Because malformin stimulates ethylene production in green cuttings[11], and ethylene inhibits extension growth, we determined ethylene production by etiolated cuttings treated with malformin (Table II). Malformin stimulated ethylene production in etiolated cuttings and stimulated elongation despite these higher ethylene levels.

Although cuttings from green seedlings elongate only slightly we examined the effect of malformin on their growth increment in similar experiments. After 4 days in the light the growth increment of cuttings in water, malformin 10^{-5} *M*, and malformin 10^{-6} *M* was 1.09, 0.61, and 1.44 cm, respectively (average 70 cuttings). Malformin (10^{-6} *M*) stimulated the elongation of green cuttings; however, the absolute increase in growth increment was less than that of etiolated cuttings.

Table II. Effect of malformin on ethylene production by stem segments from etiolated cuttings of *Phaseolus vulgaris* in the light

Treatment	Length of treatment (h)		
	24	48	72
	Ethylene (nl/h/g fresh wt.)		
H_2O	1.23	1.69	1.14
Malformin 10^{-5} *M*	2.96	3.52	1.65
Malformin 10^{-6} *M*	2.78	1.48	1.24

Average 3 determinations, cv. Harvester. Approximately 2.0 cm sections were excized from the hypocotyl hook region, sealed in syringes, incubated 2 to 4 h, and analyzed for ethylene by gas chromatography as described[14].

Light inhibition of etiolated stem elongation is a process mediated by phytochrome[15]. Stimulation of stem elongation by malformin on etiolated cuttings subsequently maintained in light, but not in the dark, suggests an effect of malformin on this process. Until the effect of malformin on other phytochrome mediated responses is known, we are unable to offer an explanation for malformin-induced growth stimulation[16].

Zusammenfassung. Das Wachstum etiolierter, wurzelfreier Bohnenkeimlinge von *Phaseolus vulgaris* war mit Malformin A im Licht, nicht aber in der Dunkelheit stimuliert, und zwar obwohl die Äthylenproduktion durch Malformin-Behandlung gesteigert wird.

W. W. John and R. W. Curtis

Department of Botany and Plant Pathology, Lilly Hall of Life Sciences, Purdue University, West Lafayette (Indiana 47907, USA), 22 July 1974.

[14] R. W. Curtis, Pl. Physiol. *43*, 76 (1968).
[15] P. Ray, *The living plant* (Holt, Reinhart and Winston, Inc., New York 1972).
[16] Journal Paper No. 5589 of the Purdue Agricultural Experiment Station. Supported in part by grant No. GB-23540 from the National Science Foundation.

On the Absence of Chitin in the Endosternite of Chelicerata

The endosternite of chelicerates is an internal skeletal support for some of the prosomal muscles. Firstman[1] has an excellent review of the structure and derivation of this organ in the various chelicerate classes and orders. It has been contended since Lankester[2] that the endosternite, notably of *Limulus*, contains chitin. However, other authors[3] were unable to find chitin in the endosternites of chelicerates, including *Limulus*. An examination of Lankester's paper[2] shows that the determination of chitin was made by a Prof. Schäfer (footnote on pages 133–134), and that he was cautions in his determination. At one point Prof. Schäfer states, '... I am not prepared to say positively that the substance is 'chitin' or even that it is chiefly chitin; but considering its solubilities, or rather insolubilities, it is probably either that substance or a mixture of that and a substance allied to keratin'. Despite this circumspect comment, Lankester states that chitin is definitely present in the endosternite and draws upon this to make other far-reaching conclusions. Lankester's conclusions were agreed to by Halliburton[4]. However, neither Schäfer's nor Halliburton's methods are currently accepted as documentation for the presence of chitin[5]. Reexamination seemed desirable.

We took endosternites from as many different chelicerate groups as we could muster, and subjected them to the standard van Wisselingh qualitative test for chitin. The specimens were heated in saturated KOH solution in sealed glass tubes at 160 °C for 30 min. Any particulate matter remaining was washed first in 50% ethanol, then in distilled water. In all cases, except for the solpugid apodeme and the positive control, testing ended at this point because the material had entirely dispersed. The positive control and the apodeme gave the characteristic violet color with iodine solution, followed by slow dispersion in 75% H_2SO_4; dispersion in weak acetic acid, followed by precipitation with dilute H_2SO_4; to indicate the presence of chitosan (see page 27[5] for further details).

