

by a methanolic solution of hyamine hydroxide according to a procedure similar to that of PASSMANN et al.¹², and subsequently counted. It was found that 10% of the metabolized gramine was incorporated into expired CO₂.

When the above experiment was repeated with the grain remains of five 62-day-old plants, no radioactive CO₂ was detected. Likewise no radioactivity was observed in the expired CO₂ when radioactive skatole¹³ (3-methyl-indole) was provided to barley under identical conditions to those described above.

The above experiments suggest that the alkaloid gramine is metabolized by excised barley shoots (grown in the dark), and that one of its metabolic pathways¹⁴ is the oxidation of its methylenic side chain to carbon dioxide. We are currently attempting to isolate intermediates of this oxidative pathway¹⁵.

Résumé. Nous avons observé que quand la gramine marqué au carbone attaché au noyau hétérocyclique a été administrée à des pousses d'orge âgées de 60 jours

(dans l'obscurité), 10% de la radioactivité a passé au CO₂ expiré et 0.4% au tryptophane.

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¹² J. M. PASSMANN, N. S. RADIN and J. A. D. COOPER, *Analyt. Chem.* 28, 484 (1956).

¹³ Radioactive skatole, tagged in the carbon attached to the ring, was synthesized from radioactive gramine⁸ according to the method of A. P. TERENT'EV, N. A. DZBANOVSKII and N. A. FAVORSKAYA, *Zh. Obshch. Khim.* 23, 2035 (1953), through *Chem. Abstr.* 49, 3124a.

¹⁴ Very little incorporation of gramine into the lipid fraction of the plant was observed.

¹⁵ This investigation was supported by a grant from the University Medical Research Fund for which one of us (G.A.D.) is grateful. We are grateful to Dr. C. I. ABOU-CHAAR for useful discussions.

Test-Tube Fertilization of Ovules in *Melandrium album* Mill. with Pollen Grains of Several Species of the *Caryophyllaceae* Family¹

The further improvement of the technique of test-tube fertilization may be useful to plant breeders in their efforts to obtain hybrids in those cases where the 2 parents do not cross due to certain obstacles in the path of the pollen tube^{2,3}. The present report concerns the development of hybrids derived from the ovules of *Melandrium album* fertilized with pollen grains of 5 different species.

Female flower buds, from which ovules were to be obtained for culture work, were bagged 4 days before pollination. Pistils with a short peduncle were sterilized in saturated chlorine water for 20 min and then rinsed 4 times with autoclaved water. Later the style and the ovary wall were removed and the ovules along with the placenta were inoculated on the medium consisting of WHITE's minerals⁴, WHITE's vitamins⁴ and 2% sucrose.

Anthers of the flower buds still closed (24 h before anthesis) were excised and kept for 2–4 h in the sterile inoculation chamber. Later the pollen grains were scooped out and dusted on the surface of the ovules. Anthers from the following species have been used: *Melandrium album*, *M. rubrum*, *Silene schafta*, *S. tatarica* and *Dianthus carthusianorum*.

The pollen grains of all species started to germinate within 8–12 h and later on the pollen tubes were entering the micropyle (Figure 1). During the first 3 days of culture the fertilized ovules enlarged, became turgid and in another 7 days they turned white (Figure 2). Dissections of seeds from 14-day-old cultures revealed normally differentiated embryos (Figure 3) and a perisperm fully packed with starch grains. When pollen grains of *Melandrium album*, *M. rubrum* and *Silene schafta* were used, mature embryos were dissected and cultured on a fresh medium. They started to germinate after 2 days and healthy seedlings were obtained by the fourth week (Figure 4). The 10-week-old seedlings, after thorough washing in water, were transferred to soil in pots and raised in the culture room. After another 12–15 weeks, regular flowers had been produced (Figures 5 and 6). Thus fully formed plants with flowers were obtained.

