

DISCOVERY AND BIOASSAY OF A KAIROMONE
FROM WATERHYACINTH, *EICHHORNIA CRASSIPES*^{1,2}ERNEST S. DEL FOSSE³ AND B. DAVID PERKINSDepartment of Entomology, University of Florida, Gainesville,
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ABSTRACT

Adult mottled waterhyacinth weevils, *Neochetina eichhorniae* Warner (Coleoptera: Curculionidae), were tested in an olfactometer and in 1-liter jars for their response to a chemical (or complex of chemicals) produced by young, growing tissue of waterhyacinth, *Eichhornia crassipes* [Mart.] Solms-Laubach. A significant number ($P \leq 0.001$) were attracted to this chemical, which is properly termed a kairomone. Significantly high oviposition ($P \leq 0.05$) and feeding ($P \leq 0.001$) also occurred on waterhyacinth tissue with the higher levels of the kairomone.

This kairomone apparently concentrates waterhyacinth weevils (both *N. eichhorniae* and *N. bruchi* Hustach) and waterhyacinth mites, *Orthogalumna terebrantis* Wallwork (Acari: Galumnidae), around fresh arthropod feeding on waterhyacinth. Part of the chemical complex apparently is used as a phagostimulant and oviposition stimulant for *Neochetina* spp.

Semiochemicals induce or mediate chemical interactions between individual organisms (Law and Regnier 1971) and are divided into intraspecific pheromones (Karlson and Luscher 1959) and interspecific allelochemicals (Whitaker 1970a,b). Allelochemicals are further divided into allomones, kairomones, synomones and apneumones (Nordlund and Lewis 1976), depending upon whether the producer, receiver or both benefit from the interaction. Kairomones (Brown et al. 1970, Nordlund and Lewis 1976), are chemicals produced or acquired by an organism which, when they are perceived by an individual of another species in the natural context, evoke a reaction adaptively favorable to the receiver but not the emitter.

The same chemical may act as a kairomone if it is a phagostimulant or an allomone if it is a feeding deterrent (Nordlund and Lewis 1976), i.e., the functions are not mutually exclusive (Brown et al. 1970). Most known kairomones are chemical messengers produced by phytophagous arthropods and serve to attract their parasitoids (Lewis et al. 1975). Del Fosse (1975) and Perkins et al. (1976) determined the presence of a condition or chemical which attracted and concentrated adult *Neochetina eichhorniae* Warner under field conditions when waterhyacinth (*Eichhornia crassipes* [Mart.] SolmsLaubach) plants were broken. This chemical is a kairomone *sensu* Nordlund and Lewis (1976), because it attracts *N. eichhorniae* and *Orthogalumna terebrantis* Wallwork (and probably *N. bruchi* Hustache) to waterhyacinth (Del Fosse 1975, Perkins et al. 1976). The arthropods then feed upon waterhyacinth and cause considerable damage to the plant (Perkins 1973a, 1974, Del Fosse et al. 1975, Cordo and DeLoach 1975, Del Fosse 1977 and unpublished data).

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These experiments were designed to determine if there is a kairomone present in waterhyacinth pseudolaminae and petioles which is released after feeding by *N. eichhorniae* (or possibly *N. bruchi*) or *O. terebrantis*.

METHODS AND MATERIALS

AGGREGATION TESTS: Previous tests with an olfactometer (Del Fosse, unpubl. data) indicated that both *N. eichhorniae* and *N. bruchi* were attracted to a kairomone in waterhyacinth. This olfactometer (Fig. 1) was then used to test the attraction of *N. eichhorniae* to an unidentified chemical (or complex of chemicals) present in young, growing waterhyacinth pseudolaminae (leaves) and petioles.

Crushed waterhyacinth petioles and ground pseudolaminae were added to the left-hand box on the olfactometer (Fig. 1). A whole, mature pseudolamina of equal biomass was added to the right-hand box; the cut end was sealed with silicone glue (non-toxic to weevils or mites) to prevent escape of the kairomone. (An equal amount of glue was placed in the "ground-tissue" box.) Twenty-seven adult *N. eichhorniae* were added to the shell vial (Fig. 1) and left overnight. The experiment was replicated 3 more times, alternating the samples between boxes each time. Both this experiment, and oviposition and feeding tests (below) were conducted in closed rooms with artificial light located ca. 2-3 m above the olfactometers. *N. eichhorniae* is essentially nocturnal and negatively phototactic (Perkins 1972), so the olfactometers were placed in a position that would not influence weevil movement or other activities. A 12:12 photoperiod was used. No air flow was maintained through the olfactometers. (Air flow was maintained through the olfactometers in 3 earlier replicates. This caused waterhyacinth tissue to dry out overnight, resulting in death of weevils.) The number of weevils in each box was noted after 24 h. Results were analyzed statistically using the chi-square test.

