# CORRELATION OF CHEMICAL CONSTITUTION AND PHYSICAL PROPERTIES OF FATTY ACID ESTERS WITH OVIPOSITION RESPONSE OF *AEDES AEGYPTI*[1]

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The physical and chemical factors underlying the attraction of *Aedes aegypti* and other mosquito species to oviposition sites have been the subject of numerous investigations.

Among the physical factors studied, the selection of an oviposition site by gravid females has been found to be influenced by the color of breeding water (Williams and DeLong, 1961; Field and Takaji, 1965) or more precisely by the degree of light reflection from the water surface (Jobling, 1935; Kennedy, 1942; O'Gower, 1957).

Other environmental stimuli affecting egg deposition by several aedine species include texture of breeding container surfaces and, especially, the preference of a moist porous surface over a free water surface (O'Gower, 1955, 1957, 1963).

Odors released by various waters as well as chemicals added to larval breeding foci are known to influence the selection of oviposition sites (Wallis, 1954; Gjullin and Johnsen, 1965; Gjullin *et al.*, 1965). In nature, degree of water or soil salinity and pH (Woodhill, 1941; Chapman, 1960; Kliewer *et al.*, 1964; Knight, 1965; Petersen and Rees, 1966), extent of organic pollution (de Zulueta, 1950; Manefield, 1951), variations in water and soil temperatures and in organic constituents of breeding waters (Bast, 1963) play important roles in establishing habitat preferences.

A concerted effort to integrate the various correlative factors into a single study with one species led O'Gower (1963) to investigate the influence of humidity, visual, tactile, chemotactile, and olfactory stimuli, acting simultaneously, on the oviposition behavior of *Aedes aegypti* var. *queenslandis*.

The recently initiated campaign to eradicate *Aedes aegypti* from continental U.S.A., Puerto Rico, and the Virgin Islands (Schliessmann, 1964) has necessitated a search for more rapid and sensitive surveillance methods for detecting low population densities of the vector in suspected areas.

A simple and effective detection method based on oviposition preferences of female *Aedes aegypti* has been described by Fay and Perry (1965). In agreement with other investigators, the factors found to exert the most influence on the selection of oviposition sites include texture, color, and shape of the artificial container, as well as the odor and taste of its contents.

The present investigation, an extension of the above work, deals specifically with an attempt to correlate the chemical structure and physical properties of homologous series of certain fatty acid esters with the attraction of *Aedes aegypti* females to preferred oviposition sites.

MATERIALS AND METHODS. All the chemicals tested were commercially available compounds of ACS grade or better. On two occasions when technical grade chemicals were used for comparison, they were so designated.

Homologous series of acetic, propionic, and butyric acid esters were tested as dilute solutions in water or as the undiluted chemicals in small shell vials suspended on the inside wall of the container. The

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water insoluble compounds were emulsified by the addition of 10 mg. of sodium lauryl sulfate per liter of water.

Oviposition response was measured by comparing the number of eggs laid in 2 jars containing the test chemical with those laid in 2 control jars containing distilled water only. At least 8 replications were made with each test and the results were computed as averages ± standard deviation. The total number of eggs per replicate ranged from 500 to 2000. Where fewer than 500 eggs were laid the test was rerun. Thus,

$$\text{Oviposition response} = \frac{\text{No. of eggs in test chemical}}{\text{Total no. of eggs in chemical} + \text{control}} \times 100.$$

It should be emphasized that the containers used as controls consisted of the same attractive qualities as the test jar in terms of size, color, texture and oviposition surface. Hence, the net response must be due to the effect of the chemical alone. Details of the method of exposure and other pertinent information have been given in an earlier paper (Fay and Perry, 1965).

Results and Discussion. Selection of the test chemicals was based primarily on their occurrence in nature or on their suspected presence in preferred mosquito habitats as by-products of organic decomposition.

