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EFFECT OF TANNIC ACID CONCENTRATION ON DEVELOPMENT OF THE WESTERN TREEHOLE MOSQUITO, *Aedes sierrensis* (Diptera: Culicidae)

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Abstract—Populations of *Aedes sierrensis* (Ludlow) completed development in tannic acid solutions over a wide concentration range (i.e., 0–1.0 g/liter) in artificial microcosms exposed to field conditions. The most notable effects of high tannic acid concentration were to slow larval developmental rates and to reduce numbers of adults produced; adult size and sex ratio were minimally affected. Vector potential of the western treehole mosquito is discussed in terms of tannin concentration.

Key Words—Treehole mosquito, tannin, *Aedes sierrensis*, Diptera, Culicidae, treehole, insect development, water chemistry.

INTRODUCTION

Aedes sierrensis (Ludlow) is the dominant treehole mosquito of the Pacific coastal range and the foothills of the Sierra Nevada (Bohart and Washino, 1978; Washburn and Anderson, 1986). Adults are aggressive biters and vectors of two filarial worms (Weinman et al., 1973; Walters and Lavoipierre, 1981). Larvae hatch when desiccation-resistant eggs are inundated with treehole water following fall or winter rains, and larvae browse on treehole detritus and filter-feed on microorganisms in the water. Intraspecific competition among larvae for food is often severe (Broadie and Bradshaw, 1991; Washburn et al., 1991) and prolonged development during winter months includes a fourth-instar diapause. Pupation and adult eclosion occur in spring and early summer. After mating and blood-feeding, females lay eggs on and above receding water surfaces as treeholes dry out during the summer.

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Tannins are water-soluble, polyphenolic compounds extractable from oak foliage and bark, major constituents of treehole litter throughout the range of *Ae. sierrensis*. Tannins are structurally diverse but functionally similar in their ability to bind proteins. Tannins may protect plants from vertebrate herbivores (Austin et al., 1989; Mole et al., 1990), invertebrate herbivores (Bernays, 1981; Berenbaum, 1983), and disease (Schlosser, 1980; Walkinshaw, 1989). They may also regulate decay processes and nutrient cycling for the benefit of plants (reviewed by Tiarks et al., 1989; see Haslam, 1988 for an alternative viewpoint). Insect herbivores exploit, tolerate, or succumb to tannin-rich diets, depending upon their adaptations to dietary tannins (Steinly and Berenbaum, 1985; Martin et al., 1987; Schultz, 1989).

Although they are inconsequential to their host trees, inhabitants of water-filled treeholes must survive in varying concentrations of soluble allelochemicals. For example, tannins in treehole waters may limit rates of litter decomposition, bind dissolved proteins, affect growth rates of microorganisms, or directly affect mosquitoes themselves.

Studies suggest that tannin concentration in the larval habitat affects mosquito production. Pospisil and Broche (1981) demonstrated that a laboratory population of *Culex quinquefasciatus*, a foul-water mosquito, did not survive exposure to 0.1 g/liter tannin; development was slowed at lesser tannin concentrations relative to the control. Mitchell and Rockett (1981) found five Great Lakes region mosquito species in treehole waters with tannin–lignin concentrations up to 0.45 g/liter; four of five species were common in treeholes ranging from 45 to 450 mg/liter tannin–lignin. Bradshaw and Holzapfel (1986, 1988) demonstrated high correlations between tannin–lignin concentration (plus eight other chemical and physical properties of treehole habitats) and the distribution of mosquito species in European and Florida treeholes but did not report concentrations. Mercer (1991, 1992) reported that solutions of commercial tannic acid (a hydrolyzable tannin) and solutions of tannin purified from loblolly pine foliage (a mixture of condensed tannins) influenced survivorship of *Ae. sierrensis* populations reared under non-diapause-inducing conditions.

Here I describe a field experiment designed to test the effects of tannic acid on *Ae. sierrensis* growth and survivorship.