OVIPOSITION AND FEEDING TESTS: Two replicates each of 10 males alone, 10 females alone, and 5 males: 5 females were added to separate 1-liter jars containing a whole, fresh pseudolamina-petiole unit (with cut end sealed) or crushed waterhyacinth pseudolamina and petioles. Numbers of eggs and feeding spots were counted after 24 h. The experiment was replicated 4 times and results were analyzed statistically using the chi-square test.

RESULTS

AGGREGATION TESTS: After 24 h, 97 of the 108 weevils (89.82%), a significant ($P \leq 0.001$) number, were attracted to the box containing the kairomone (Table 1). Male and female weevils were attracted equally well. Within 1 minute after being placed in the shell vial, some weevils oriented to the tube containing crushed waterhyacinth tissue. The existence of a chemical that attracts weevils to broken and crushed waterhyacinth pseudolaminae and petioles is thus strongly suspected.

OVIPOSITION AND FEEDING TESTS: Weevils laid significantly ($P \leq 0.05$) more eggs in the presence of the kairomone (Table 2). This occurred both when 10 mated females were tested alone, and when a 1:1 sex ratio was tested. In other laboratory tests (Del Fosse 1977) *N. eichhorniae* laid more

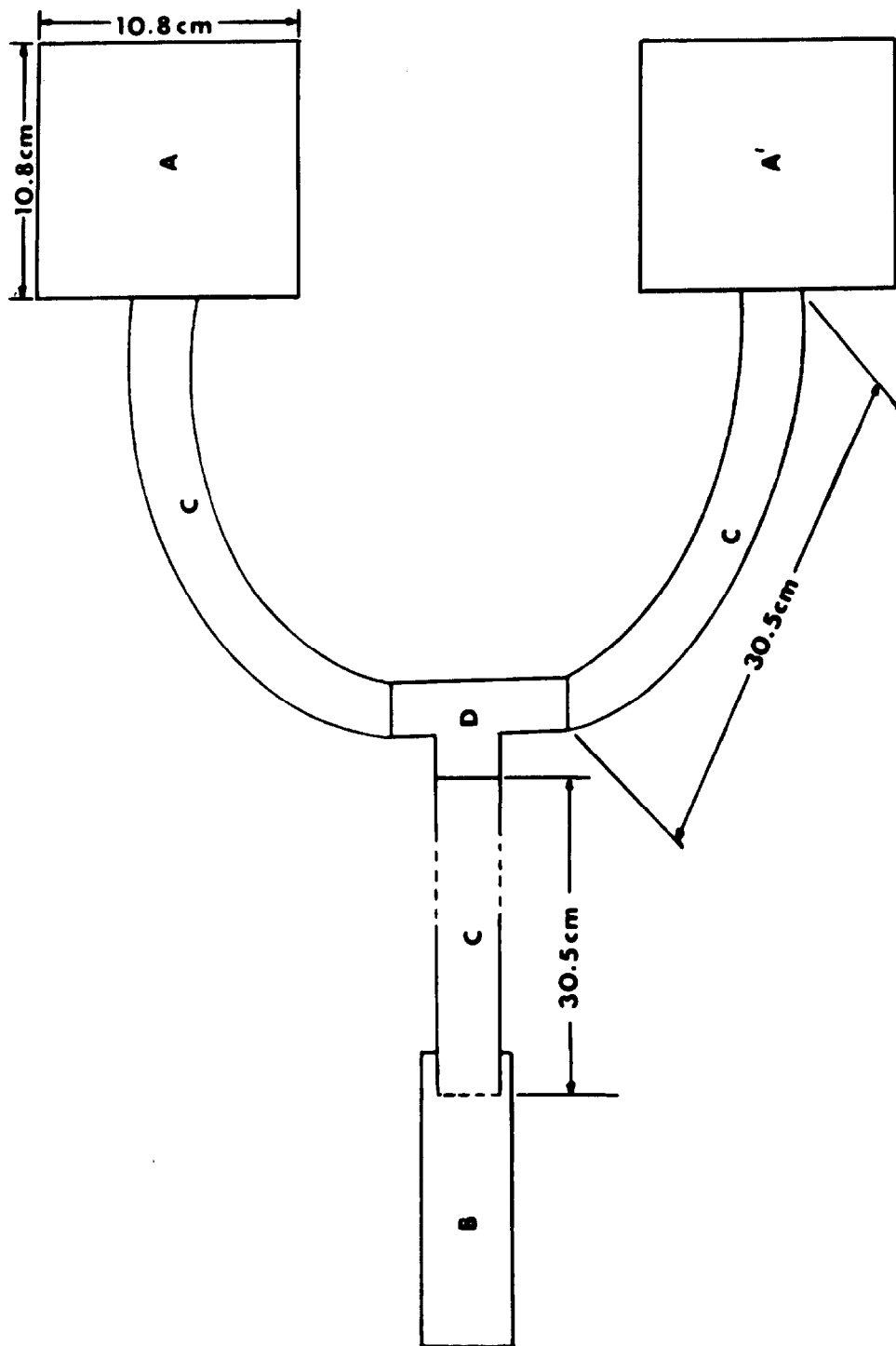


Fig. 1.—“Y”-shaped olfactometer used to bioassay the kairomone produced in waterhyacinth, *Eichhornia crassipes* (Mart.) Solms-Laubach, tissue. Either crushed or whole waterhyacinth pseudolaminae and petioles were added to box A or A'. Mottled waterhyacinth weevils, *Neochetina eichhorniae* Warner, were then placed immediately in the 6-dram glass shell vial (B) and allowed to move down 1.9 cm dia. clear plastic tubing (C) and through the copper “T” (D) to the boxes.

TABLE 1. ATTRACTION OF *Neochetina eichhorniae* WARNER TO WATERHYACINTH (*Eichhorniae crassipes* [MART.] SOLMS-LAUBACH) TISSUE. EACH MEAN REPRESENTS AN AVERAGE OF 48 READINGS.

Type of Tissue	$\bar{X} \pm 1 \text{ SD}$	
	Number of Weevils Attracted	Percent
Crushed	*24.25 \pm 0.96	89.82 \pm 3.54
Whole	2.75 \pm 0.96	10.18 \pm 3.54

*P \leq 0.001

TABLE 2. OVIPOSITION IN WATERHYACINTH (*Eichhorniae crassipes* [MART.] SOLMS-LAUBACH) TISSUE BY *Neochetina eichhorniae* WARNER AT VARIOUS SEX RATIOS. EACH MEAN REPRESENTS AN AVERAGE OF 48 READINGS.