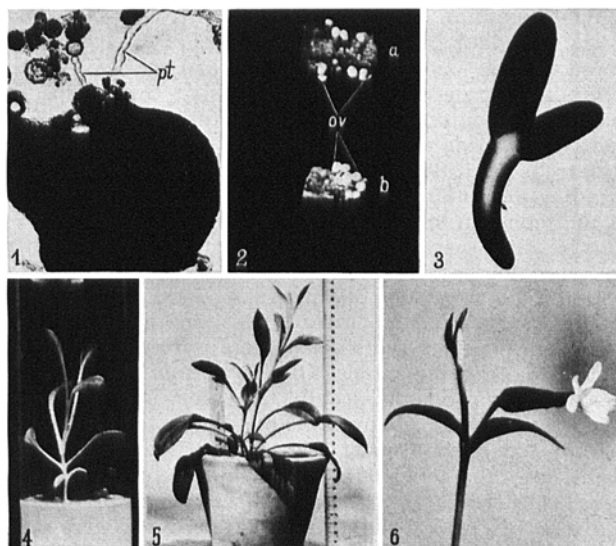


Fig. 1. Whole mount of ovule from 1-day-old culture; 2 pollen tubes (pt) are seen at the micropyle. Pollen grains of *Silene schafta*. $\times 50$.

Fig. 2. 10-day-old culture; developing ovules (ov) are situated on the placenta. (a) ovules fertilized with pollen grains of *Melandrium rubrum*. (b) ovules fertilized with pollen grains of *Dianthus carthusianorum*. $\times 1,2$.

Fig. 3. Whole mount of an embryo dissected from an ovule 14 days after culturing. Ovules fertilized with pollen grains of *Melandrium rubrum*. $\times 14$.

Fig. 4. A hybrid seedling obtained from a test-tube embryo (ovules were fertilized with pollen grains of *Silene schafta*) after 4 weeks of culture. $\frac{1}{2}$ natural size.

Fig. 5. A hybrid plant (*Melandrium* \times *Silene schafta*) after 10 weeks of growth in a soil where initially they were transferred from the culture tubes. $\frac{1}{16}$ natural size.

Fig. 6. A fully developed female flower from a hybrid plant (*M. album* \times *S. schafta*) after 12 weeks of growth. $\frac{1}{2}$ natural size.

¹ Dedicated to the memory of the late Professor P. Maheshwari who taught me and inspired me to undertake this work.

² K. KANTA, N. S. RANGASWAMY and P. MAHESHWARI, *Nature* 194, 4835 (1962).

³ M. ZENKTELER, *Naturwissenschaften* 52, 23 (1965).

⁴ N. S. RANGASWAMY, *Phytomorphology* 11, 1, 2 (1961).

When pollen grains of *Silene tatarica* were used, mature and normal embryos developed but they were not transferred to a fresh medium and their further growth was not examined.

A few of the ovules dusted with pollen grains of *Dianthus carthusianorum* were fertilized and young embryos (in the stage of young cotyledons) were developed. Due to a shortage of this material and high percentage of infections, we did not succeed in obtaining mature embryos. Further efforts will be undertaken in order to culture mature embryos and seedlings.

Detailed embryological and cytological studies of the above described experiments are carried out and the results will be reported elsewhere⁵.

Zusammenfassung. Samenanlagen von *Melandrium album* wurden mit Pollenkörnern der folgenden Arten

befruchtet: *Melandrium album*, *M. rubrum*, *Silene schafta*, *S. tatarica*, *Dianthus carthusianorum*. Es wird die weitere Entwicklung der Embryonen beschrieben.

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⁵ Acknowledgments: I wish to thank the head of the Department of General Botany Doc. Dr. A. SZWEYKOWSKA for the facilities provided during the course of this work and her interest in these investigations.