METHODS AND MATERIALS

Field Experiment. I measured developmental rates, survivorship, and adult size of *Ae. sierrensis* mosquitoes reared in solutions of commercial tannic acid at five concentrations plus one control. Two of the concentrations exceeded the range of total phenolic concentrations I previously measured in California treeholes (i.e., 0.01–0.5 g/liter). Rainwater, collected as trunk-wash from six oak trees during early autumn 1988, was filtered and used to prepare the concentration series: 0, 0.1, 0.25, 0.5, 1.0, and 2.0 g/liter tannic acid. Five replicates of each concentration were prepared; 300 ml of the appropriate tannic acid solution were poured into narrow-mouthed 0.5-liter plastic bottles (7 cm diameter) to simulate treeholes. One hundred newly hatched *Ae. sierrensis* larvae and 0.09 g ground rat chow were added to each microcosm, which was then covered with screening. The 30 bottles were arranged haphazardly in a large basin partially filled with water to standardize temperature fluctuations, and the basin was placed in an enclosure under an oak–madrone canopy at the Hopland Field Station (Mendocino County, California) on November 30, 1988.

Each week (except weeks 1, 9, and 26) after beginning the experiment, I emptied the contents of each replicate into an enamel pan and determined numbers and developmental stages of surviving mosquitoes. I noted but did not remove dead mosquitoes or fungal growths. Rat chow was added to each replicate when particulate matter appeared to be limiting in any replicate (0.09 g during weeks 2, 8, and 12; 0.15 g during week 14); I did not inspect the microcosms for microorganisms that serve as food for larvae. Following weekly scoring, the contents of each replicate were returned to the appropriate container and the bottles were arranged haphazardly in the basin and returned to the enclosure. No additional water was added to any microcosm during the course of the experiment.

Emergent adults were aspirated into 70% ethanol for number, gender, and size determinations. Partially eclosed mosquitoes were not included in adult counts or size determinations. Wing length was measured for each adult using a stereo dissecting microscope (30×) with a micrometer scale.

At the end of the experiment, the pH of experimental solutions, original stock solutions (kept refrigerated during the experiment), and fresh solutions of tannic acid prepared with trunk-wash rainwater were measured.

Oviposition Experiment. Gravid female *Ae. sierrensis* were allowed to choose between tannic acid solution or distilled water in a laboratory oviposition choice test. Ten to 15 inseminated and blood-fed females plus 20–25 males were kept in gallon-sized cardboard cylinders and provided with sugar and water. Three cylinders were placed in a ventilated, darkened enclosure. A plastic cup lined with a paper towel wick and containing 15 ml 2.0 g/liter tannic acid solution and an identical cup containing distilled water were placed in each cylinder. Cups with fresh solutions were exchanged and the number of eggs oviposited in each solution determined daily. At the end of the experiment, equal numbers of eggs from each treatment were immersed in 0.1% sodium sulfite solution to stimulate hatching; egg hatch success was determined by counting the number of larvae for each treatment.

Statistical Treatment. Significant differences in survivorship were determined with an epidemiological cohort life-table analysis (Dawson-Saunders and

Trapp, 1990). By using this analysis, I sacrificed variation due to replication and calculated variance instead as a cumulative conditional probability of survival for each treatment. Numbers of surviving mosquitoes were pooled for the five replicates of each treatment; 95% confidence intervals were calculated at intervals for each treatment throughout development.

Developmental rates, proportion of females, and mean male and female wing lengths were determined for each replicate and used to calculate treatment means for each tannic acid concentration. One-way ANOVA was used to determine differences among treatment means if normally distributed or among log-transformed means if treatment variances were unequal (i.e., female wing length) (Sokal and Rohlf, 1981). Multiple comparisons among pairs of means or log-transformed treatment means were done with the T-method (Sokal and Rohlf, 1981). A *t* test for paired comparisons was used to compare oviposition in tannic acid and distilled water (Sokal and Rohlf, 1981).

RESULTS

Field Experiment. Mosquitoes in high tannic acid concentration treatments experienced high initial mortality (Figure 1). All larvae exposed to 2.0 g/liter tannic acid died before the first assessment. Survivors among larval populations exposed to 0.5 and 1.0 g/liter tannic acid solutions were significantly fewer than populations exposed to more dilute solutions by week 2. The number of survivors in the 0.1 g/liter treatment was significantly higher than in the control by week 13 and remained higher for the remainder of the experiment. Survivorship was highest for larvae exposed to 0.25 g/liter tannic acid.

