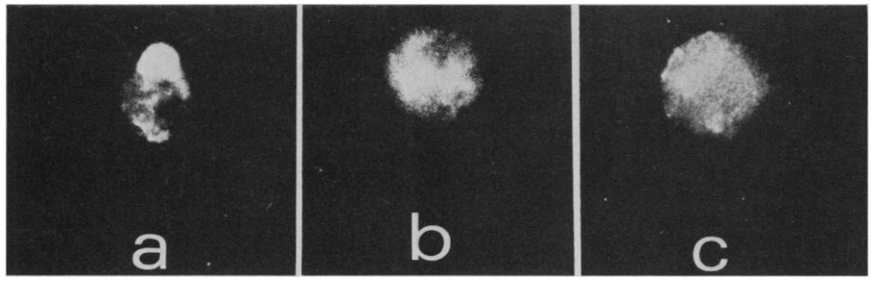

Fig 1. Concanavalin A receptor site distribution on the surface of a micromere, mesomere, and a macromere. Gametes of the sea urchin *Strongylcentrotus purpuratus* were obtained by injection of 0.55*M* KCl. The egg suspensions were treated and fertilized as described (*8*). The zygotes were distributed into beakers and allowed to develop at 17°C until the 32- to 64-cell stage was reached. Zygotes were collected and washed three times in CMF-SW with 1 percent Ficoll. Each 3 ml of packed cells was incubated for 10 minutes in 2 ml of 0.01*M* EGTA [ethylene-bis-(oxyethylenenitrilo)tetraacetate] in CMF-SW with 1 percent Ficoll and gently aspirated to complete dissociation. This cell suspension was diluted with 10 ml of CMF-SW with 1 percent Ficoll and layered over a 5 to 15 percent Ficoll discontinuous gradient made in a beaker. The beaker was placed in a 17°C incubator for 3 hours in order to separate out any cell debris, unfertilized eggs, or undissociated embryos. Cells were removed from the gradient, washed twice, and treated with FITC–Con A (750 μg/ml; Miles-Yeda) for 10 minutes in a rotating suspension at 17°C and washed in CMF-SW with 1 percent Ficoll. The cells were then fixed with 4 percent formaldehyde in CMF-SW with 1 percent Ficoll for 20 minutes, washed twice, and mounted on slides. (a) Micromere; (b) mesomere; (c) macromere. The experiment was repeated four times with different batches of cells. In all cases 95 percent of the micromeres appeared capped or highly clustered under the above conditions, while the mesomeres and macromeres displayed random site distributions.

cells and cell complexes may be due to differential cell surface properties (*4*). These morphogenetic movements, especially during gastrulation, of migratory embryonic cells represent a behavior pattern that is similar to invasiveness characteristic of malignant cells. Moscona has suggested that the Con A receptor sites may be associated with the capacity of malignant and embryonic cells to migrate and infiltrate (*5*). Dissociated sea urchin embryo cells and chick embryo cells are more agglutinable with Con A at early stages of development than at later stages (*6*). The decrease in Con A agglutinability occurs at a time when there is also a decrease in migratory activity.

By the use of a quantitative agglutination assay (*7*) we have shown that the micromere is the only cell type at the 32- to 64-cell stage of the sea urchin embryo that is agglutinable with Con A (*8*). Macromeres and mesomeres, the other cell types present, are not significantly agglutinable with Con A (*8*).

We now report a striking difference between the visual display patterns of the Con A receptor sites of the micromere and those of the nonmigratory embryonic cell types (macromeres and mesomeres).

Embryos were prepared and dissociated as described (*8*). The dissociated sea urchin embryo cells were fixed for 20 minutes in 4 percent formaldehyde in calcium-magnesium-free seawater (CMF-SW) with 1 percent Ficoll. Fluorescein isothiocyanate conjugated Con A (FITC–Con A, Miles-Yeda) was added to cell suspensions at a concentration of 750 μg/ml. Suspensions were rotated for 10 minutes at 17°C and washed twice in CMF-SW with 1 percent Ficoll before mounting on slides. When these cells were examined under the Leitz Orthoplan phase contrast fluorescence microscope (with incident illumination from a xenon lamp) the distribution of Con A receptor sites appeared to be random on all three embryonic cell populations. However, if the cells were treated with FITC–Con A before fixation the receptor sites on the micromeres were capped or highly clustered on about 95 percent of the cells (Fig. 1). The distribution of Con A receptor sites on the mesomeres and macromeres remained random under these procedures (Fig. 1). The fluorescence observed did not appear to be due to the uptake of FITC–Con A by the cells because at least 95 percent of the fluorescence was removed by incubating the FITC–Con A treated cells in 0.4*M* α-methyl-D-glucoside solution for 20 minutes. No fluorescence was visible to the eye, in the latter experiment.