Orthomorpha sp. - a New Predatory Millipede on *Achatina fulica* in Andamans

While working on the biological control of *Achatina fulica*, the giant African snail, a species of millipede was found to be in association with snails at Port Blair in the South Andamans. It was suspected that the millipedes may be feeding on the snails; to investigate this, specimens of millipede were brought to the Indian Agricultural Research Institute, New Delhi. The millipede started breeding and multiplying in large numbers on the onset of the monsoon in July, in our snail-rearing laboratory. This breeding season actually coincided with the breeding season of the snails. A number of empty shells of *Achatina* (especially of the young ones) were observed to be present in the snail-rearing laboratory. This was not a normal feature in previous years when there were no millipedes in the snail-rearing rooms. One day, from a particular spot which was demarcated, all the empty shells of giant African snail were removed. Three days later it was observed that even at that spot from where the empty shells had been removed, several empty *Achatina* shells were lying - many of them full of millipedes of various age groups. Three snails, about 8 months old, were seen in a partly eaten state. This gave the clue that millipedes feed upon *Achatina*.

Laboratory experiments were laid out in jars: one jar containing soil, roots, leaves, millipedes and snails of about 1 cm shell length, and the other containing everything as in jar one except millipedes. This experiment was repeated 3 times and several replications were made. The number of millipedes and snails in each case were 50 and 5 respectively. It was observed that within 5 days the snails were eaten away.

After this, several 18 × 15.5 cm jars, open at both ends, were placed 6 cm deep in the soil and 100 millipedes were released on the soil surface enclosed by these jars in such a way that every alternate jar was without millipedes, to serve as a check. It was observed that even big-sized snails fell victim to the attack of millipedes, but they were never found attacking the actively moving snails. Certain millipedes are known to secrete poisonous chemicals like phenols¹ and hydrocyanic acid² from their stink glands, whose importance in the defence mechanism of the animal is already known. It was observed that when the snails were resting on the soil surface, the millipedes attacked them from below many at a time. It appears

that since large numbers of millipedes attack the snail several times, the secretion of the stink glands is enough to inactivate the snails to such an extent that ultimately they are not able to repulse the attacks, and the millipedes start feeding on them by scraping their body surfaces. It was further observed that in some cases the millipedes not only feed upon the snails but also make the shells their regular abode after they have fed upon the animal contained therein. Millipedes have been said to feed on the 'underground part of plants'³ and 'on decaying vegetable matter'⁴. They have also been said to be 'largely herbivorous'⁵, 'but also will eat animal matter'⁶ and sometimes dead worms, molluscs, insects, etc.⁷.

The millipede has been identified as *Orthomorpha* sp. (Order Proterospemphora, Family Strongylosomidae). Secretions of the stink gland have stood the sodium picrate and copper acetate-benzidine acetate tests for hydrocyanic acid. Details of the secretion and the mechanism of killing are under study⁸.

Résumé. Un mille-pieds, *Orthomorpha* sp., a été observé se nourrissant d'*Achatina fulica*. Ces Myriapodes injectent la sécrétion toxique de leur glande fétide dans les limaçons pour les immobiliser.

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³ M. S. MANI, *Introduction to Zoology* (Malhotra Bros. 60, Daryaganj, Delhi 1950), p. 341.

⁴ J. N. COMSTOCK, *Introduction to Entomology* (Comstock Publ. Co. Inc., Ithaca, N.Y. 1950), p. 17.

⁵ P. B. WEISZ, *The Science of Biology* (McGraw-Hill Book Co. Inc., New York, Toronto, London 1959), p. 750.

⁶ T. I. STORER and R. L. USINGER, *General Zoology* (McGraw-Hill Book Co. Inc., New York, Toronto, London 1957), p. 454.

⁷ J. C. CLOUDESLEY-THOMPSON, *Spiders, Scorpions, Centipedes and Mites* (Pergamon Press, London, New York 1958), p. 26.

⁸ The authors wish to express their gratitude to S. PRADHAN and N. C. PANT, Head of the Division of Entomology and Professor of Entomology respectively, for their keen interest in the progress of these investigations.