We conducted our tests in the field when red-banded leaf roller moths were in flight. Wicks containing one test chemical each were placed in traps made from pint ice-cream cartons coated with sticky polybutene. In a preliminary study in which traps contained RiBLuRe (*cis*-11-tetradecenyl acetate) and one of five other chemicals in equal amounts (Table 1), we found that *trans*-11-tetradecenyl acetate, the geometrical isomer of RiBLuRe, and *cis*-9-tetradecenyl acetate strongly inhibit male response to RiBLuRe. It was also surprising to find that dodecyl acetate is strongly synergistic to RiBLuRe (this will be the subject of a future report).

Table 1. FIELD RESPONSE TO COMBINATIONS OF RIBLURE (*cis*-11-TETRADECENYL ACETATE) AND ONE TEST CHEMICAL*

Test chemical	Total males	Moths per trap†
Dodecyl acetate	538	21·5
Hexadecyl acetate	293	11·7 ‡
Tetradecyl acetate	233	9·3 ‡
cis-9-Tetradecenyl acetate	51	2·0 ‡
trans-11-Tetradecenyl acetate	19	0·8 ‡

* Thirty female equivalents (10 μg) of each placed on a new wick each day. Traps placed in complete randomized block design.
† Honestly significant difference = 6·81 moths ($\alpha = 0{\cdot}05$).
‡ RiBLuRe control alone in same orchard attracted 7·95 moths per trap.

Table 2. PRELIMINARY RANKING OF RIBLURE INHIBITORS

Strong inhibitors (moths per trap ~0)	Questionable inhibitors (moths per trap ~3)
trans-11-Tetradecenyl acetate	*cis*-9-Hexadecenyl acetate
cis-11-Tetradecenyl alcohol	*cis*-7-Tetradecenyl acetate
cis-9-Tetradecenyl alcohol	*cis*-7-Hexadecenyl acetate
11-Tetradecynyl alcohol	2(11-Tetradecynyloxy)tetrahydropyran
11-Tetradecynyl acetate	*cis*-7-Dodecenyl acetate
	10-Undecenyl acetate
Medium inhibitors (moths per trap ~1)	**Non-inhibitors (moths per trap ~10)**
	Methyl *cis*-11-tetradecenoate
trans-9-Tetradecenyl acetate	*cis*-6-Hexadecenyl acetate
cis-9-Tetradecenyl acetate	*cis*-5-Dodecenyl acetate
trans-7-Tetradecenyl acetate	*cis*-7-Decenyl acetate
trans-5-Tetradecenyl acetate	*cis*-5-Tetradecenyl acetate
cis-11-Hexadecenyl acetate	Tetradecyl alcohol

To check the inhibition observations further, a larger number of chemicals were used alone and in combination with RiBLuRe. None of the test chemicals, other than RiBLuRe, was found to be attractive to male red-banded leaf roller moths, although varying degrees of inhibition were found with the combinations. Low infestations were encountered in these tests, but a preliminary order of inhibitors can be set up from the data (Table 2). The red-banded leaf roller male response can be characterized as follows. (1) Any tetradecyl acetate or alcohol with unsaturation in the 11-position (other than RiBLuRe itself) is strongly inhibitory. (2) In general, the *trans* configuration is more inhibitory than the corresponding *cis* configuration with RiBLuRe odd-numbered positional isomers. (3) Alcohols seem to be more inhibitory than corresponding acetates (also suggested in reports dealing with the gypsy moth, *Porthetria dispar*, which claim that 7 per cent rincinoleyl alcohol inactivates gyplure[2], 12-acetoxy-*cis*-9-octadecen-1-ol, while 20 per cent of the *trans* geometrical isomer is required to effect strong inhibition[11]). (4) Inhibitory effects decrease as the position of unsaturation is moved from carbon 11 to lower odd-numbered carbons.

These generalities suggest that the strongest inhibitor for RiBLuRe would be *trans*-11-tetradecenyl alcohol (as yet untested). It may be possible to develop a low-cost inhibitor which is sufficiently active to be used in programmes of mass inhibition insect control.

Compounds in the strong inhibitor group could not be ranked within the group because of the high ratio (1 : 1) of inhibitor to attractant used. A separate test showed that 5 per cent *trans*-11-tetradecenyl acetate would inhibit the response to 10 μg of RiBLuRe. Because the *trans* geometrical isomer does inhibit in low concentrations, it is questionable whether a bioassay with a synthetic *cis* : *trans* mixture (1 : 1)[1] of a proposed attractant could prove anything about the ability of either isomer to function as an attractant. Furthermore, it is possible that common laboratory bioassays and electrophysiological studies do not differentiate between the real attractant response and excitatory responses to other chemicals which do possess an affinity for the receptor sites. In field bioassays low concentrations elicit flight towards the attractant source and higher concentration gradients nearer the trap effect final orientation and other precopulatory responses. This should be the most proper behavioural test for sex attractancy.

