

earlier ages that there were few left for tests at the older ages. *A. stimulans*, *A. trichurus* and *A. excrucians* also showed a similar pattern of biting activity that was not affected by the presence of a permanent alternative food source. With these mosquitoes there was probing activity first followed by engorgement several hours later. Once biting had started, only individual mosquitoes took blood meals at irregular intervals.

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THE EFFECT OF ODORS RELEASED BY VARIOUS WATERS ON THE OVIPOSITION SITES SELECTED BY TWO SPECIES OF *CULEX*

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Some mosquitoes are known to choose specific types of water for oviposition. Wallis (1954) showed that *Aedes aegypti* (L.) preferred a 0.25 percent salt solution to distilled water. O'Gower (1963) found that the choice of oviposition site by this species was influenced by at least five different stimuli. Gjullin and Johnsen (1964) showed that distilled water could be made more attractive to *Culex quinquefasciatus* Say by adding different chemicals.

Culex quinquefasciatus and *C. tarsalis* Coquillett usually lay their eggs on waters containing grass, logs, sewage, and other organic matter. These materials give off various gases as they break down. This paper reports the results of tests conducted to demonstrate the effects of gases recovered from such waters and of other

gases known or suspected of occurring in these waters on these two mosquito species.

MATERIALS AND METHODS. Waters used as a source of odors for these tests were obtained from log ponds or from grass infusions. The infusions were prepared from dried grass and tapwater in the ratio of 1 gram of grass to 82 ml. of water.

Odors were recovered from a glass vessel partially filled with the grass infusion or log pond water. The vessel was closed except for a small air inlet at the top and a glass tube which terminated an inch or two above the water surface. Air drawn through this tube passed through a fritted glass tube placed in a test tube of distilled water.

The 75-ml. distilled water samples

through which the air had been bubbled for 18 to 24 hours were tested in a 12×12×12-inch screen cage fitted with a sleeve on one side. The attractiveness of the water sample to gravid *Culex* females was determined by the number of egg rafts laid on it and on a similar distilled water sample through which air only had been bubbled. The beakers containing the water samples were placed in opposite sides of the cage and rotated daily during the 3-day test period. A 10 percent sugar water solution was available as food in all cages.

The cages were held at 76–80° F. and 55–70 percent relative humidity in a room with an exhaust fan, which provided a continuous low volume intake of fresh air. The room was kept in darkness after the test beakers had been placed in the cages except during the period when the egg rafts were being counted and removed.

The methane test samples were obtained by bubbling the gas through water. The furfural was added directly to the water.

RESULTS AND DISCUSSION. The relative attractiveness of odors from log pond water, distilled water, and grass infusion waters to females of *Culex quinquefasciatus* and *C. tarsalis* is demonstrated in Table 1. Waters taken from log ponds were very attractive to gravid females of *C. quinquefasciatus*. During cage tests when females were provided both log pond and distilled water for oviposition, they laid 100 percent of their egg rafts on the log pond water. The far greater attractiveness of distilled water containing the odors recovered from log pond waters than of untreated distilled water was apparent since 91 percent of the eggs were laid on the water containing the odor. However, when females had a choice between log pond water and distilled water treated with log pond water odors, they laid only 14 percent of the egg rafts on the distilled water. The same egg-laying pattern was observed when grass infusion waters, distilled water containing infusion odors,

and distilled water alone were tested with this species.

The effect of odors from grass infusion waters and log pond waters was also tested on gravid females of *C. tarsalis*. The numbers of egg rafts laid by this species on distilled water treated with grass infusion odor were not significantly different from those laid on untreated distilled water. Log pond water was more attractive than either distilled water or distilled water plus log pond odor.

TABLE 1.—Attractiveness of various odors to females of *Culex quinquefasciatus* and *C. tarsalis* as indicated by the numbers of egg rafts laid. Three replications.

| Type of water | Egg rafts laid | |
|--|----------------|---------|
| | Total number | Percent |
| <i>Culex quinquefasciatus</i> | | |
| (Grass infusion) | | |
| Distilled water plus grass infusion odor | 42 | 87 |
| Distilled water | 6 | 13 |
| Grass infusion | 73 | 94 |
| Distilled water | 2 | 6 |
| Grass infusion | 58 | 81 |
| Distilled water plus grass infusion odor | 13 | 18 |
| (Log pond water) | | |
| Distilled water plus log pond odor | 33 | 91 |
| Distilled water | 3 | 9 |
| Log pond water | 68 | 100 |
| Distilled water | 0 | 0 |
| Long pond water | 59 | 86 |
| Distilled water plus log pond odor | 10 | 14 |
| <i>Culex tarsalis</i> | | |
| (Grass infusion) | | |
| Distilled water plus grass infusion odor | 25 | 52 |
| Distilled water | 24 | 48 |
| (Log pond water) | | |
| Log pond water | 37 | 63 |
| Distilled water | 21 | 37 |
| Long pond water | 42 | 67 |
| Distilled water plus log pond odor | 22 | 33 |

Egg deposition on the distilled water media was similar.

Culex tarsalis may be present in large numbers in some log ponds during the summer season. The log pond water samples tested in this experiment were collected in the fall and winter months. The percentages of egg rafts laid on these waters and on distilled waters were 63 percent and 37 percent, respectively. More attractive odors may be produced by organic materials in these ponds during summer months.

Several other stimuli may also be expected to influence the choice of oviposition site by these species of *Culex*. O'Gower has shown that egg deposition by *Aedes aegypti* variety *queenslandis* Theobald was influenced by chemotactile, humidity, visual, olfactory, and tactile stimuli.