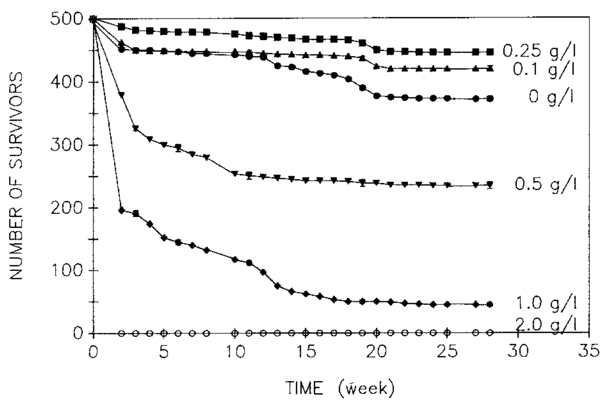

FIG. 1. Survival curves for *Aedes sierrensis* populations developing in six treatments of tannic acid under field conditions. Error bars indicate 95% confidence intervals; treatment error bars that overlap are not significantly different.

Adult eclosion began during week 18 and was completed by week 28 (June 1989). The number of adults produced and the cumulative conditional probability for each treatment are represented, respectively, by end points and error bars of the survival curves in Figure 1. Significantly more adults eclosed from two intermediate tannic acid treatments (0.25 and 0.1 g/liter) than the tannin-free control or the treatments of higher tannic acid concentrations (Figure 1).

Excess food accumulated in the 0.5 and 1.0 g/liter treatments, and fungal clumps appeared within two weeks in the 1.0 g/liter treatment. All populations were minimally affected by freezing of experimental solutions between weeks 8 and 10. Solution volume decreased less than 5% for any microcosm; solutions with tannic acid became increasingly dark through time.

Generally, larval populations developed more slowly as tannic acid concentration increased. Means of median developmental times (i.e., time required for half the surviving population of a replicate to reach a developmental stage) are listed in Table 1 for each tannic acid concentration. Mosquitoes developing in the 0 and 0.1 g/liter tannic acid treatments reached the second and third instars earlier than those in other treatments; likewise, larvae developing in 0.5 or 1.0 g/liter tannic acid reached the third and fourth larval instars significantly more slowly than in other treatments. However, with the exception of the 1.0 g/liter treatment, mosquitoes pupated and emerged as adults at the same time regardless of tannic acid concentration.

Tannic acid concentration had no effect upon the sex ratio of adults produced from experimental populations and had only limited influence upon adult size. Slightly more than half the 1518 emergent adults were female (overall females = 51.3%; range among treatment means = 46.5–59.9% females). The mean proportion of females did not vary significantly among tannic acid treatments ($F_{4,20} = 1.974$, $P = 0.137$). Mean male wing lengths differed significantly among tannic acid treatments ($F_{4,20} = 33.62$, $P < 0.001$), while mean

TABLE 1. MEDIAN DEVELOPMENTAL TIME (MEAN TIME IN WEEKS) FOR MOSQUITO POPULATIONS IN TANNIC ACID SOLUTIONS[a]

Concentration (g/liter)	Second instar	Third instar	Fourth instar	Pupa	Adult
0	2.0a	4.0d	10.4h	19.0k	20.0m
0.1	2.0a	4.8de	10.0h	19.0k	20.0m
0.25	3.0b	6.2e	11.0h	19.0k	20.0m
0.5	3.2b	9.2f	13.6i	19.0k	20.0m
1.0	4.6c	15.0g	18.6j	21.4l	23.0n

[a]Values followed by the same letters within a column are not significantly different (T-method of multiple comparisons; Sokal and Rohlf, 1981).

female wing lengths did not ($F_{4,20}$ = 1.23, P = 0.33). The smallest males eclosed from the 1.0 g/liter treatment. The relationships between tannic acid concentration and mean male and female wing lengths are illustrated in Figure 2A and B.

The pH values of the experimental tannic acid solutions changed through time. At the end of the experiment, experimental solutions were less acidic than either the original stock solutions or freshly prepared tannic acid solutions. The exception was the experimental 2.0 g/liter treatment, which was removed from the field at week 2 and kept refrigerated until the end of the experiment.

Oviposition Experiment. In three tests, gravid *Ae. sierrensis* females oviposited similarly in 2.0 g/liter tannic acid solution (443 eggs) and distilled water (626 eggs) (t test: t_2 = 0.983, NS). Equal proportions of eggs hatched following collection from the two solutions: 78.9 and 78.5% for the tannic acid and distilled water treatments, respectively.