As a confirmatory test, selected specimens were placed in 1 *N* NaOH for 24 h at 60 °C. This is based on the Hackman and Goldberg[6] procedure for purifying chitin. No structure containing a significant amount of chitin should be dispersed by this method, yet only the positive control retained its structural identity; the others had completely dispersed, with one exception. The exception was the piece of *Limulus* endosternite, which had swollen and started to peel off flakes, but had not completely dispersed. After 72 h the *Limulus* endosternite had dispersed, while the positive control had not changed in appearance, except that it became lighter colored.

Specimens tested by the van Wisselingh method were the endosternites of *Argas persicus* (Oken), *Amblyomma americanum* (L.) (Acarina); *Tarantula* sp. (Amblypygi); *Argiope argentata* (Forskal), *Dictyna* sp., *Dysdera crocata* C. L. Koch, *Lycosa frondicola* Emerton, *Pellenes hoyi*

[1] B. Firstman, J. Arachnol. *1*, 487 (1973).
[2] E. R. Lankester, Q. Jl. microsc. Sci. *24*, 129 (1884).
[3] A. P. Mathews, Z. physiol. Chem. *130*, 69 (1923).
[4] W. D. Halliburton, Q. Jl. microsc. Sci. *25*, 173 (1885).
[5] A. G. Richards, *The Integument of Arthropods* (Univ. Minnesota Press, Minneapolis 1951).
[6] R. H. Hackman and M. Goldberg, J. Insect Physiol. *2*, 221 (1958).

(Peckhams), *Aphonopelma* sp. (Araneae); *Chelifer cancroides* (L.) (Chelonethida); *Leiobunum ventricosum* (Wood) (Phalangida); *Odontobuthus doriae* (Thorell), *Diplocentrus* sp., *Uroctonus* sp., *Vejovis spinigerus* (Wood) (Scorpionida); *Mastigoproctus giganteus* (Lucas) (Uropygi); and *Limulus polyphemus* L. (Merostomata). Also tested were the internal prosomal apodeme of *Eremobates* sp. (Solpugida) and the ventral nerve cord, ganglia and mesenteries of *Phoxichilidium femoratum* (Rathke) (Pycnogonida). As positive control the movable cheliceral finger of *Eremobates* sp. was used; as negative control a piece of muscle from *Limulus*. For dissolution in warm NaOH, we used endosternites from *Amblyomma americanum, Argiope argentata, Leiobunum ventricosum, Centruroides vittatus* (Say) (Scorpionida) and *Limulus polyphemus* with the same positive and negative controls.

It is our conclusion, based on currently accepted methods for chitin determination, that chitin is absent from the endosternite of chelicerates[7,8].

Zusammenfassung. Die Endosternite von 18 Arten aus den drei lebenden Klassen von Cheliceraten wurden nach der Methode von van Wisselingh auf Chitin geprüft, wobei alle Proben negative Ergebnisse für die echten Endosternite ergaben. Nur die innere prosomische Apophyse einer Solpugide enthält Chitin.

B. CUTLER and A. G. RICHARDS

1747 Eustis Street, St. Paul (Minnesota 55113, USA); and Department of Entomology, Fisheries, and Wildlife, University of Minnesota, St. Paul (Minnesota 55108, USA), 8 July 1974.

[7] Paper No. 8721, Scientific Journal Series, Minnesota Agricultural Journal Series, Minnesota Agricultural Experiment Station, St. Paul, Minnesota 55108, USA.

[8] We wish to thank the following for providing specimens for this study: D. B. RICHMAN, Florida; P. J. CLAUSEN, E. F. COOK, W. S. HERMAN, and R. D. PRICE, Minnesota; M. H. MUMA, New Mexico; and J. K. HALLAN, Texas.