The relative oviposition response elicited by compounds tested as 0.1 percent aqueous solutions is shown in Table 1. Data on the physical properties of these compounds were taken from standard reference handbooks. Based on these results, the best oviposition attractants were found to be the lower members of homologous series of acetic, propionic, and butyric acid esters. Of practical significance are ethyl acetate, methyl propionate, and methyl butyrate (methyl acetate was not included in the tests because of its high vapor pressure). In addition to the compounds mentioned in Table 1 the following esters tested as 0.1 percent suspensions elicited less than 20 percent oviposition response: methyl esters of octanoic, decanoic, undecylenic, lauric, linoleic, benzoic and anisic acids.

Since the above tests did not clearly indicate that the attraction was strictly the result of olfactory stimulation, the same compounds were tested undiluted as vapors. These results (Table 1) showed a trend similar to that obtained with the 0.1 percent aqueous solutions but there was less discrimination between the lower and higher members of the propionate and butyrate homologous series. Thus, aqueous solutions of the fatty acid esters containing butyl and amyl moieties elicited a lower response than the same chemicals used as vapors. It is possible that greater discrimination was due to stimulation of tactile chemoreceptors in addition to olfactory receptors, but it is more likely that acuity of response was the result of a higher molecular concentration of the chemical in the air in the vicinity of the oviposition container.

Boiling Point. The data shown in Table 1 relating the physical properties of the chemicals with the biological response of the mosquito were plotted graphically. Figure 1 shows the relationship between boiling point and oviposition response. If a line is drawn across the graphs to intersect the 50 percent oviposition response it becomes clear that high activity correlates favorably with lower boiling point (between 75° and 125° C.). It is apparent, however, that this relationship is not a continuous one and that there is a sharp break in the curve at a certain boiling point which is characteristic for each homologous series.

Water Solubility. Odors must penetrate the mucous layer of the olfactory epithelium and the lipoid covering of the olfactory end organ as well (Dethier, 1947). Hence, an effective olfactory stimulant must have both water solubility and lipid solubility. A plot of water solubility of the homologous series against oviposition response (Fig. 2) shows a sharp initial rise in activity followed by a much shallower slope as water solubility increases. Again, the fatty acid esters containing

Table 1.—Relationship between certain physical properties of homologous series of acetic, propionic and butyric acid esters and the oviposition response of *Aedes aegypti*.

| Compound | Molecular weight | Boiling point (°C.) | Vapor pressure at 25° C. (mm) | Solubility in $H_2O$ (gm./100 ml.) | % Oviposition response ± std. dev.* | |
|---|---|---|---|---|---|---|
| | | | | | 0.1% Solutions | Vapors |
| Ethyl acetate | 88.1 | 77.1 | 92 | 8.60 | 62.5±6.7 | 62.8±8.2 |
| Isopropyl acetate | 102.1 | 89.0 | 64 | 3.09 | 63.5±8.9 | 53.1±10.1 |
| Propyl acetate | 102.1 | 101.6 | 34 | 1.89 | 59.1±4.3 | 53.3±11.5 |
| Butyl acetate | 116.1 | 112.5 | 22 | 0.50 | 52.9±9.2 | 52.1±17.2 |
| Amyl acetate | 130.1 | 148.0 | 7.2 | 0.18 | 49.0±2.1 | 49.3±10.9 |
| Methyl propionate | 88.1 | 79.9 | 84 | 6.50 | 67.1±7.7 | 55.0±11.0 |
| Ethyl propionate | 102.1 | 99.1 | 37 | 2.40 | 62.4±15.1 | 64.4±12.2 |
| Propyl propionate | 116.1 | 123.4 | 14 | 0.50 | 60.1±8.2 | 54.0±2.1 |
| Butyl propionate | 130.1 | 145.4 | 7.8 | 0.10 | 48.8±10.4 | 52.3±15.5 |
| Amyl propionate | 144.1 | 165.0 | 3.6 | 0.03 | 21.1±10.3 | 50.0±8.1 |
| Methyl butyrate | 102.1 | 102.3 | 32 | 1.56 | 66.2±7.6 | 75.3±3.9 |
| Ethyl butyrate | 116.1 | 121.3 | 18 | 0.68 | 63.3±8.6 | 55.6±7.3 |
| Isopropyl butyrate | 130.1 | 143.0 | 7.2 | 0.16 | 59.8±13.5 | 40.1±7.7 |
| Butyl butyrate | 144.1 | 166.4 | 4.0 | 0.08 | 36.5±12.1 | 51.5±7.4 |
| Amyl butyrate | 158.1 | 185.0 | 1.3 | 0.05 | 31.3±19.1 | 44.8±16.3 |
| Sodium acetate ($3H_2O$) | 136.1 | ..... | .... | 125 | 49.2±2.9 | .. |
| Sodium propionate | 96.1 | ..... | .... | 100 | 46.8±3.6 | .. |
| Sodium butyrate | 110.1 | ..... | .... | >50 | 45.0±7.5 | .. |