The Con A–induced clustering and capping of the receptor sites on the micromeres indicates that the lateral mobility of these sites is similar to that observed in malignant cell types (*2*, *3*). The greater lateral mobility of the Con A receptor sites on migratory cell types such as embryonic cells, lymphocytes, and neoplastic cells may be due to (i) a greater intrinsic fluidity of the lipid bilayer; (ii) a structural modification of the Con A receptor sites; or (iii) alterations in the structure of microtubules and microfilaments attached to the inner membrane surface, which might effect the mobility of membrane proteins and glycoproteins. These possibilities and others have been discussed by Nicolson (*3*).

MARIE ROBERSON
ANTHONY NERI
STEVEN B. OPPENHEIMER
Department of Biology, California State University, Northridge 91324

References and Notes

1. N. Sharon and H. Lis, *Science* **177**, 949 (1972).
2. G. L. Nicholson, *Nat. New Biol.* **239**, 193 (1972); *ibid.* **243**, 218 (1973); M. Inbar and L. Sachs, *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **32**, 124 (1973); J. Z. Rosenblith, T. E. Ukena, H. H. Yin, R. D. Berlin, M. J. Karnovsky, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 1625 (1973).
3. G. L. Nicolson, *Int. Rev. Cytol.* **39**, 89 (1974).
4. P. L. Townes and J. Holtfreter, *J. Exp. Zool.* **128**, 53 (1955).
5. A. A. Moscona, *Science* **171**, 905 (1971).
6. S. W. Krach, A. Green, G. L. Nicolson, S. B. Oppenheimer, *Exp. Cell Res.* **84**, 191 (1974); S. J. Kleinschuster and A. A. Moscona, *ibid.* **70**, 297 (1972).
7. S. B. Oppenheimer and J. Odencrantz, *ibid.* **73**, 475 (1972).
8. M. M. Roberson and S. B. Oppenheimer, *ibid*, **91**, 263 (1975).
9. We thank D. L. Morton and W. D. Winters for the use of facilities and the fluorescence microscope. This research was supported by PHS research grant CA 12920 (S.B.O.).
* Address reprint requests to S.B.O.

17 March 1975; revised 23 April 1975

Juvenile Hormone Analogs: Detrimental Effects on the Development of an Endoparasitoid

Abstract. *A high incidence of mortality of the endoparasitoid* Aphidius nigripes *was observed when its host,* Macrosiphum euphorbiae, *was treated with juvenile hormone analogs. Larval and pupal stages of the parasitoid were susceptible. Off-target effects on natural enemies may seriously limit the use of juvenile hormone analogs, especially in integrated control programs.*

The potential of juvenile hormone analogs, the so-called "third generation" insecticides, as a means of controlling insect pests has been well documented. Observed effects include increased mortality (*1*), reduced fecundity (*2*), altered mating behavior (*3*), inhibition of dispersal due to abnormal wing development (*4*), and termination of diapause during periods of adverse climatic conditions (*5*). However, I believe that far too little consideration has been given to the possible undesirable effects on natural enemies, especially endoparasitoids. Two studies (*6*) of host-parasitoid complexes indicate that parasitoid development and fecundity are unaf-

fected when the host is treated with juvenile hormone analogs. On the basis of their findings, these investigators (*6*) suggest that integrated control programs may be developed with the use of juvenile hormone analogs and natural enemies. However, in both cases, the hosts were treated prior to parasitization. Normally, when an insect pest population is treated with any form of chemical control agent, several, if not all stages of the life cycle of the pest's parasitoids are present concurrently. In order to conduct a more realistic test, I investigated the effect of several juvenile hormone analogs applied to different developmental stages of a parasitoid within the host. I used the potato aphid, *Macrosiphum euphorbiae* (Homoptera: Aphididae), and its endoparasitoid, *Aphidius nigripes* (Hymenoptera: Braconidae), as experimental animals.