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Chemical Control of Moulting in Arthropods

INSECTS are the only group of arthropods in which the control of moulting is well understood. A group of steroids —the ecdysones, thought to be produced by the prothoracic or ecdysial glands—cause moulting in almost all insect groups in which they have been tested[1]. Ecdysones isolated from a crustacean[2] and from several different plants[3] were active in insects[4,5]. No reports exist of moulting induced by ecdysones in arthropods other than insects, but ecdysterone shortened the period between moults in crayfish which had been induced to moult by removal of their eyestalks[6].

We have shown that the ecdysones control moulting in three diverse classes of arthropods besides insects.

As experimental animals, we selected representatives of the principal arthropod classes. *Limulus polyphemus*, the horseshoe crab, was chosen because it is one of the few survivors of the Merostomata, a marine class dating back to the Cambrian whose closest relatives were the eurypterids and trilobites. The spider, *Araneus cornutus*, and two different members of the Crustacea were examined. The terrestrial isopod, *Armadillidium vulgare*, was chosen as a crustacean whose ecology and physiology bear the closest resemblance to that of insects. The freshwater crayfish, *Procambarus* sp., represented the decapods.

The ecdysone used in most experiments was ecdysterone (20-hydroxyecdysone, β-ecdysone, crustecdysone), which has been isolated from both Crustacea[2] and insects[7]. It was isolated from a plant source (Rohto Pharmaceutical Company, Osaka), but it is identical to the ecdysterone

Table 1. EFFECTS OF ECDYSTERONE ON MOULTING IN ARTHROPODS

Animals and treatment	Number	Cumulative No. that moulted within 3 weeks	Per cent that moulted within 3 weeks	
Subphylum Chelicerata				
Class Merostomata				
Limulus polyphemus (30–50 g)				
Uninjected controls	50	1	2	Only partial moulting
Injection of 8 μl./g of crustacean Ringer	6	0	0	
Injection of 40 μg/g ecdysterone in crustacean Ringer	5	5	100	Complete moulting, but all had to be peeled
Class Arachnida				
Araneus cornutus (0·1–0·4 g)				
Uninjected controls	15	1	7	After 25 days 7 per cent had moulted
Injection of 4 μl. of insect Ringer per animal	19	3	16	After 25 days 15 per cent had moulted
Injection of 20 μg ecdysterone in insect Ringer per animal	24	14	56	After 25 days 75 per cent had moulted. Similar results were obtained with 10 μg of ecdysterone per animal
Subphylum Mandibulata				
Class Crustacea				
Armadillidium vulgare (0·1–0·2 g)				
Uninjected controls	22	6	27	None moulted until after 15 days
Injection of 20 μl. insect Ringer per animal	16	2	12	None moulted until after 15 days
Injection of 20 μg ecdysterone in 20 μl. of insect Ringer per animal	15	12	80	73 per cent moulted between 12–15 days. Similar results were obtained with 5 μg of ecdysterone per animal
Dipped in absolute methanol for 5 s	10	1	10	
Dipped in a solution of 0·5 per cent ecdysterone in absolute methanol for 5 s	8	4	50	
Topical application of 20 μl. of 0·5 per cent ecdysterone in absolute methanol	7	4	57	
Class Crustacea				
Procambarus sp. (3–7 g)				
Uninjected controls	15	0	0	
Injection of 7 μl./g of 10 per cent ethanol	10	0	0	
Injection of 3 μg/g ecdysterone in 10 per cent ethanol	7	6	86	Moulting occurred between 10–14 days
Injection of 6 μg/g ecdysterone in 10 per cent ethanol	10	10	100	Moulting occurred between 7–10 days
Injection of 12 μg/g ecdysterone in 10 per cent ethanol	10	10	100	Moulting occurred between 7–10 days

obtained from insects and Crustacea[8]. We also used a synthetic α-ecdysone (obtained from Dr P. Hocks, Schering AG, Berlin, and Hoffmann and LaRoche, Basle), inokosterone (Rohto) and ponasterone *A* (obtained from Dr J. Pollard, Calbiochem). The hormones were dissolved in an appropriate Ringer solution or in an ethanolic solution. The volumes injected were always small compared with the volume of the animal (1–5 per cent).