* Values based on 8 replicates, 500–2,000 eggs per replicate.

butyl and amyl moieties and having the lowest water solubility also were the least attractive. All these compounds, however, have a high lipid solubility. When the sodium salts of acetic, propionic and butyric acid were substituted for their respective alkyl esters (cf Table 1) the activity dropped markedly. The sodium salts have high water solubility but very low lipid solubility. It is evident that some water solubility is essential for stimulation of olfactory receptors but high lipid solubility is more important since the molecules must first penetrate the lipid barrier before reaching the mucous layer. Undoubtedly, oil-water partition coefficients are more accurate measures of activity than water solubility alone, but these were not determined.

EFFECT OF CONCENTRATION. Three oviposition attractants, i.e., ethyl acetate, methyl propionate, and methyl butyrate, were used to study the effect of concentration on oviposition response. The results plotted in Figure 3 show that as the concentration of the chemical in water exceeds that necessary to stimulate olfactory chemoreceptors, oviposition response increases. A further increase in concentration results in a reversal of re-

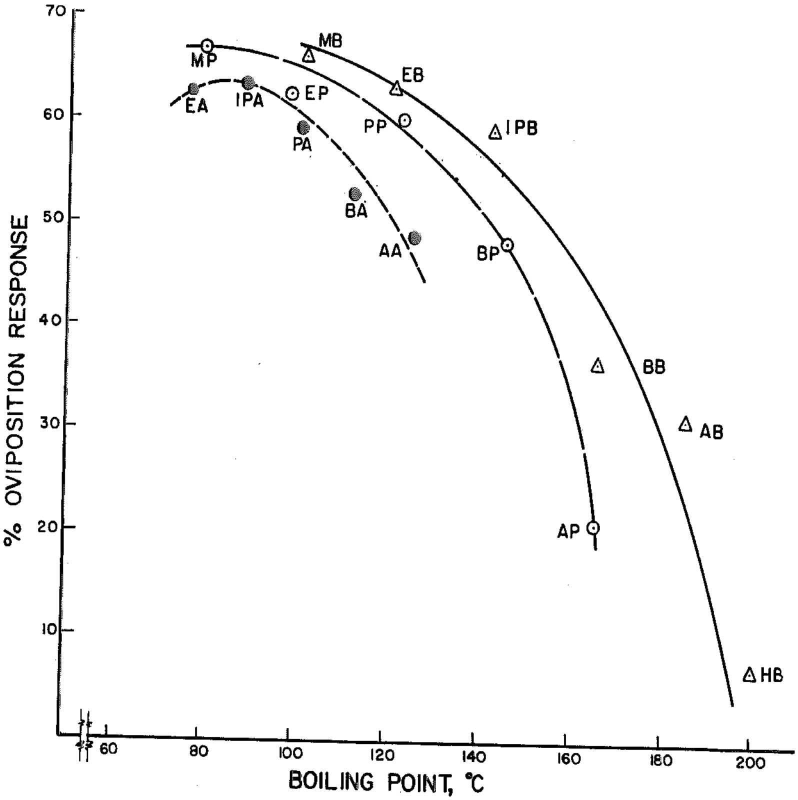

FIG. 1.—Relationship between boiling point of esters of acetic, propionic, and butyric acids and the oviposition response of *Aedes aegypti*.