Third instar aphid nymphs were individually exposed to *A. nigripes*, and after parasitization were placed on small potato seedlings held at 21°C, with a photoperiod of 16 hours of light and 8 hours of darkness. These aphids were then treated either 1, 3, 6, or 8 days after parasitization, coinciding, respectively, with the 1st, 2nd to 3rd, and 4th larval and pupal stages of *A. nigripes* development. Individual plants, each with ten parasitized aphids, were sprayed, until runoff was imminent, with Altozar [ethyl (2**E**,4**E**)-3,7,11-trimethyl-2,4-dodecadienoate], ZR-619 [ethyl (2**E**,4**E**)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienethiolate], and ZR-777 [prop-2-ynyl (2**E**,4**E**)-3,7,11-trimethyl-2,4-dodecadienoate] (see Fig. 1), at three concentrations, 0.01, 0.05, and 0.10 percent. This range of concentrations was selected as it had previously been used in a study in which similar juvenile hormone analogs were tested directly on an aphid (*7*). The solvent used was water with 0.1 percent Tween 20.

Aphid mortality, caused by A. *nigripes*, occurs just before parasitoid pupation. At the time of death virtually all that remains of the host is its cuticle which turns from green to golden-brown. At this time the parasitized aphid is said to be mummified. Aphid mortality occurring prior to mummification resulting from treatment with juvenile hormone analogs was recorded (Table 1). Little or no host mortality occurred when parasitized aphids were treated with 0.01 and 0.05 percent solutions of the juvenile hormone analogs. Host mortality observed when 0.10 percent juvenile hormone analog solutions were applied varied considerably, depending on treatment time; however, at no time did this mortality exceed 56.1 percent. On the other hand, the mortality of parasitoids developing in surviving, treated hosts was extremely high, even at the lowest concentration of juvenile hormone analogs tested (Table 2). The difference in the parasitoid mortality observed in treated and control (solvent only) groups demonstrates the marked susceptibility of the different developmental stages of A. *nigripes* to all concentrations of the chemicals tested. At the end of the experiment, all mummified aphids from which no parasitoids had emerged were dissected. I observed that all parasitoids treated with 0.01 and 0.05 percent juvenile hormone solutions died as pupae regardless of the time of treatment. In the case of those treated with 0.10 percent solutions, death occurred in either the late larval or pupal stages. However, certain individuals (5 to 15 percent) exhibited both larval and pupal characteristics (particularly those treated 6 days after parasitization just prior to parasitoid pupation).

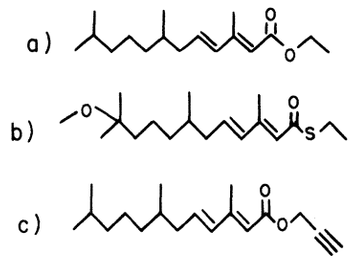

Fig. 1. Chemical structures of (a) Altozar, (b) ZR-619, and (c) ZR-777.

Vinson (*8*) observed that the developmental time of *Cardiochiles nigriceps* (Hymenoptera: Braconidae), an en-

Table 1. Mortality of *Macrosiphum euphorbiae* (parasitized by *Aphidius nigripes*) resulting from treatment with three juvenile hormone analogs in solutions at concentrations of 0.01, 0.05, and 0.10 percent.

Treatment time*	Analog	Concentration (% solution)					
		0.01		0.05		0.10	
		Treated (No.)	Mortality (%)	Treated (No.)	Mortality (%)	Treated (No.)	Mortality (%)
1	Altozar	30	0.0	29	0.0	34	11.8
1	ZR-619	29	0.0	29	0.0	35	11.4
1	ZR-777	31	0.0	29	0.0	35	14.3
		(Control: No. treated 37; % mortality 0.0)					
3	Altozar	30	0.0	29	0.0	42	27.9
3	ZR-619	30	0.0	28	0.0	63	50.8
3	ZR-777	30	0.0	31	0.0	66	56.1
		(Control: No. treated 32; % mortality 0.0)					
6	Altozar	39	0.0	42	4.8	32	12.5
6	ZR-619	30	0.0	41	0.0	36	13.9
6	ZR-777	41	0.0	43	2.3	51	31.4
		(Control: No. treated 40; % mortality 0.0)					

*Time in days after parasitization. Hosts treated 8 days after parasitism were mummified and therefore not included in this table.