Sex ratio: Type of tissue	$\bar{X} \pm 1 \text{ SD}$	
	Number of eggs	Percent
10 Mated females alone:		
Crushed	*2.50 \pm 3.00	100.00 \pm 0.00
Whole	0.00 \pm 0.00	0.00 \pm 0.00
5 Males: 5 Females:		
Crushed	*4.00 \pm 2.00	92.38 \pm 19.63
Whole	0.33 \pm 0.58	7.62 \pm 18.08

*P \leq 0.05

(P \leq 0.05) eggs in the presence of *O. terebrantis*. This may be due to release of a higher titre of the kairomone when there is mite feeding on the plant.

Weevils also produced significantly more (P \leq 0.001) feeding spots/insect on crushed waterhyacinth tissue than those attracted to whole pseudolaminae with sealed ends (Table 1), so a phagostimulant for *N. eichhorniae* is likely associated with waterhyacinth. Combined male and female weevils created an average of more feeding spots than either sex alone (Table 3); perhaps copulation makes them hungry.

DISCUSSION

The waterhyacinth mite concentrates around and in fresh weevil feeding spots (Bennett 1968a,b, Perkins 1973b, Cordo and DeLoach 1975, Del Fosse 1975). This may be due to high titres of the kairomone around areas of fresh feeding, concentrating weevils and mites until plants become unsuitable for feeding (Del Fosse and Perkins, unpubl. data).

Thus, not only are *Neochetina* spp. tied to waterhyacinth in the weevil's pupal stage (because root hairs are needed for pupation) (DeLoach and Cordo 1976), but the presence of a kairomone that stimulates oviposition, feeding and aggregation on injured plants due to release of a kairomone strengthens the host specific relationship.

TABLE 3. *Neochetina eichhorniae* WARNER FEEDING SPOTS PRODUCED ON WATERHYACINTH (*Eichhornia crassipes* [MART.] SOLMS-LAUBACH) TISSUE AT VARIOUS SEX RATIOS. EACH MEAN REPRESENTS AN AVERAGE OF 48 READINGS.

Sex Ratio: Type of Tissue	$\bar{X} \pm 1 \text{ SD}$	
	Number of Feeding Spots	Percent
10 Males Alone:		
Crushed*	**278.00 \pm 47.64	72.77 \pm 7.21
Whole	104.00 \pm 37.80	27.23 \pm 7.21
10 Females alone:		
Crushed*	**265.00 \pm 23.80	58.11 \pm 1.32
Whole	191.00 \pm 22.02	41.89 \pm 1.32
5 Males: 5 Females:		
Crushed*	**292.50 \pm 27.54	67.16 \pm 4.83
Whole	143.00 \pm 40.84	32.84 \pm 6.17

*Some pseudolaminae almost completely eaten. Count was estimated by dividing total surface area eaten by surface area of 1 average feeding spot.