*Abbreviations:* Prefixes: E=ethyl; IP=isopropyl; P=propyl; B=butyl; A=amyl; H=hexyl: Suffixes: A=acetate; P=propionate; B=butyrate. Thus, EA=ethyl acetate; BP=butyl propionate, etc.

sponse as indicated by lower oviposition values. Although discriminating concentrations were not used in these tests, it appears that there definitely is a critical concentration for ethyl acetate, methyl propionate, and methyl butyrate at which the acceptance threshold changes into a rejection threshold. This is characteristic of most olfactory attractants reported in the literature.

EFFECT OF BILATERAL ANTENNECTOMY. The experiments of Roth (1951) and Rahm (1958) with *Aedes aegypti* had pointed out that the antennae are the sites of the sensory organs responsible for the attraction of females to their hosts. Investigations with other insects have also established the presence of olfactory sensillae on the antennae (see Dethier, 1947, 1963 for details and references). It was of interest to us to supplement our data with observations on the oviposition preferences of antennectomized females.

*Aedes aegypti* females were removed from the colony cage in groups of 10 each and were lightly anesthetized with carbon dioxide. Both antennae were amputated with fine surgical micro dissecting scissors under a binocular microscope. Eleven flagellar segments were removed from each antenna leaving the scape, pedicel, and stumps of the first two flagellar segments. According to Roth (1948, 1951) continuous $CO_2$ anesthesia for as long as 60 minutes has no effect on the feeding behavior and recovery of *Aedes aegypti* females. The time required for the operation was 4 to 5 minutes per 10 insects and the process was repeated until the antennae of 100 females had been amputated. The antennaeless insects were allowed to recover for at least 1 hour before transfer to the experimental cage.

The controls consisted of: (a) 100 females treated with $CO_2$ in the same

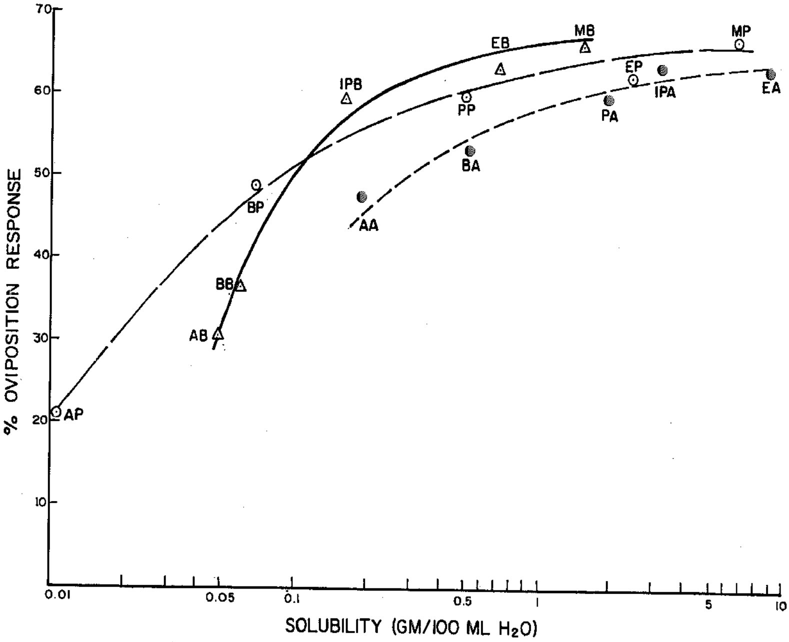

FIG. 2—Relationship between water solubility of esters of acetic, propionic, and butyric acids and the oviposition response of *Aedes aegypti*. For explanation of abbreviations see Fig. 1.