Table 2. Mortality of the endoparasitoid *Aphidius nigripes* in hosts (*Macrosiphum euphorbiae*) treated with three juvenile hormone analogs in solutions at concentrations of 0.01, 0.05, and 0.10 percent.

Treatment time*	Analog	Concentration (% solution)					
		0.01		0.05		0.10	
		Treated (No.)	Mortality (%)	Treated (No.)	Mortality (%)	Treated (No.)	Mortality (%)
1	Altozar	30	100.0	29	96.6	30	100.0
1	ZR-619	29	100.0	29	100.0	31	100.0
1	ZR-777	31	55.0	30	90.0	30	100.0
		(Control: No. treated 37; % mortality 10.8)					
3	Altozar	30	93.3	29	100.0	31	100.0
3	ZR-619	30	100.0	28	100.0	31	100.0
3	ZR-777	30	55.3	31	96.8	29	100.0
		(Control: No. treated 32; % mortality 12.5)					
6	Altozar	39	100.0	40	97.5	28	100.0
6	ZR-619	30	100.0	41	97.6	31	100.0
6	ZR-777	41	73.2	42	92.9	35	100.0
		(Control: No. treated 42; % mortality 4.8)					
8	Altozar	30	100.0	28	100.0	25	100.0
8	ZR-619	31	100.0	30	100.0	25	100.0
8	ZR-777	29	100.0	31	100.0	26	100.0
		(Control: No. treated 40; % mortality 20.0)					

*Time in days after parasitization.

doparasitoid of the tobacco budworm, *Heliothis virescens* (Lepidoptera: Noctuidae), was significantly prolonged when its host was treated with ENT-70221 (5 or 50 mg). He also noted that the sex ratio of parasitoid populations emerging from treated as compared to untreated hosts differed significantly. My results and those of Vinson (*8*) demonstrate that endoparasitoids may be seriously affected when they develop in hosts treated with juvenile hormone analogs.

If juvenile hormone analogs can significantly reduce the reproductive potential of certain insect pests (*2*), it is reasonable to assume that similar reductions might also occur in the reproductive potential of parasitoids developing in treated hosts. The possible changes in parasitoid biology caused by juvenile hormone analogs could seriously limit the role of parasitoids in the natural regulation of insect pests.

The usefulness of juvenile hormone analogs in integrated control programs utilizing biological control agents is questionable, particularly if, as shown in my study, parasitoid mortality far exceeds that of its host (the insect pest) even at very low rates of application. Comprehensive studies are essential to evaluate fully the off-target effects of these "third generation" insecticides prior to their use on a commercial scale in natural ecosystems. These considerations are particularly important in view of the undesirable side effects already observed (*9*) when host-parasitoid complexes are disrupted by the application of organochlorine hydrocarbon, organophosphorus, and carbamate insecticides.

JEREMY MCNEIL
Département de Biologie,
Université Laval,
Québec, Canada G1K 7P4

References and Notes

1. J. E. Cummings and B. McKague, *Can. Entomol.* **105**, 509 (1973); J. Granett, *ibid.* **106**, 695 (1974); A. A. Guerra, D. A. Wolfenbarger, R. D. Garcia, *J. Econ. Entomol* **66**, 833 (1973).
2. C. E. Richmond, *J. Econ. Entomol.* **65**, 950 (1972); W. G. Wellington, *Can. Entomol.* **101**, 1163 (1969).
3. I. Outram, *J. Econ. Entomol.* **66**, 1033 (1973).
4. L. Dessureault and J. M. Perron, *Nat. Can.* **100**, 107 (1973); G. Tamaki, *Can. Entomol.* **105**, 761 (1973).
5. W. H. Reissig and J. A. Kamm, *J. Econ. Entomol.* **67**, 181 (1974).
6. J. D. Wilkinson and C. M. Ignoffo, *ibid.* **66**, 643 (1973); J. E. Wright and G. E. Spates, *Science* **178**, 1292 (1972).
7. S. G. Nassar, G. B. Staal, N. I. Armanious, *J. Econ. Entomol.* **66**, 847 (1973).
8. S. B. Vinson, *ibid.* **67**, 335 (1974).
9. R. van den Bosch and P. S. Messenger, *Biological Control* (Intext Press, New York, 1973), pp. 119–127.
10. Support provided by C.R.A.Q. grant L.S.-73-558. I thank the Zoecon Corporation for providing the chemicals and my students for their technical assistance. I am indebted to Drs. A. Anderson and J. McLeod for their helpful criticism of the manuscript.

