OVIPOSITION DETERRENTS FOR *AEDES AEGYPTI*[1] IN EXTRACTS OF *LEMNA* MINOR[2]

G. J. R. JUDD AND J. H. BORDEN
Pestology Centre
Department of Biological Sciences
Simon Fraser University
Burnaby, B.C., Canada
V5A 1S6

ABSTRACT

Dual choice bioassays demonstrated that water and methanolic extracts of *Lemna minor* L., in aqueous solution, deterred oviposition by *Aedes aegypti* L. The methanolic extracts were active at concentrations of 1000 and 10,000 PPM but were inactive at 1, 10, and 100 PPM. Pentane extracts and *L. minor* culture water showed no activity. None of the extracts bioassayed were biologically active against *Culex pipiens* L. Experiments in which physical contact with the extracts was prevented, provide evidence that a volatile chemical emanating from the extract is responsible for the deterrency.

INTRODUCTION

Investigations into the possible insecticidal properties of plants have disclosed that certain aquatic plants are detrimental to the development of mosquito larvae, while others can apparently prevent oviposition (Matheson and Hinman 1929; Matheson 1930; Furlow and Hays 1972; Angerilli 1977). Among the latter are several species of Lemnaceae, the duckweeks. In field experiments almost no volunteer egg rafts or larvae occurred in artificial ponds containing *Lemna minor* L., and in the laboratory, extracts of *L. minor* deterred oviposition by *Aedes aegypti* L. (Angerilli 1977).

Our objectives were: to confirm the oviposition deterrent activity of *L. minor* under laboratory conditions to *A. aegypti* and *Culex pipiens* L., to measure the sensitivity of any biologically active fractions, and to determine if the activity is the result of volatile or non volatile chemicals.

METHODS AND MATERIALS

Extraction of Plant Material

Samples of *L. minor* were collected from a drainage ditch in Cloverdale, B.C., thoroughly washed in tap water and cultured in laboratory aquaria filled with distilled water.

Water extracts were prepared by grinding 1 g (wet wt.) samples of the plant in an electric blender and suspending the ground material in 100 ml of distilled water. This solution was filtered once through a Whatman No. 1 filter paper disk (4.5 cm dia.) in a Buchner funnel to remove any unsoluble material. Culture water was taken from the aquaria and filtered as above for use in bioassays.

For the organic solvent extractions, plants were ground in the electric blender, frozen at -60 C and freeze dried. Plant powder in 10 g (dry wt.) quantities, was extracted with 300 ml of pentane followed by extraction with 300 ml of methanol for 24 h/solvent in a Soxhlet apparatus (reagent grade solvents, Fisher Scientific Co.). Solvents were removed from the extracts using a rotary evaporator. Methanolic extract residues were weighed and dissolved in distilled water to produce a stock solution of 1 g extract/100 ml water or 10,000 PPM of crude extract. A portion of this solution was serially diluted with distilled water producing concentrations of 1, 10, 100 and 1000 PPM. Pentane extracts were weighed and dissolved in 10 ml of a 1% (by wt.) Tween 80 emulsifying solution (Fisher Scientific Co.), and then diluted with distilled water to 10,000 PPM crude extract. These solutions were filtered as before and all were stored in stoppered vials at 4 C until needed for bioassays.

Bioassay Procedures

A. aegypti and *C. pipiens* were obtained from colonies maintained at Simon Fraser University and reared by the methods of Gerberg (1970), Gillespie and Belton (1980) and McLintock (1960) since 1966 and 1974, respectively. Bioassays with *A. aegypti* were performed in 15 x 15 x 17 cm cages, with four wooden sides, screen rears and Plexiglas fronts; *C. pipiens* were bioassayed in 25 x 25 x 45 cm wooden frame screen cages with Plexiglas fronts because they will not oviposit when kept in cages of less than 28,000 cm^3 (Gerberg 1970). Mosquitoes in each bioassay were reared from the same batch of eggs. At approximately 10 days of age, adult mosquitoes were blood fed on a caged guinea pig and then released into test cages. A 10% sucrose solution in a 25 ml Erlenmeyer flask stoppered with a dental

[1]Diptera: Culicidae.
[2]Arales: Lemnaceae.
[3]Research supported by the British Columbia Ministry of Agriculture.