The effect of distillation on the attractiveness of grass infusions to ovipositing females of *C. quinquefasciatus* was studied. Three lots of grass infusions of different ages were distilled. Egg deposition by the females on the first distilled fraction of 75 cc and on distilled water was compared in this way. Similar comparative tests were made of a 75-cc heated but undistilled fraction and of a nontreated sample.

In these tests the distilled fractions appeared to be no more attractive than distilled water to ovipositing females. Slightly lower egg deposition occurred on the heated undistilled residual fraction than on an untreated infusion sample. Results of these tests are shown in Table 2.

The effect of low temperature on the volume of attractive oviposition odors released from grass infusions was also studied. A portion of a grass infusion, which had been held at room temperature for 10 days or more, was placed at 33-35° F. for 3 days. Air from above the water surface of this cold vessel was then drawn for 24 hours through a fritted glass tube placed in a tube of distilled water. A similar volume of air from the

TABLE 2.—Effect of distillation on attractiveness of grass infusion to ovipositing *Culex quinquefasciatus*. Three replications.

| Type of water | Total number of egg rafts laid |
|------------------------------------|--------------------------------|
| 1st distilled fraction | 22 |
| Distilled water | 17 |
| 2nd distilled fraction | 13 |
| Distilled water | 30 |
| Heated but undistilled fraction | 41 |
| Distilled water | 22 |
| Untreated and undistilled infusion | 57 |
| Distilled water | 13 |

vessel holding the other half of the grass infusion at 68-75° F. was simultaneously drawn through another tube of distilled water.

Water samples containing odors collected at 68-75° F. were approximately twice as attractive to gravid females of *C. quinquefasciatus* as those in which odors were collected at 33-35° F. The total number of egg rafts laid at the low and high collecting temperatures was 15 and 32, respectively, in a series of three tests.

Methane and furfural were also tested with these species. Methane, or "marsh gas," is produced by the anaerobic decomposition of cellulose and other organic matter in swamps, marshes, and sewage ponds. Furfural is produced commercially by hydrolysis of oat hulls, corncobs, or straw. The decomposition of similar organic matter in permanent or semipermanent waters might also be expected to produce this material.

In tests with the methane, the gas was first bubbled through distilled water for 6 hours. This water was placed in the mosquito cage. A water source outside of the cage through which methane was bubbled was continuously exchanged with the water in the cage by siphon to maintain the methane level. Methane had an attractive effect since more eggs were laid on methane-treated distilled water than on distilled water alone when both were tested against log pond water. In similar tests with *C. tarsalis* to compare methane-

TABLE 3.—The effect of methane and furfural on the oviposition responses of *Culex quinquefasciatus* and *Culex tarsalis*. Three replications.

| Type of water | Egg rafts laid | |
|--------------------------------------|----------------|---------|
| | Number | Percent |
| <i>Culex quinquefasciatus</i> | | |
| (Methane) | | |
| Log pond water | 59 | 67 |
| Distilled water plus methane | 30 | 33 |
| Log pond water | 76 | 87 |
| Distilled water | 11 | 13 |
| (Furfural) | | |
| Distilled water plus 25 ppm furfural | 20 | 91 |
| Distilled water | 2 | 9 |
| Distilled water plus 25 ppm furfural | 3 | 4 |
| Log pond water | 66 | 96 |
| <i>Culex tarsalis</i> | | |
| (Methane) | | |
| Distilled water | 36 | 49 |
| Distilled water plus methane | 37 | 51 |
| (Furfural) | | |
| Distilled water plus 25 ppm furfural | 35 | 70 |
| Distilled water | 14 | 29 |
| Distilled water plus 25 ppm furfural | 14 | 25 |
| Log pond water | 42 | 75 |
| Distilled water ^a | 22 | 37 |
| Log pond water | 38 | 63 |

^a Data from Table I.

treated water directly with distilled water, the methane had no attractive effect.

The most attractive strength of furfural to *C. quinquefasciatus* and to *C. tarsalis* was determined by replicated cage tests in which the females had a choice of waters containing 5, 25, or 50 p.p.m. of this chemical. For *C. quinquefasciatus* the 25-p.p.m. strength was 29 percent more attractive than the 50-p.p.m., and 100 per-

cent more attractive than the 5-p.p.m. For *C. tarsalis* the 25-p.p.m. strength was 23 percent more attractive than the 50-p.p.m., and 50 percent more attractive than the 5-p.p.m.

When tested at 25 p.p.m., furfural was found to be about 10 times as attractive as distilled water to *C. quinquefasciatus* and a little more than twice as attractive to *C. tarsalis*. In these 3-day cage tests, its attractiveness was greatly reduced after 24 hours, and fresh solutions were therefore added daily. In comparative tests of log pond water and furfural at 25 p.p.m. with *C. quinquefasciatus* and *C. tarsalis*, the furfural was far less attractive. The results of these tests are shown in Table 3.

SUMMARY. Odors from grass infusions and log pond waters collected in distilled water were attractive to gravid females of *Culex quinquefasciatus* Say. These odors were not attractive to females of *C. tarsalis* Coquillett. However, log pond water was more attractive to this species than either distilled water or distilled water plus log pond odors. Distilled water saturated with methane was also attractive to gravid females of *C. quinquefasciatus* but not to those of *C. tarsalis*. Gravid females of both species were more attracted to distilled water treated with 25 p.p.m. of furfural than to waters treated with 5 or 50 p.p.m.

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