was added. This cytokinin is approximately 10 times more active than kinetin⁹. The experiment also included 1 control group without any addition of test extract or 2 iP. Extracts of sea water collected during different seasons were tested, but only extracts from the samples collected during October had cytokinin-like activity (Table).

Callus grown on medium with sea-water extract had a larger number of shoots and bud-like proliferations than callus grown on medium without such additions. The proliferations contained tracheids and were covered with trichomes similar to normal tobacco leaves. Small but well-developed shoots were observed only in the series with highest amount of extract and in the series with 2 iP. The experiment was repeated and the same tendency was observed. It is well known that cytokinins induce shoot formation in tobacco callus? and the present results give evidence for cytokinin-like activity in the extracts used.

The effect of sea-water extract (October) on growth and bud formation of tobacco callus

Treatment	Fresh weight (mg)	Dry weight (mg)	No. of buds or bud-like projections	Cultures showing differ- entia- tion (%)
No additions	1230 ± 122	82 ± 7.6	0.5ª	40
0.1 ml extract	1710 ± 131	118 ± 11.0	1.1 b	60
0.5 ml extract	1690 ± 170	124 ± 14.5	1.0b	60
1.0 ml extract	1580 ± 148	112 ± 13.8	2.5 °	70
$5\times 10^{-7}M$ 2 iP	3020 ± 269	233 ± 20.8	More than 10°	100

The amounts of extract was added to 50 ml medium. Mean values from 10 cultures. *Only projections. *Projections, leaves and abnormal shoots. *Projections, leaves and abnormal and well developed shoots.

Ectocarpus confervoides grown in a bacteria-free culture liberates extracellular α -amino-nitrogenous compounds 10 . A series of papers provide data on the ability of the littoral algae to exude organic matter $^{11-13}$ Kinetin is needed for normal growth of Ectocarpus fasciculatus and Pylaiella littoralis in a defined medium 14 . These observations together with the results reported here, suggest that some part of the highly stimulating effect of sea water from the Fucus-Ascophyllum zone on algal growth depends on its content of naturally occurring cytokinins. Probably the cytokinins of the sea water are exuded from the littoral algae. Sea water collected during April and May, when the Fucus-Ascophyllum zone is poorly developed, contained no cytokinin-like activity.

Further work is now in progress to identify the naturally occurring cytokinins of the sea water from the *Fucus-Ascophyllum* zone.

Résumé. Les résultats indiquent la présence de cytokinines dans l'eau de mer de la zone de Fucus-Ascophyllum. Des cultures de tissus de Nicotiana ont été employés pour prouver l'activité des cytokinines.

Marianne Pedersén and G. Fridborg

Institute of Physiological Botany, University of Uppsala, Uppsala (Sweden), 10 May 1971.

- ⁹ F. Skoog, H. Q. Hamzi, A. M. Szweykowska, N. J. Leonard, K. L. Carraway, T. Fujii, J. P. Helgeson and R. N. Loeppky, Phytochemistry 6, 1169 (1967).
- ¹⁰ G. E. Fogg and G. T. Boalch, Nature, Lond. 181, 789 (1958).
- ¹¹ J. S. Craigie and J. McLachlan, Can. J. Bot. 42, 23 (1964).
- ¹² K. M. KHAILOV, Dokl. Akad. Nauk. SSSR 147, 1355 (1963).
- ¹³ І. МсN. Sieвurth, J. exp. mar. Biol. Ecol. 3, 290 (1969).
- ¹⁴ M. Pedersén, Nature, Lond. 218, 776 (1968).
- ¹⁵ This investigation was supported by grants from the Swedish Natural Science Research Council to Dr. Lisbeth Fries and to Dr. G. Fridborg.

Studies on Plant Extracts With Juvenile Hormone Activity. Effects of *Iris ensata* Thamb. (Iridaceae) on *Dysdercus koenigii* F. (Pyrrochoridae)

Recent progress in endocrinology has revealed that the insects can also be controlled by the use of hormones (WILLIAMS ^{1,2}), and the possibility of plants being one of the various sources of these third generation pesticides is rated high.

Although SCHMIALEK³ made the first observations regarding juvenile hormone activity of natural products, search for juvenile hormone analogues of plant origin received attention only after the discovery of an unknown factor with juvenile hormone activity in certain American paper products against the European bug, *Pyrrhocoris apterus*⁴. Later the activity was found to be due to some lipid soluble materials of certain pulp trees, chiefly the wood of Canadian balsam fir *Abies balsamea*^{5,6}. The active principles were isolated by Bowers et al.⁷, from Canadian balsam fir and Cerny et al.⁸ from Czechoclavakian balsam fir. Positive effects of coniferous plants on insect metamorphosis has also been reported by Carlisle and Ellis⁹, and high juvenile hormone activity has been

found in plants like Abies nordmanniana, Pseudotsuga menziesii glauca, Tsuga canadensis. 6 out of 52 plants reported by Williams and Robins 10, and 2 out of 60 plants tested by Slama 4, exhibited appreciable juvenile

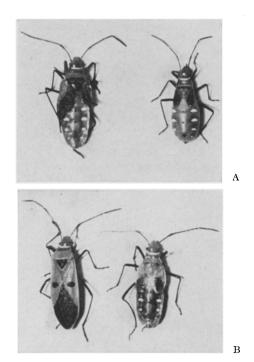
- ¹ C. M. WILLIAMS, Nature, Lond. 178, 212 (1956).
- ² C. M. WILLIAMS, Scient. Am. 217, 13 (1967).