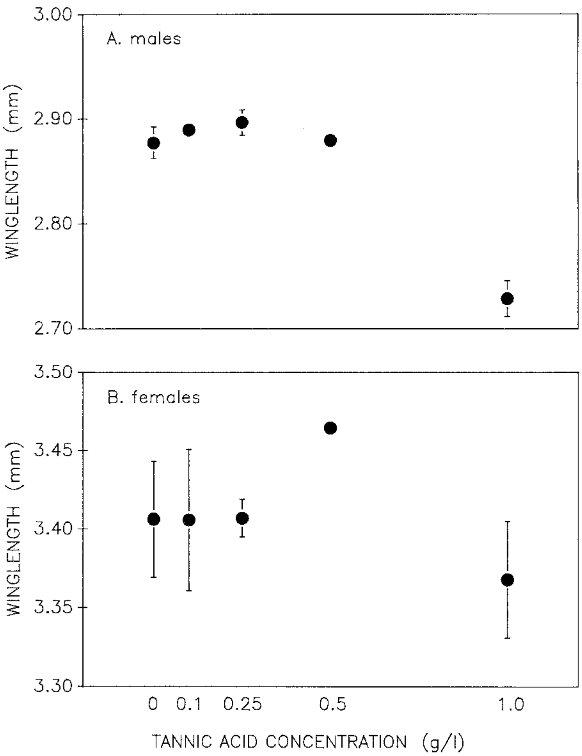

FIG. 2. Mean wing length (± 1 SEM, in mm, N = 5) of (A) male and (B) female *Aedes sierrensis* eclosing from solutions of tannic acid.

DISCUSSION

The contribution of each treehole habitat to the biting population of mosquitoes depends upon the number, size, and proportion of females among emergent adults. For many natural populations of mosquitoes, female size is correlated with fecundity and survival, and thereby the opportunity for disease transmission (Hawley, 1985a,b). In this experiment, tannic acid concentration affected numbers of adults produced but neither the proportion nor the size of female mosquitoes. Numbers of adults produced may have been influenced by rates of larval development and intraspecific competition.

Control populations gained no advantage by hastening through larval molts relative to the populations at intermediate concentrations. The end of fourth-instar larval diapause is triggered by a critical photoperiod coincident with warmer treehole water (Jordan and Bradshaw, 1978). Thus all populations that had developed sufficiently produced adults once these external conditions were met. Faster developmental rates in control populations simply meant more time spent in larval diapause.

Tannic acid did not appear to inhibit all decay-causing organisms; fungus accumulated on excess food in the 0.5 and 1.0 g/liter treatments. Food bound by tannins may have been of lower quality for larvae in these treatments. Tannin binding was indicated by the dark tan color of food in these treatments for which mosquito survivorship was low (0.5 and 1.0 g/liter).

Exposure to 1.0 g/liter tannic acid had a pronounced effect on mosquito development; pupation and adult eclosion were significantly later in this treatment than in lower tannin treatments. Slow development caused by tannins may be important in natural treeholes, which may dry before adults are produced. *Aedes sierrensis* are protandrous, and in this experiment males eclosed before females in all treatments (data not shown), although approximately equal numbers of males and females were produced. Delays in eclosion may be reflected in male bias for a given treehole that dries before female eclosion (Washburn et al., 1989). High tannin concentrations may contribute to such delays.

Some early larval mortality may have been due to acidity at high tannic acid concentration. However, most of the initial values fell within the range determined for 81 natural treeholes inhabited by *Ae. sierrensis* (pH range: 3.9–9.4; Washburn and Anderson, 1986). By the end of the experiment, experimental solutions had become less acidic; the greatest pH changes were for the 0.5 and 1.0 g/liter solutions, which had the highest mosquito mortality and the greatest accumulation of food residue and fungus.

Ovipositing *Ae. sierrensis* females did not discriminate against tannic acid solutions sufficiently concentrated (i.e., 2.0 g/liter) to cause complete mortality among their progeny. However, eggs laid in these solutions experienced no loss in viability (e.g., due to tannic acid binding with egg proteins). Gravid *Ae.*

sierrensis females may be unable to predict the quality of treehole habitats during the following developmental season (Hawley, 1985b); through bet-hedging, they may ensure the survival of their progeny by dispersing many, small eggs in different treeholes.

Treehole mosquitoes and other inhabitants must tolerate the variety of tannins in treehole waters and their varying concentrations during the season. Under winter conditions, *Ae. sierrensis* may benefit from intermediate tannin concentrations, thus maximizing adult production. Following infrequent summer rains when opportunistic cohorts of *Ae. sierrensis* develop in warmer, more productive treehole waters without a fourth-instar larval diapause, lower tannin concentrations likewise may allow optimal adult production. In either case, tannin concentration may be an important factor in the high variability in mosquito production from treehole breeding habitats.

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