** $P \leq 0.001$

Plans for isolating, identifying and producing the chemical(s) from waterhyacinth involved in these biotic interactions are now underway. The waterhyacinth kairomone may be utilized to concentrate adult weevils or mites for collection (Perkins et al. [1976] have demonstrated the potential of this collection method) and in an integrated control program of waterhyacinth. In the latter use, weevils could theoretically be concentrated on one area of the waterhyacinth mat and another control technique (e.g. a low level of chemical herbicides) would be applied to another area of the mat, thus maximizing the effects of both techniques.

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LITERATURE CITED

- BENNETT, F. D. 1968a. Insects and mites as potential controlling agents of waterhyacinth (*Eichhornia crassipes* [Mart.] Solms.) Proc. Brit. Weed Contr. Conf. 9:832-5.
- BENNETT, F. D. 1968b. Investigations of insects attacking waterhyacinth in Florida, British Honduras and Jamaica, 1968. Mimeo. Rep. 9p.
- BROWN, W. L., JR., T. EISNER AND R. W. WHITTAKER. 1970. Allomonas and kairomones: transpecific chemical messengers. Bio. Sci. 20:21-2.
- CORDO, H. A., AND C. J. DELOACH. 1975. Ovipositional specificity and feeding habits of *Orthogalumna terebrantis* in Argentina, a biological control agent of waterhyacinth. Environ. Ent. 4:561-5.

- DEL FOSSE, E. S. 1975. Interaction between the waterhyacinth mite, *Orthogalumna terebrantis* Wallwork, and the mottled waterhyacinth weevil, *Neochetina eichhorniae* Warner. Doctoral Dissertation, Univ. of Fla., Gainesville, 193 p.
- DEL FOSSE, E. S. 1977. Effect of *Orthogalumna terebrantis* Wallwork on *Neochetina eichhorniae* Warner eggs and oviposition. Entomophaga 22(4): (In Press).
- DEL FOSSE, E. S., H. L. CROMROY, AND D. H. HABECK. 1975. Determination of the feeding mechanism of the waterhyacinth mite. Hyacinth Contr. J. 13:49-51.
- DELOACH, C. J., AND H. A. CORDO. 1976. Ecological studies of *Neochetina bruchi* and *N. eichhorniae* on waterhyacinth in Argentina. J. Aquatic Plant Manage. 14:53-9.
- KARLSON, P., AND J. LUSCHER. 1959. Pheromones, a new term for a class of biologically active substances. Nature, Lond. 183:155-6.
- LAW, J. H., AND F. E. REGNIER. 1971. Pheromones. Annu. Rev. Biochem. 40:533-48.
- LEWIS, W. J., R. L. JONES, H. R. GROSS, JR., AND D. A. NORDLUND. 1975. The role of kairomones and other behavioral chemicals in host finding by parasitic insects. Behav. Biol. 16:267-89.
- NORDLUND, D. A., AND W. J. LEWIS. 1976. Terminology of chemical releasing stimuli in intraspecific and interspecific interactions. J. Chem. Ecol. 2:211-20.
- PERKINS, B. D. 1972. Host specificity and biology studies of *Neochetina eichhorniae* Warner, an insect for the biological control of waterhyacinth. Tech. Rep., U.S. Army Corps of Engineers, 35 p.
- PERKINS, B. D. 1973a. Preliminary studies on a strain of the waterhyacinth mite. Proc. Intern. Symp. Biol. Contr. Weeds. Rome. 1971. 2:176-84.
- PERKINS, B. D. 1973b. Release in the United States of *Neochetina eichhorniae*, an enemy of waterhyacinth. Proc. Annu. Meet. S. Weed Sci. Soc. 26:368 (Abstr.)
- PERKINS, B. D. 1974. Arthropods that stress waterhyacinth. PANS 20:304-14.
- PERKINS, B. D., M. M. LOVARCO, AND W. C. DRUDEN. 1976. A technique for collecting adult *Neochetina eichhorniae* Warner (Coleoptera: Curculionidae) for waterhyacinth control. (Note). Fla. Ent. 59:352.
- WHITTAKER, R. H. 1970a. The biochemical ecology of higher plants. pp. 43-70. In E. Sandheimer and J. B. Simeone, eds., Chemical Ecology. Academic Press. N.Y.
- WHITTAKER, R. H. 1970b. Communities and Ecosystems. MacMillan Co., N.Y.