TABLE I. Effects of extracts and culture water of ***Lemna minor*** on oviposition rates in ***Aedes aegypti*** and ***Culex pipiens.***

Exper. No.	No. of Replicates	No. of Insects / Replicate	Treatment Choices	Extract Concentration (PPM)	$\bar{X}$ No. of Eggs *A. aegypti*[a]	$\bar{X}$ No. of Egg Rafts *C. pipiens*[a]
1	10	5	Culture water	--	106.0 ns	.6 ns
			Control		153.4	.8
2	10	10	Water Ext.	10,000	39.5***	1.5 ns
			Control		574.4	.2
3	5	5	Pentane Ext.	10,000	8.0 ns	---
			Control		50.4	
4	10	5	Methanolic Ext.	10,000	2.5***	.4 ns
			Control		136.6	1.5
5	5	5	Methanolic Ext.	10,000	.6***	---
			Control		119.4	
	5	5	Methanolic Ext.	1,000	26.2**	---
			Control		149.8	
	5	5	Methanolic Ext.	100	60.4 ns	---
			Control		54.2	
	5	5	Methanolic Ext.	10	66.6 ns	---
			Control		92.8	
	5	5	Methanolic Ext.	1	62.2 ns	---
			Control		121.8	

[a]Significant difference (t-Test) between paired experimental and control means indicated by: *** = $P < 0.001$; ** = $P < 0.01$; ns = no significant difference.

cotton wick provided a food source throughout the bioassays. Insects were held in test cages for 4-5 days preceding experiments to assure their ovipositional readiness. All tests were carried out in an environmental chamber at 23 C, 30-40% RH, and a 16:8 h light:dark photoperiodic regime. The cages were evenly spaced on a 1 x 2.3 m shelf, 60 cm below 3 banks of fluorescent lights.

Test solutions were bioassayed for possible oviposition deterrency in a dual choice system. Distilled water served as a control in all tests. Oviposition containers consisted of 50 ml pyrex beakers lined with paper towelling and wrapped in black vinyl tape. The 2 containers were positioned in the centre of each cage 10 cm apart, and their positions were randomized in each replicate cage. Tests were concluded after 48 h and the mean number of eggs or egg rafts per treatment were calculated.

Experiments

Three sets of experiments were performed. The first series tested the oviposition deterrent activity of water, methanol, and pentane extracts of *L. minor*, as well as *L. minor* culture water to both *A. aegypti* and *C. pipiens*.

To determine the sensitivity of the methanolic extract, solutions containing 1, 10, 100, 1000 and 10,000 PPM of the crude extract were bioassayed with *A. aegypti*.

To investigate the role of olfaction in oviposition deterrency, methanolic extracts, in 20 ml aliquots, were presented to test insects in 3 types of oviposition containers, each exposing the extract to different degrees. The first was a completely exposed, open beaker as described above. The second was identical with the addition of a gauze barrier placed 1 cm above the extract surface. In the third, extracts were placed in 25 ml gauze covered vials, then set singly in the centres of the open type oviposition containers. The outer beakers were filled with distilled water to the same height as in the absence of the inner vial. Control beakers with distilled water were treated in the same way, for the respective treatments.

RESULTS AND DISCUSSION

A water extract of *L. minor* significantly deterred oviposition by *A. aegypti*, but was ineffective against *C. pipiens* (Table 1). The culture water was biologically inactive against either mosquito species (Table 1). These results indicate that while the active fraction is water soluble, it is either absent from the culture water, or is at concentations too low to be effective. More time for a culture to develop, or physical damage to the plants may be necessary to make water inhabited by *L. minor* unsuitable for oviposition in the field as found by Angerilli (1977).

In Experiments 3-4 (Table 1) oviposition by *A. aegypti* was deterred by the methanolic extract but the pentane extract showed no significant activity. The activity of the water and methanolic extracts demonstrate the polar nature of the active principle(s). The methanolic extract was inactive against *C. pipiens*. Variability between replicates contributed to the non significant results obtained for *C. pipiens*. A treatment effect for the methanolic extract was significant at concentrations of 1000 and 10,000 PPM (Table 1, Exp. 5). The rate of oviposition was negatively correlated with increasing extract concentration ($r = -0.899$, $P < 0.05$).

If the deterrent mechanism is an olfactory response, a treatment effect should occur when the extract cannot be contacted by the test insects. This effect was demonstrated (Table II). However, the extract's deterrent activity was increasingly apparent as exposure increased, suggesting the probable involvement of gustatory, tactile or visual cues in the insects' response. Using Dethier *et al.*'s (1960) terms and definitions, *L. minor* extracts which cause a negative oviposition response can be referred to as oviposition deterrents, although this last experiment suggests they may also act as oviposition repellents.

These experiments do not totally exclude the possible involvement of microorganisms in producing oviposition repellents in the extract solutions (Kramer and Mulla 1979). It may be that chemicals responsible for the observed results are produced by certain microorganisms that utilize organic matter in the test solutions as a substrate. To eliminate any effect that microorganisms may have, bioassays could be conducted for a shorter length of time at periods of peak oviposition.

L. minor clearly deters *C. pipiens* infestation of ponds in nature (Angerilli 1977). However, the lack of activity from any of the extracts against *C. pipiens* in this study, indicates that chemical deterrents probably play a minor role in this deterrency. Light intensity, water surface reflectance and surface texture can all influence natural mosquito oviposition behavior (Belton 1967; Snow 1971). Furthermore, Furlow and Hays (1972) concluded that species of Lemnaceae prevent oviposition of mosquitoes by forming a continuous surface mat.