 ³ P. SCHMALEK, 7. Naturforsch, 16h, 461 (1961).
- ³ P. Schmialek, Z. Naturforsch. 16b, 461 (1961).
- ⁴ K. Slama, Entomologia exp. appl. 12, 721, (1969).
- ⁵ K. Slama and C. M. Williams, Proc. natn. Acad. Sci., USA *54*, 411 (1965).
- ⁶ K. Slama and C. M. Williams, Biol. Bull. 130, 235 (1966).
- ⁷ W. S. Bowers, H. M. Fales, M. J. Thompson and C. Uebel, Science 154, 1020 (1066).
- ⁸ V. Cerny, L. Dolejs, L. Labler, F. Soim and K. Slama, Tetrahedron Lett. 12, 1053, (1967).
- ⁹ D. B. Carlisle and P. E. Ellis, Bull. ent. Res. 57, 405 (1967).
- ¹⁰ C. M. WILLIAMS and W. E. ROBBINS, Science 18, 791 (1968).
- ¹¹ K. N. SAXENA and C. B. WILLIAMS, Nature, Lond. 210, 441 (1966).

hormone activity. This paper reports the presence of juvenile hormone activity in a new plant called *Iris*

Material and methods. The plant was collected from Kashmir valley and dried in shade at a temperature of 25–30 °C for 30 days. Pseudobulbs along with roots were soxlet extracted in acetone. On concentration under reduced pressure, the extract yielded 3.7% ambercoloured, semi-solid residue. Similarly the leaf extract yielded 5.6% green residue.

Dysdercus koenigii F. was chosen as test insect and these were reared in 15 \times 12 cm glass jars containing water soaked cotton seeds in a 25° \pm 1°C control room programmed for a daily illumination of 14 h. For tests fifth instar nymphs were isolated soon after moulting and given no food except distilled water until used for testing.



Effect of acetone extract of *Iris ensata* on *Dysdercus koenigii* nymphs. A. Showing normal 5th instar nymph and adultoid. B. Adult and 6th instar nymph.

The test insects were treated with 10, 20, 40 and 80 µg doses of the total extracts of both the roots and leaves separately in acetone. The solution was applied with micro-applicator on mid dorsal region of the nymphs by the method of SAXENA and WILLIAMS ¹¹. All treatments were replicated. Food and water provided soon after the treatment to induce development.

Results and discussion. 98% of the individuals treated with 10 µg dose of the root extract moulted into adults while some of them appeared with crippled wings failing to cover the abdomen. 50% nymphs moulted to adultoids under the effect of 20 µg while 30% moulted into 6th instar nymphs under the effect of 40 µg. Under the influence of 80 µg, 70% nymphs moulted into sixth instar, 20% died during moulting and 10% adultoids were found. The development pattern in the sixth instar nymphs of treated individuals (Figure) showed resemblance with the pattern found by Bowers et al. 4 in their experiments with Pyrrhocoris apterus. The female adultoids failed to mate with adultoid males but mated with normal males although the egg laying in these cases was also abnormal, as 10-15 eggs were laid by a single female once or twice only. However, these eggs were found to be viable and the development of the nymphs hatched out was also normal. The leaf extract proved to be ineffective, even at higher concentrations.

From the above studies it is evident that *Iris ensata* contains principles with juvenile hormone activity. This plant is promizing for large scale trials and the raw material is available in plenty throughout the Kashmir valley.

Zusammenfassung. Acetonextrakt aus Wurzeln und Rhizomen von Iris ensata hemmen die Metamorphose von Dysdercus koenigii. Adultoide sind schwach fortpflanzungsfähig.

B.P. Saxena and J.B. Srivastava 12

Regional Research Laboratory, Jammu-Tawi (India), 20 April 1971.

¹² Acknowledgments: The authors are thankful to Dr. K. Ganapathi, Director, and Dr. A. Husain, Chairman, Discipline of Agricultural Sciences, Regional Research Laboratory, Jammu-Tawi, for the facilities provided for this work. Thanks are also due to Mr. Y. K. Sarin, Incharge, Plant Collection Unit, of the same laboratory for the identification and collection of the plant material.

Influence of Growth-Regulating Substances on Phymatotrichum omnivorum (Shear) Dugg

The importance of growth-regulating substances and their response in fungi have been studied by earlier workers, and they found considerable variation in stimulation as well as inhibition 1-3. Leonian and Lily 4, Bhargava 5 and Leelavathi 6 reported that inhibitory or toxic effect was found at higher concentrations of growth-regulating substances while there was no effect or stimulation at lower concentrations. The present study reports the effect of different concentrations of the three major groups of growth regulators on the vegetative growth of *Phymatotrichum omnivorum*.

The fungus was grown in the dark at 28°C in 125-ml Erlenmeyer flasks containing 50 ml of synthetic medium as a stationary culture?. There major groups of growth-

regulating substances namely indole acetic (IAA), gibberellic acid (GA) and kinetin (K) was used to study its influence on vegetative growth of the organism. Acetone was used as solvent for the growth regulators and added to the warm sterilized medium. Equal quantity of

- ¹ M. S. Murdia, Curr. Sci. 6, 362 (1939).
- ² R. R. Richards, Bot. Gaz. 100, 523 (1948).
- 3 A. R. WEINHOLD, F. F. HENDRIX and R. D. RABAE, Phytopathology 52, 757 (1962).
- ⁴ L. H. LEONIAN and V. G. LILY, Am. J. Bot. 24, 135 (1937).
- ⁵ K. S. Bhargava, Lloydia 9, 13 (1946).
- ⁶ K. M. LEELAVATHI, Can. J. Microbiol. 15, 713 (1969).