development in the tires were recorded. Pupae were picked daily and the resultant adults identified to species. The temperature data for the test period were obtained from the U. S. Weather Bureau, Savannah, Georgia.

RESULTS AND DISCUSSION. In previous laboratory studies, full rations of a finely ground standard laboratory chow were designated as 0.15 mg. and 0.30 mg. per larva on days 0 and 1, respectively, and 0.60 mg. per larva daily thereafter. Larvae were also reared at ½ and ¼ of these amounts. Using these food regimens, larval development times were determined at constant temperatures of 60°, 70°, 80° and 90° F. and at fluctuating 24-hr cycles of 50° to 70°, 60° to 80°, 70° to 90° and 80° to 100° F. (Keirans and Fay, 1968). An estimate of the accumulated food supply in the tires was then possible by comparing the larval development periods from the laboratory and field tests using comparable temperature conditions.

In tires seeded with larvae immediately (0 weeks), the time required to initial pupation varied from 12 to 18 days and pupation varied from 0 to 140 percent (Table 1). No pupation occurred in one tire, and chemical analysis indicated the presence of soap, which is often used as a lubricant to fit tubeless tires to the wheel rims. The 140 percent pupation was due to additional eggs deposited by naturally occurring *Ae. aegypti.* Larvae added to tires at 1 and 2 weeks varied in initial pupation time from 6 to 9 and 6 to 8 days, respectively. In tires aged 3 weeks, the first pupae appeared on days 5 through 14 (Table 1). In the latter case, since the 14-day figure is so obviously at variance with all the pupation times in the other 3-week-old tires (Table 1), it was not included in the totals. Initial pupation in the 4-week-old tires varied from 5 to 10 days. Tires at Site A, situated in a roadside ditch, were flooded out.

In tires exposed for 0 weeks the average values for initial pupation and temperature at the four sites were 13.3 days and 73° F. In the laboratory the nearest correspond-

TABLE 1.—Initial pupation (days) for 25 first instar *Aedes aegypti* placed in pairs of auto tires previously exposed in four field environments for 0, 1, 2, 3 and 4 weeks, respectively. Values for percent pupation shown in parentheses.

Field Site	Replicate	Exposure Period (Weeks) 0	1	2	3	4
A	1	12 (32)	9 (76)	6 (12)[b]	6 (92)	flooded
	2	18 (76)	9 (60)	8 (68)	9 (80)	flooded
B	1	.. (0)[a]	8 (80)	6 (92)	5 (76)	5 (84)
	2	13 (68)	8 (92)	6 (96)	7 (96)	9 (396)
C	1	13 (140)	9 (68)	6 (152)	.. (0)[c]	7 (176)
	2	13 (104)	8 (68)	7 (112)	14 (120)	7 (172)
D	1	12 (88)	6 (116)	6 (92)	5 (100)	5 (72)[d]
	2	12 (80)	6 (100)	7 (104)	9 (92)	10 (104)

[a] Soap in tire.

[b] Three *Toxorhynchites rutilus* larvae (Diptera: Culicidae) and several *Copelatus* sp. (Coleoptera: Dytiscidae) larvae and one adult ♀ present. Both predaceous on *Ae. aegypti* larvae.

[c] Probable malathion contamination confirmed.

[d] Stolen after 18 larvae had pupated.

ing pupation time under similar temperatures of 70° F. or 60° to 80° F. (Table 2) occurred on day 11 at ¼ food ration. Thus, on the average, these tires contained less than ¼ of the designated standard food supply. Average pupation times in tires aged for 1 week and in the laboratory tests were approximately equal but the average field temperature was 2° F. cooler than the 80° F. in the laboratory, indicating slightly more than ¼ food ration available to the tire-reared larvae. Similarly, with tires at 2, 3 and 4 weeks, a comparison of temperatures and initial

TABLE 2.—Estimated food accumulation in tires exposed to field conditions based on average temperature–time factors to initial pupation and controlled laboratory studies.

Field: Tire Exposure Period (Weeks)	Average Temp. (° F.)	Average Initial Pupae (Days)	Est. Food Regimen	Laboratory: Daily Temp. Cycle (° F.)	Average Initial Pupae (Days)	Actual Food Regimen
0	73	13.3	−¼	60°–80° [a]	11	¼
1	78	7.9	+¼	70°–90° [a]	8	¼
				70°–90° [a]	7	½
2	80	6.5	+½	70°–90° [a]	7	½
				70°–90° [a]	6	Full
3	79	6.8	+½	70°–90° [a]	7	½
				70°–90° [a]	6	Full
4	74	7.2	+Full	60°–80° [b]	9	Full

[a] Time to average initial pupation for a constant median temperature for the range shown fell on the same day.

[b] Time to average initial pupation for a constant median temperature for the range shown fell on day 10.

pupation times in the field and laboratory with indicated rations in the laboratory shows better than ½ food rations at 2 and 3 weeks and better than full food in tires aged for 4 weeks.

Other mosquitoes were found in the tires. *Culex* sp. were found at all four sites but not in all tires. A total of 4,384 *Culex* larvae were counted but some were not identified to species. At Site B, 1,770 *Culex* larvae were found in one tire that contained a dead sparrow. The next most numerous species found in the tires was *Orthopodomyia signifera;* 40 larvae were collected from two tires at Site A. Thirteen *Aedes triseriatus* were found in one tire at Site D, and five *Toxorhynchites rutilus* larvae were collected, three at Site A and two at Site D.

Organic material available as larval food in addition to debris from the previously mentioned vegetation included Diptera (excluding Culicidae) and Hymenoptera (mostly ants) present in tires at all four sites; Orthoptera, Thysanoptera, Lepidoptera and Aranaea at three sites; Collembola, Odonata, Corrodentia, Hemiptera, Neuroptera, and Coleoptera at two sites, and at only one site (D) Isopods were found. All of the above were found dead and were assumed to be an available source of food for the larvae.

The data gathered from this study show that a freshly discarded auto tire containing water is capable of supporting larval development of *Ae. aegypti.* Under natural conditions there would be approximately a week's accumulation of organic matter in the water before any larvae could be found, since a freshly discarded tire would contain no eggs. This fact would make it even easier for larvae to survive, since more than ¼ food ration is available after 1 week. In addition, as can be seen from the percent pupation figures in Table 1, tires of any age containing water are capable of supporting and bringing through to pupation large populations of mosquito larvae.

SUMMARY. Evaluations were made of the capability of auto tires of various ages to support the development of *Ae. aegypti* following exposure to field conditions for 0, 1, 2, 3 and 4 weeks. The criteria for food supplies were based on the time to initial pupation and comparisons with laboratory tests using known amounts of food. At 0 weeks, the used auto tires contained less than ¼ the optimum ration of food per larva but were capable of supporting larval development. At 1, 2, 3 and 4 weeks, the food supplies in the tires were +¼, +½, +½ and +full, respectively.

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