A NEW MEDIUM FOR REARING THE MOSQUITO *PSOROPHORA CONFINNIS* [1]

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INTRODUCTION. The authors have in progress an ecological study of the temporary-water mosquito, *Psorophora confinnis,* in the Coachella Valley, California. One aspect of this study is a detailed observation of the rate of development of *P. confinnis* in nature, at prevailing summer temperatures, and an experimental study of the effect of temperature on development under controlled conditions in the laboratory.

Our first attempts to culture *P. confinnis* immediately revealed two problems. First, the media that are commonly used for rearing mosquito larvae (Trembley, 1955), prepared from dog biscuit, yeast, and/or bread crumbs, frequently form scums and become foul. This often proves fatal to the larvae, or necessitates frequent changes of the medium. Second, development in these artificial media is slower, often much slower, than in nature. This has usually not been recognized in experimental studies of the effect of temperature on development of mosquitoes (such as Huffaker, 1944; Bar-Zeev, 1958), possibly because good comparative data on the rate of development in nature are not available.

Since *P. confinnis* larvae are usually most abundant in shallow waters that temporarily flood areas grown up to grasses and forbs (Horsfall, 1955; Al-Azawi and Chew, 1959), it was decided to incorporate an homogenate of fresh grass into several different standard media. The following medium was developed, and it proved to be highly successful for *P. confinnis.* With it, there is little or no scum formation, and the rates of development approximate those in the field under similar temperature conditions. The medium has the technical advantages that it is easy to prepare in a relatively standardized manner, it requires only a short incubation period, and it does not have to be changed for developmental periods ranging from 14.3 days at 65° F. to 3.6 days at 95° F.

METHODS. Annual rye grass is seeded in flats two weeks prior to use. The grass is watered as needed, but in any case is watered 24 hours prior to use. Ten grams of freshly cut grass are homogenized in 200 ml. of water (five 30-second periods of homogenization in a semi-micro Waring blender), and the homogenate is filtered through glass wool. Forty ml. of filtrate are added to 300 ml. of tap water in a one-liter beaker; 0.1 gm. of powdered dog biscuit (black biscuits of the Fives brand) is then stirred into the medium and it is incubated for 24 hours at 80–85° F.

Embryonated eggs of *P. confinnis* are hatched in a 1:200 solution of corn broth (from cream-style canned corn) warmed to 85° F. Only those larvae that hatch within one-half hour are used. Twenty-five larvae are placed in each 340 ml. of incubated medium; this number is reduced to 15 at the start of the second instar. An additional 0.05 gm of powdered dog biscuit is added during the third instar of development. The state of development is noted at 3- or 6-hour intervals.

RESULTS AND DISCUSSION. Table 1 compares the rate of development in this grass-biscuit medium, with that in the same medium, except with the grass homogenate omitted. The 50 percent endpoint of each developmental stage is taken as the midpoint in the period prior to the observation when the majority of individuals were of the next instar.

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The obvious difference in this comparison is in the first instar, which was completed 24 percent faster in the grass-biscuit medium than in the biscuit-only medium. The larvae in the grass-biscuit medium were more robust.

In the comparison in Table 1, the only one in which accurate observations were made on a 3-hour schedule, the biscuit-only medium was unusually free from scum formation, and the rates of development and survival in this culture were unusually close to those in the grass-biscuit medium. In other grosser comparisons, development in biscuit-only media, or in yeast media, was much slower, and often failed in the first or second instar due to scum formation.

Table 2 compares the developmental rates at constant temperature in the laboratory with those at variable temperatures in the field. The laboratory rates vary from 27 percent slower at the lowest temperature to 5.5 percent faster than the field rates at the highest temperature. Variation of temperature, as occurs on a 24-hour pattern in the field, is known to increase the rate of development of numerous organisms over the rate at a constant temperature providing the same number of degree hours (see Huffaker, 1944, for example). This may well account for differences shown in Table 2 between field and laboratory rates.

Afterwards it was discovered that several other workers have utilized grass as a part of a medium for culturing mosquito larvae, although none used it as an homogenate. Roy (1931) solved his difficulty in rearing *Anopheles stephensi* by adding "ordinary grass . . . with roots attached," to his medium of "mud and other organic

Table 1.—Duration of developmental stages.

Developmental stage	Temp.	Grass homogenate-biscuit medium	Biscuit-only medium
1st larval instar	85° F.	19.5 hrs. (79%) [1]	25.5 hrs. (68%) [1]
2nd larval instar	85° F.	16.3 hrs. (86%)	15.5 hrs. (91%)
3rd larval instar	85° F.	15.9 hrs. (82%)	17.5 hrs. (97%)
4th larval instar	90° F.	22.3 hrs. (87%)	24.4 hrs. (97%)
pupal stage	95° F.	18.0 hrs. (100%)	16.8 hrs. (99%)
hatching to emergence		92.0 hrs. (3.8 days)	99.7 hrs. (4.1 days)

[1] The percentage of individuals that survived to complete that stage of development.

Table 2.—Developmental time, hatching to emergence, in the field (diel temperature fluctuation) and in the laboratory (constant temperature). Field data July–August 1963; laboratory data August 1963.

Field data [1]			Laboratory data [2]	
Temperature	Broods	Developmental time	Temperature	Developmental time
73–77° F.	1	108 hrs.	75° F.	137 hrs.
78–82° F.	2	114 hrs.	80° F.	123 hrs.
83–87° F.	7	90 hrs.	85° F.	99 hrs.
88–92° F.	1	96 hrs.	90° F.	91 hrs.
85–85–85–90–95° F. [3]	...	86 hrs.	85–85–85–90–95° F.	92 hrs.

[1] The field data are arranged according to the 5-degree class into which the mean temperature for the developmental period fell.

[2] There were 13 replicates of one brood in each laboratory experiment, at each temperature.

[3] Composite of field data for instars developing at different temperatures, for comparison with the laboratory data in Table 1.

substances" in "unfiltered water of Calcutta." The tips of the blades of grass were left projecting from the water. He observed that the grass "prevents the formation of scum." Shute (1936) was not able to raise normal *A. maculipennis* on artificial media of yeast, hay infusions, and other materials, but he did succeed with a medium containing only narrow slices of grass sod in rainwater. When this medium was kept in sunlight so that the grass continued to grow, it could be used for weeks without changing. Vollmar (1936) successfully used the same type of medium for this species.

The reasons for the value of the grass incorporated into mosquito culture media are not known. In homogenized form, grass obviously provides a variety of foods for bacteria and protozoa. The homogenate could contain bacteriostatic substances. Both grass homogenate and fresh blades could provide growth-promoting factors. For example, the stimulating effect of estrogenic substances in new-growth grass is known for certain vertebrate species.

The pH of the media in Table 1 was measured. When they were first prepared, the grass-biscuit medium had a pH of 7.9 and the biscuit-only medium pH 8.0. After one day of incubation both media were nearly neutral (pH 7.18 and 7.26 respectively); then both gradually became more basic (by the fifth day, pH 8.32 and 8.50 respectively). The grass-containing medium was consistently less basic. The significance of pH to *P. confinnis* is not known. The natural waters in which this species is abundant in the Coachella Valley vary from pH 5.5 to 8.0, but are usually between 6.4 and 7.0 (Al-Azawi and Chew, 1959).

SUMMARY. *Psorophora confinnis* larvae and pupae develop at rates approximating those in nature, in an artificial medium of an homogenate of young grass blades plus dog biscuit.

Since *Anopheles* spp. have also been reared in media incorporating grass, such media may have wider applicability.

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