Groups of experimental animals were selected in which spontaneous moulting was minimal. They were maintained in the laboratory. In some groups, such as crayfish, in which moulting is seasonal, animals were collected after the moulting season. In others, such as *Limulus* and spiders, mature animals which moulted infrequently were used. Animals were usually divided into three groups: those which received an injection of hormone in an appropriate solvent; controls injected with solvent only; and uninjected controls. In some experiments, a methanolic solution of ecdysterone was applied topically, or animals were dipped in a methanolic solution of ecdysterone. This method was first used successfully in applying ecdysones to the rice stem borer *Chilo simplex*[9] and subsequently to other insects (for example, *Galleria mellonella*) in our laboratory. Animals were observed for up to 6 weeks after treatment and examined for signs of moulting. Sometimes the experimental animals shed their old cuticle and moulted normally. Sometimes, they secreted a new cuticle, but did not shed the old one. The old cuticle was always only loosely attached and could easily be peeled away. These animals always died.

To ensure an adequate level of hormone, large doses of ecdysterone were used initially. In some animals, the effects of a wide variety of doses were studied and we estimated the minimum effective dose.

Some typical results are shown in Table 1. Ecdysterone stimulated moulting in all of the arthropods examined. In *Limulus*, the doses we used induced moulting in all treated animals whereas only one of fifty-six controls showed any signs of moulting. In the spider, *Araneus*, 75 per cent of the experimental animals but only 15 per cent of the controls moulted after 25 days. Soon after they were injected with ecdysterone the spiders ceased to spin their usual webs and spun instead a moulting pad[10]. Some days later signs of moulting could be detected. Apparently ecdysterone induced a behavioural and a moulting response.

Moulting occurred almost synchronously in many of the animals treated with hormone. In *Armadillidium*, 75 per cent of the animals which received about 150 μg/g of ecdysterone moulted between 12 and 15 days after injection. In *Procambarus*, all the animals which received 6 μg/g of ecdysterone moulted between 7 and 10 days after the injection.

Response was proportional to dose. This was studied most carefully in *Procambarus*, where 3 μg/g of ecdysterone was the minimum effective dose to initiate secretion of a normal cuticle. Ecdysis occurred 10–14 days after a single injection. If the dose were increased to 6 or 12 μg/g, cuticle secretion and ecdysis occurred within 7 to 10 days. After being given higher doses of ecdysterone, they were unable to shed the old cuticle without assistance and usually died. Similar results were obtained with insects[4,5].

Table 2 shows the effects of four different ecdysones on moulting in crayfish. All four were about equally effective in inducing moulting and, at the concentrations used, they promoted the regeneration of appendages.

The ecdysones may induce moulting in these diverse arthropods by stimulating, directly or indirectly, the ecdysial glands to release a moulting hormone. But, if the situation is analogous to that in insects, this explanation is unlikely. It seems far more probable that the ecdysones induce moulting in crustaceans and arachnids as they do in insects, by acting directly on the chitogenous epithelium.

Chemical control of moulting probably evolved simultaneously with the development of a chitinous exoskeleton. Ecdysones were probably used by the common ancestor of the major arthropod groups in the late pre-Cambrian period. Once the ecdysones had evolved, both they and their primary receptor sites appear to have undergone little

Table 2. EFFECTS OF FOUR ECDYSONES ON MOULTING IN *Procambarus* sp.

	No.	Per cent which moulted 7–10 days	10–14 days	14–21 days
Uninjected controls	25	0	0	0
10 per cent ethanol controls	10	0	0	0
Ecdysterone	7	14	86	0
α-Ecdysone	15	56	7	7
Inokosterone	14	21	56	7
Ponasterone A	15	14	35	0

Animals weighed 3 to 7 g. α-Ecdysone was dissolved in 50 per cent ethanol; all other ecdysones were dissolved in 10 per cent ethanol.
The dose was 3 μg/g. Ethanol controls received 7 μl./g of 10 per cent ethanol.

change during evolution, because both recent groups of arthropods, such as insects, and ancient groups, such as horseshoe crabs, respond to the same molecules.

Our results may have some practical consequences. Ecdysones act topically on a wide variety of arthropods and may cause abnormal moulting and death, so ecdysone analogues may be useful not only as insecticides but also miticides. Ecdysones could also be used in the commercial production of "soft-shelled" crabs.

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Note added in proof. Ecdysterone induces moulting in the fiddler crab *Uca pugilator* and the tarantula *Dugesiella hentzi*.