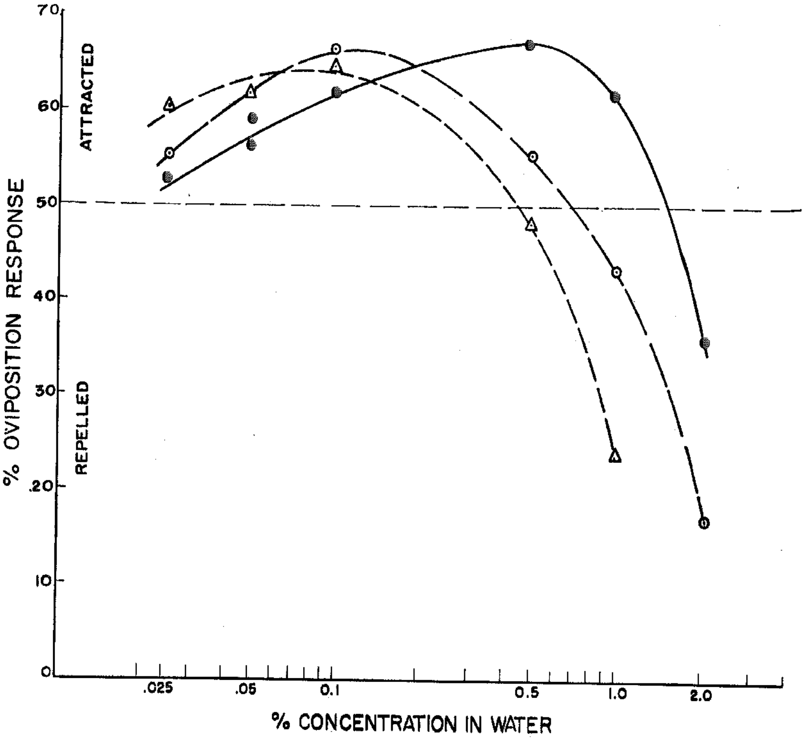

Fig. 3.—Effect of concentration of ethyl acetate, methyl propionate, and methyl butyrate on the oviposition response of *Aedes aegypti*. —●—●—●— ethyl acetate; —⊙—⊙—⊙— methyl propionate; —△—△—△— methyl butyrate.

manner but with intact antennae, and (b) 100 untreated females (no $CO_2$ anesthesia) with intact antennae. The results are shown in Table 2. Under the experimental conditions, $CO_2$ anesthesia had no deleterious effect on the recovery and subsequent oviposition activity of the mosquito. Antennectomy seems to have caused a definite reduction in oviposition in the test chemicals but a statistical analysis of the data in Table 2, using arc sine transformation of percentage values, revealed little or no significant difference in oviposition response between antennectomy, $CO_2$ anesthesia only, and no treatment. The reason for this lack of a clear-cut difference is indicated in the highly variable results obtained with antennectomized females. High variability, in this case, is tantamount to saying lack of positive response. We, therefore, conclude that antennectomy caused a definite shift from positive olfactory response to lack of chemical orientation.

A conspicuous feature of these results concerns the number of eggs laid on free water compared with eggs laid on the oviposition surface provided. As shown in Table 2, the control mosquitoes deposited 1–10 percent of the total number of eggs on free water whereas the antennectomized females laid 43, 25, 40, 26, 29 and 5 percent of the eggs (in individual tests) on free water. The reason for this anomaly is not clearly understood. However, since the vapor concen-

TABLE 2.—Effect of bilateral antennectomy and $CO_2$ anesthesia on oviposition response of *Aedes aegypti*.

| Test chemical | 0.1% Aqueous solutions | | | Vapors | | |
|---|---|---|---|---|---|---|
| | Mosquito treatment | % Eggs on oviposition surface[a] | % Eggs on free water | Mosquito treatment | % Eggs on oviposition surface[a] | % Eggs on free water |
| Ethyl acetate | None | 61[c] | 3 | None | 62[c] | 5 |
| | $CO_2$ | 58[b] | 1 | $CO_2$ | 57[c] | 10 |
| | $CO_2$ + antennectomy | 49 | 5 | $CO_2$ + antennectomy | 43 | 29 |
| Methyl propionate | None | 62[c] | 6 | None | 66[c] | 9 |
| | $CO_2$ | 65[c] | 2 | $CO_2$ | 56[b] | 2 |
| | $CO_2$ + antennectomy | 56 | 26 | $CO_2$ + antennectomy | 55 | 40 |
| Methyl butyrate | None | 66[c] | 8 | None | 57 | 5 |
| | $CO_2$ | 64[b] | 6 | $CO_2$ | 68[b] | 9 |
| | $CO_2$ + antennectomy | 58 | 25 | $CO_2$ + antennectomy | 57 | 43 |