27 January 1975; revised 2 April 1975

Swarming Behavior: Evidence for Communication in Social Wasps

Abstract. *Behavior of wasps at sites around swarms and along emigration routes suggests the use of odor marks. Wasps perform breaking runs through swarms, resulting in dispersal of clustered wasps. Orientation in flight of swarm mates to specific trail sites facilitates swarm emigration to the new nest.*

Swarming among social insects serves to establish new colonies or move a colony to a new nest site. Of necessity, it requires communication among individuals so that they cluster together and emigrate more or less as a group. In highly social bees, swarming is well known and some of the behavioral and chemical mechanisms are known in the honey bee (*1*). However, in other groups such as neotropical social wasps (*2*), generally regarded as primitively social, the mechanisms of swarming in relation to emigration and nest-founding are not understood.

While studying social wasps as prey of army ants in the rain forests of Ecuador and Panama, I observed swarms of 15 species of wasps (*3*). The swarms consist of one or more queens accompanied by many workers. The evacuation of the nest, formation of the swarm cluster, emigration of the swarm to a new nest site, and construction of the new nest are distinctly separate processes rapidly executed. The orientation of the swarming wasps, the selection of the new nest site, and the emigration of the swarm to the site appear to be facilitated by both communication within the swarm and orientation to marked trail sites.

Although swarming is primarily thought of in association with colony reproduction, swarms are of two distinct types: founding swarms, consisting of queens and workers that leave the mother colony in response to cyclic intracolony factors; and absconding swarms, consisting of the entire adult population of a colony that leaves its nest upon the destruction of the nest or brood (*4*). The swarms discussed here are absconding swarms, formed after loss of the nest to army ants, particularly *Eciton hamatum* and *E. burchelli*.

After an attack on the nest by *Eciton*, the entire adult population flies for only a short time; small, scattered clusters of wasps form immediately on nearby ant-free vegetation, uniting within an hour into a single, large swarm cluster on a leaf or branch near the old nest. Large populations may form two or three separate swarm clusters, but small species or colonies may form a single swarm on the underside of one large leaf. There is usually no strong defense of the nest and no attempt to reoccupy it even after the ants have left.

In these early stages of swarm formation, some of the flying wasps hover or land at specific sites on leaves and stems about the nest area. Close observation at these sites showed that some wasps made straight, short runs while dragging the ventral surfaces of their gasters on the substrate (Fig. 1), but others merely landed and antennated the site (Fig. 2). All wasps landing at a site responded in either of these two ways, dragging or antennating, while still others hovered near the sites momentarily. These behavior patterns, seen in five species (*5*), suggest that the dragging of the gaster served to deposit a mark, even though no substance could be seen on the substrate.

The gastral sternites that are pressed against the substrate during the runs have already drawn the attention of investigators (*6*). Richards has noted a subcuticular gland of unknown function on the fifth gastral sternite of four genera of social wasps, and I have seen a similar gland in *Stelopolybia myrmecophila*. Jeanne (*7*) has associated a small tuft of hair on the terminal gastral sternite of *Mischocyttarus drewseni* with the rubbing application of ant repellant on the nest petiole. It is probable that dragging the gaster serves to lay down a glandular secretion which other wasps, that is, antennating wasps, use for orientation.

Dragging *Angiopolybia pallens* and *Leipomeles dorsata* make upward runs several centimeters long on nearly vertical surfaces. *Polybia catillifex* runs were short and performed slowly on the large horizontal leaves under which they nest. When running wasps drag or rub their gasters on a narrow substrate such as a stem, the gasters move back and forth in a conspicuous lateral wagging movement. During the run, the wings are either held up at an angle or are moved rapidly. The antennae may touch the substrate or be held above it.

Antennating wasps when visiting sites walk slowly forward; the abdomen is held high and still, antennae are in contact with the substrate, and the wings are held up but are never moved rapidly. Their visits are shorter, and they sometimes move only a few millimeters forward before flying on.

The dragging behavior is often seen during and immediately after swarm formation but as flight activity around the forming swarm decreases, it becomes less evi-