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Cellulase in *Nereis virens*

IN their communication about direct uptake of organic solutes, Chapman and Taylor[1] commented on the contradictory published accounts of the food and feeding of the polychaete *Nereis virens* Sars. Verrill[2] stated that it is carnivorous, while Gross[3] suggested that it is almost entirely herbivorous. Turnbull[4] regarded the animal as omnivorous. The principal recognizable remains that we found in the guts of *N. virens* collected at Southend, Essex, were shells of the small gastropod *Hydrobia*, small fragments of small cockle (*Cardium edule*) shells, and the alga *Enteromorpha intestinalis*. When kept in aquaria, *N. virens* readily ate the flesh of cockles, mussels (*Mytilus edulis*), and shore crabs (*Carcinus maenas*), when these were placed near the entrances to the burrows. The shell-valves of cockles, approximately 5 mm in diameter, were broken by the jaws of the worms and ingested. Large cockles and mussels were eaten only when they died and gaped open. Moribund cockles and mussels occur in large numbers at Southend, resulting both from natural mortality and the activities of the "bait-diggers", who accidentally bury these molluscs in their search for *Nereis*. *Enteromorpha* was also readily ingested, but it was observed during these experiments that the faecal pellets of the worms frequently contained strands of apparently undigested algae. This posed the question whether *Nereis* can digest the cell walls of algae which it has eaten or whether it can only utilize contents of cells broken mechanically.

Cellulolytic activity has been recorded previously in several polychaete worms, including *Loimia medusa* and *Sabellastarte indica*, whereas reputedly carnivorous forms, such as *Glycera chioni*, do not seem to possess a cellulase. We have investigated cellulase activity in *N. virens* by

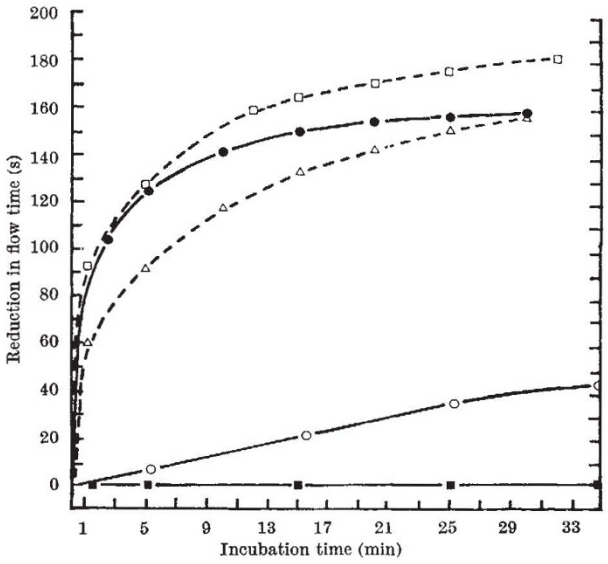

Fig. 1. Production of cellulase by gut of *Nereis* after different feeding regimes. ●—●, Entire gut, algae fed; ○—○, entire gut, starved 6 weeks; △ - - - △, washed gut wall, algae fed; □ - - - □, gut contents, algae fed; ■—■, heat treated control.

a technique similar to that described by Yokoe and Yasumasu[5]. A known wet weight of gut was ground in ten times (weight/volume) of 0·1 M sodium citrate/phosphate buffer at *p*H 7·0. The mixture was then centrifuged at 3,000*g* for 10 min. A sample (2·5 ml.) of the supernatant was then added to 30 ml. of 1 per cent sodium carboxymethyl cellulose (CMC), and run in an Ostwald viscometer at 25° C. The activity of cellulase was indicated by a reduction in flow-time when compared with a blank, in which the 2·5 ml. of supernatant was replaced by an equal volume of sodium citrate/phosphate buffer. Animals with large amounts of algae in their guts showed high cellulase activity, whereas animals which had been starved or fed on cockles showed very little cellulase activity (Fig. 1). A control was carried out with an extract of high cellulase activity which was autoclaved at 15 pounds/inch^2 for 25 min. This completely destroyed the activity. These results appear to suggest that the production of cellulases is at a very low level until induced by the presence of algae in the gut. The presence of undigested algal filaments in the faecal pellets of worms which had previously been fed on cockles and mussels would thus be accounted for.

Estimates of cellulase activity were made on gut contents, and washed gut wall of worms fed on algae; although the cellulase activity of the gut contents was higher than that of the washed gut wall, the relatively high level of activity found here suggests that the enzyme may be produced in the wall (Fig. 1). Several techniques have been used to determine whether the cellulases found in animals are produced by bacteria or by the animal itself. Antibiotics have been used to demonstrate that the cellulase from the hepatopancreas of the snail *Levantina hierosolymia* was not produced by bacteria[6]. When the insect *Ctenolepisma lineata*, which eats cellulose, was grown aseptically from the egg it produced a cellulase[7]. Workers have also demonstrated cellulase production by bacteria in the gastric juices of the crayfish *Procambarus clarkii*, which in addition produces a cellulase in its own digestive gland (ref. 8 and unpublished results of I. Yasumasu and Y. Yokoe).

In an attempt to establish with certainty whether the source of cellulase was the tissues of the worm, or its bacterial flora, worms were kept for 20 h in seawater containing the following antibiotics: 500 μg of 'Penicillin V', 250 μg streptomycin sulphate and 100 μg mycostatin/ml., no appreciable reduction in cellulase activity was