[a] indicates no significant differences between treatments of adult mosquitoes.

[b] indicates significant difference ($P = .05$) between test chemical and water only.

[c] indicates highly significant difference ($P = < .05$) between test chemical and water only. Statistical analysis was made by arc sine transformation of percentages.

tration of the chemical is highest at the water-air interface, the antennaeless insects, devoid of most olfactory chemoreceptors and other sensory receptors needed for orientation, must rely on a higher concentration of the chemical to elicit response. Since the probability of a response can be increased by increasing either the vapor concentration or the number of receptors, another explanation for the increased egg laying on free water might be that loss of hygroreceptors due to antennectomy (Roth and Willis, 1952; Bar Zeev, 1960) forces the insect to seek a higher moisture gradient for oviposition. The effect of antennectomy on threshold concentration has been studied in detail in other insects (see Dethier, 1963).

CONCLUSIONS. This study indicates that, under the present experimental conditions, the choice of oviposition sites by *Aedes aegypti* is mediated primarily by stimulation of olfactory chemoreceptors. The stimulative efficiency of acetic, propionic, and butyric acid esters correlates favorably with their chemical constitution. A combination of physical properties such as boiling point, water solubility and molar concentration determines the availability of the chemical in the atmosphere and thus influences the orientation response of *Aedes aegypti*.

SUMMARY. Homologous series of acetic, propionic, and butyric acid esters were investigated as oviposition attractants for *Aedes aegypti*. Of practical significance in these tests are ethyl acetate, methyl propionate, and methyl butyrate. Discrimination between members of the homologous series was more acute when the chemicals were used in solution than when used as vapors. The oviposition response of antennectomized females was considerably more variable than those with intact antennae, indicating loss of olfactory orientation to chemicals.

Orientation response in terms of choice of oviposition site correlates favorably with the chemical constitution of the fatty acid esters in the order butyrate > propionate > acetate. Physical properties such as boiling point and water solubility are also related to the effectiveness of the esters as attractants since these properties determine their concentration in the atmosphere at the oviposition site.

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# *SYMPHOROMYIA* BITING IN NEW YORK STATE (DIPTERA, RHAGIONIDAE)

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In western North America, biting snipe flies of the genus *Symphoromyia* may be troublesome in their attacks on man. Accounts of such attacks are well documented. Osten Sacken (1877) seems to have been the first to report such attacks which he attributed to some undescribed species in California. Aldrich (1915) records *Symphoromyia* attacking stage horses and passengers in Mount Ranier National Park in 1905. He also quotes T. D. A. Cockerell on attacks in Colorado and reports being bitten himself near Moscow, Idaho in 1913.

Other selected references to *Symphoromyia* biting humans, usually in sufficient numbers to be troublesome, include Knab and Cooley (1912) and Mills (1943) in Montana; Stanford (1931) and Knowlton and Maddock (1944) in Utah; Hearle (1929) and Ross (1940) in British Columbia; Shemanchuk and Weintraub (1961) in Alberta; Travis (1949), Sailer (1951), Frohne and Williams (1951) and Frohne (1959) in southern Alaska.

Most of the above workers tried to identify the species involved. Unfortunately the systematics of *Symphoromyia* are currently in such an unsettled state that it does not appear wise to help perpetuate the names used by repeating them. Certainly, however, more than one species was involved.

There appear to be no published records of *Symphoromyia* attacking man in eastern North America. There also appear to be no records of eastern *Symphoromyia* reaching the high population levels re-