The relatively high concentrations of crude extract needed to elicit a response from *A. aegypti* and the lack of activity against *C. pipiens* suggest that further chemical isolation and identification of the active principle(s) would probably be unwarranted.

ACKNOWLEDGEMENTS

We thank A. Syed and B. I. Gillespie for supplying the insects and Mr. R. I. Alfaro for his assistance and advice.

TABLE II. Effect of ***L. minor*** methanolic extract (10,000 PPM) exposed to varying degrees on rate of oviposition by ***Aedes aegypti.*** N = 5 replicates with 5 insects/replicate.

Oviposition Containers	$\bar{X}$ No. Eggs[a] Experimental	Control
Open beaker, with L. minor extract in water and fully exposed allowing perception by vision, olfaction, touch and contact chemoreception.	9.8***	139.2
Open beaker, with a gauze disk 1 cm above L. minor extract water solution. Olfaction and contact chemoreception of extract possible through uptake in paper towelling liner around periphery of beaker.	17.8**	119.6
Open beaker, with gauze covered L. minor extract solution in a vial in the centre, surrounded by distilled water. No contact other than olfactory with extract possible.	51.0*	95.0

[a]Significant difference (t-Test) between experimental and control means indicated by: *** = P 0.001; ** = P 0.01; * = P 0.05.

REFERENCES CITED

Angerilli, N. P. D. C. 1977. Some influences of aquatic plants on the development and survival of mosquito populations. Ph.D. dissertation. Simon Fraser University, Burnaby, B.C. 141 p.

Belton, P. 1967. The effect of illumination and pool brightness on oviposition by **Culex restuans** (Theo.) in the field. Mosq. News 27:66-68.

Dethier, V. G., L. B. Browne and C. N. Smith. 1960. The designation of chemicals in terms of the responses they elicit from insects. J. Econ. Ent. 53:134-136.

Furlow, B. M. and K. L. Hays. 1972. Some influences of the aquatic vegetation on the species and number of Culicidae (Diptera) in small pools of water. Mosq. News 32:595-599.

Gerberg, E. J. (ed.). 1970. Manual for mosquito rearing and experimental techniques. Amer. Mosq. Control. Bull. No. 5.

Gillespie, B. I. and P. Belton. 1980. Fecundity of **Aedes aegypti** (L.) as affected by rearing temperature of the larval stages. Mosq. News (in press).

Kramer, W. L. and M. S. Mulla. 1979. Oviposition attractants and repellents of mosquitoes: Oviposition responses of **Culex** mosquitoes to organic infusions. Environ. Ent. 8:1111-1117.

Matheson, R. and E. H. Hinman. 1929. Further studies on **Chara** spp. and other aquatic plants in relation to mosquito breeding. Amer. J. Trop. Med. 9:249-266.

Matheson, R. 1930. The utilization of aquatic plants as aids in mosquito control. Amer. Nat. 64:56-85.

McLintock, J. 1960. Simplified method for maintaining **Culex pipiens** Linnaeus in the laboratory (Diptera:Culicidae). Mosq. News 20:27-29.

Snow, W. F. 1971. The spectral sensitivity of **Aedes aegypti** (L.) at oviposition. Bull Ent. Res. 60:683-696.

OBITUARY

Paul M. Eide (1906-1980)

Mr. Eide, well known economic entomologist, formerly with the old Bureau of Entomology, USDA, and later with Washington State University died unexpectedly April 22nd at his home in Mt. Vernon, Washington from a heart attack. He is survived by his wife, Grace; a daughter, Judith Widen of Seattle; a brother, Dr. Carl Eide of St. Paul; a sister, Eleanor Henderson of El Paso; and several nieces and nephews.

Eide received his B.S. and M.S. degrees in entomology at WSU. While with the USDA he assisted in the development of the first use of DDT in the United States during the war years at Orlando, Florida, in cooperation with the Armed Services, researching insects attacking man and animals.Later, he was stationed at the Washington State University campus, Pullman, where he initiated research on the Cherry Fruitfly, a new pest of cherries found in the Yakima Valley. Paul was then Assistant and Associate Entomologist respectively at the Northwestern Washington Research and Extension Unit, Mt. Vernon. He retired from WSU in 1971 but maintained an office at the Research Unit and continued to serve the farmers in the area on a voluntary basis until his death.

Paul was a keen observer of insect life and had sound judgment. He was well respected by his peers and by a large following of farmers and fieldmen, especially in the Skagit Valley of Washington. He had a keen sense of humor and got along well with his colleagues. He had the amusing habit of belittling his true knowledge. When asked about an entomological problem he would first answer, "I really don't know," but when urged for a specific answer, he would then carefully and in considerable detail tell all you wanted to know.

His expertise was largely in developing controls for insect pests attacking vegetables and small fruits in northwestern Washington. Recently, at an annual Pacific Northwestern Vegetable Insect Conference at Portland, Paul received an award for the longest continued attendance — 32 years.

Horace S. Telford
Dept. of Entomology
Washington State University
Pullman, WA 99164