The Effect of Chemical Stimuli from Conspecifics on the Behavior of *Haplochromis burtoni* (Cichlidae, Pisces)

Behavioral responses of fish to chemical stimuli produced by conspecifics are well known (see, for example, PFEIFFER[1], TODD[2]). They have, however, rarely been investigated within the otherwise extensively studied family of Cichlid fish (KÜHME[3], MYRBERG[4]). This project was undertaken to analyze the effect of chemical stimuli, produced by conspecifics in a reproductive state, on the behavior of males of the African Cichlid fish *Haplochromis burtoni*.

The two stimuli used were: 1. water in which a gravid female has lived alone, 2. water in which a courting male has lived alone. Water in which no fish has previously lived was used as a control.

The experimental set-up consisted of 7 identical 60 liter aquaria. 4 were used for the test fish. The 5 th contained a single gravid female, the 6th a single male, courting the gravid female from which it was chemically, but not visually, isolated. Only individuals clearly in a reproductive state were selected. In order to ensure this, the specimens had to be replaced several times in the course of the experiment. The 7th aquarium provided the control water. The temperature was maintained in all aquaria at 26° ± 1 °C. The nets and buckets used for the manipulation of fish and water were boiled and dryed after each use to avoid contamination. The test fish were 6 adult males (7 in the gravid female situation) averaging in size approximately 15 cm long and 60 g. Before the beginning of the experiment, they were isolated from conspecifics for 10 days, with 10 young blinded Tilapia (HEILIGENBERG and KRAMER[5]). The same individuals were observed in all 3 experimental situations. The behavior recorded was that directed toward the Tilapia. Each fish was observed $1^1/_2$ h at the same time everyday.

The experiment started with a 5-day observation period. On the 6th day, 4 l of water from either the gravid female, courting male, or control water tank were syphoned into the experimental tank. These different stimuli were presented in a random order to the fish, and their source was unknown to the observer. The syphoning started at the beginning of the observation and lasted 40 min. The observations continued for 5 days to record possible long-term after-effects.

The observer, sitting behind a blind, recorded 8 behavior patterns, using a keyboard paper-tape punch (time resolution 0.5 sec) for subsequent computer evaluations (FERNALD and HEINECKE[6]).

The behavior patterns referred to are described as follows: *approaching*: the fish swims towards a Tilapia and stops nearby. *Courting*: the fish quivers sideways near a fish. This movement is normally directed to a conspecific female. *Leading*: the fish swims ostentiously, wagging its tail, in front of a fish. This is also normally directed to a conspecific female. *Attacking*: the fish bites or chases the Tilapia. *Digging*: the fish carries big amounts of gravel away from a preferred site of the aquarium. This results in a deep pit, normally used as 'spawning site'.

The greatest effects of the chemical stimuli were reflected in 3 behavior patterns: *approaching* and *courting* the Tilapia, where each occurence was recorded as single event; the presence or absence of the territorial *black eye-bar* (LEONG[7]) was scanned every 5 sec and the presence recorded as single event.

To measure the effect of the 2 stimuli, the rates of behavioral events on the 6th day and during the 5 post-test days were compared with those of the 5 pre-test days, defined as base-line activity, for each fish. For this, the data of each individual fish were normalized by subtracting the mean of an activity during the 5 pre-test days from every value of the activity observed in this fish. The mean values of these differences in all fish, and 3 times their standard error, were then plotted (see Figure) (since the intraindividual variances of the means for the 5 pre-test days were smaller than half the interindividual variances, they have been neglected in the presentation of the results).

[1] W. PFEIFFER, Experientia *19*, 1 (1963).
[2] J. H. TODD, Scient. Am. *224*, 98 (1971).
[3] W. KÜHME, Z. Tierpsychol. *20*, 688 (1963).
[4] A. A. MYRBERG JR., Z. Tierpsychol. *21*, 53 (1964).
[5] W. HEILIGENBERG and U. KRAMER, J. comp. Physiol. *77*, 332 (1972).
[6] R. FERNALD and P. HEINECKE, Behaviour *48*, 268 (1974).
[7] C.-Y. LEONG, Z. vergl. Physiol. *65*, 29 (1969).