Fig. 1. Sperm release from a single 1.5-m-long *Verongia archeri* at 49 m.

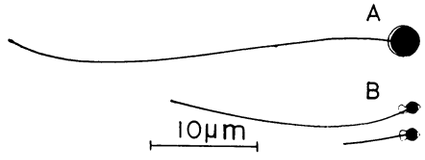

Fig. 2. Sponge sperm collected from effluent streams of (A) *Verongia archeri* and (B) *Geodia* sp. drawn to same scale.

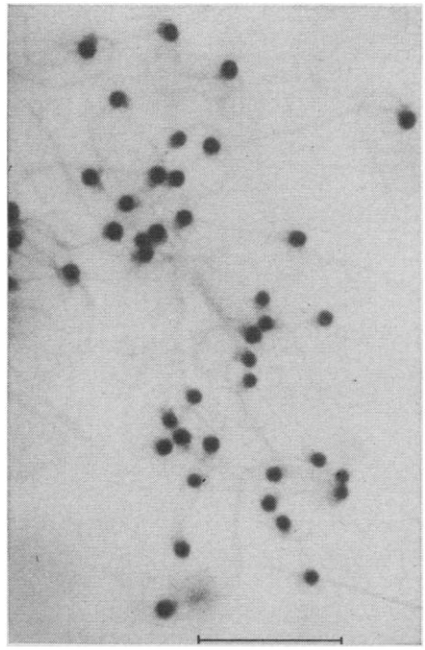

Fig. 3. Mature spermatozoa collected from effluent of *Geodia* sp. (scale, 10 μm).

samples. However, this was unquestionably another instance of sperm release. Within the area surveyed (100 by 100 m) the initiation of release spread from colony to colony down current. The activity spread at near-current velocities as sperm-laden water came into contact with successive colonies. By the end of the observational period all active colonies were still contributing to the 3-m-thick cloud which covered the entire observable reef.

The spermatozoa of *Verongia archeri* possess a head 2.6 μm in diameter which is almost completely filled by the uniformly dense-staining nucleus (Fig. 2A). This is followed by a tail 37 to 44 μm long without a recognizable midpiece intervening. The sperm of *Geodia* are slightly smaller but possess a more complicated structure (Fig. 2B). The head, 0.88 to 1.4 μm in diameter, is again almost entirely filled by the nucleus and is capped anteriorly by a short acrosomal cone. A midpiece sac of dimensions only slightly smaller is attached to the head at the junction of the 23- to 35-μm-long flagellum (*5*). No structural details are visible within the midpiece after normal staining with hematoxylin or toluidine blue.

Spectacular sudden release of huge numbers of spermatozoa seems to be normal for the Demospongiae since these observations concern species representing three orders of the two subclasses. The time of release may be related to new and full moon, but the data available are not sufficient to warrant statistical treatment. The presence of significant numbers of residual sperm in *Geodia* indicates that the sudden release of sperm is a repetitive event.

At the time of release of sperm by the *Neofibularia* population other sponges in the area were being observed over a long period for water pumping activity. The only species under constant observation, *Verongia* sp., underwent an abrupt decrease in activity to negligible values at this time. The depression continued for at least 2 days and slowly abated. The sudden release of huge numbers of gametes by large populations of sponges, as in the case of *Neofibularia* reported here and in the case of *Agelas* reported from Mexico (*2*), must constitute an environmental event of major significance to at least the other filter-feeding organisms of the reef and perhaps to the entire reef community.

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5. The released sperm described here are well within the great range of variability of sponge sperm described from tissue section materials [M. Leveaux, *Ann. Soc. Roy. Belg.* **73**, 33 (1942); O. Tuzet, *Arch. Zool. Exp. Gen.* **85**, 127 (1946); ——— and M. Pavans de Ceccatty, *Bull. Biol. Fr. Belg.* **92**, 331 (1958)]. The posterior projecting midpiece of *Geodia* sperm is somewhat similar to that reported only for the sponge *Halisarca dujardini* [C. Lévi, *Arch. Zool. Exp. Gen.* **93**, 1 (1956)].
6. The observations and laboratory work were carried out at the University of the West Indies and State University of New York Marine Laboratory at Discovery Bay, Jamaica. I thank the late Prof. T. F. Goreau and N. C. Copland for making the diving facilities and laboratory space available. I also thank Dr. W. D. Hartman for reviewing the manuscript, and R. A. Kinzie and E. A. Shinn for sperm collection and photography of the *Geodia* event. Supported by an NSF graduate student traineeship and a Sigma Xi grant in aid of research.

1 July 1970

Self-Inhibitor of Bean Rust Uredospores: Methyl 3,4-Dimethoxycinnamate

Abstract. *Two germination inhibitors from bean rust uredospores were identified as the* cis *and* trans *isomers of methyl 3,4-dimethoxycinnamate. They appear to be the "self-inhibitors" previously described from these spores.*

Uredospores of the rust fungi contain compounds which prevent germination unless removed, usually by flotation on water (*1*). These factors called "self-inhibitors" by earlier investigators were never identified (*2*). In a detailed study of the self-inhibitor from bean rust uredospores, Bell and Daly (*3*) showed that the inhibitor was extractable with water and appeared in two zones on

paper chromatograms. The partially purified inhibitor reduced germination 50 percent at a concentration of 2 μg/ml. We report here the isolation and identification of two germination inhibitors from water extracts of bean rust uredospores which have the properties of the self-inhibitors described by Bell and Daly (*3*).

Inhibitors were extracted from uredospores of the bean rust fungus *Uromyces phaseoli* (Pers.) Wint. by stirring in water (1 liter for every 10 g of uredospores). After filtration through sintered glass to remove the spores, the inhibitors were partitioned into diethyl-ether and taken to dryness on a rotary evaporator. The residue was then taken up in a small volume of ether, spotted onto thin-layer plates of silica gel, and chromatographed in a solvent consisting of benzene and ether (80 : 20 by volume). After extraction from silica gel with ether, the preparation was dried and sublimed under vacuum in a short path (5 mm) apparatus onto a condenser cooled with Dry Ice in acetone.

The active zone of inhibitor occurred on the silica-gel plates as a single ultraviolet fluorescent band at R_F 0.6 and was located by testing water extracts of 1-cm zones for the capacity to inhibit uredospore germination. Potency of the inhibitor was assayed at each step in the purification process by similar procedures.

The mass spectrum of the inhibitor showed the following diagnostic peaks [mass to charge (*m/e*); parent peak (P)]: 222 (P, base peak); 207 (P-CH_3); 191 (P-OCH_3); and 163 (P-$COOCH_3$). In addition, there were fragments at *m/e* 119, 105, 91, 77, and 39, all indicative of aromaticity. The infrared spectrum had absorption peaks at 1709 and 1692 cm^{-1} (ester carbonyl); 1590 and 1512 cm^{-1} (aromatic); 1305 cm^{-1} (methoxy); and 985 cm^{-1} (*trans*). The ultraviolet absorption spectra of the inhibitor dissolved in methanol had absorption maximums at 320, 291, and 232 nm, but the small quantities available prevented determination of extinction coefficients. The spectral data suggested that the inhibitor was the methyl ester of a dimethoxycinnamic acid. Comparison of the spectra of each of the six dimethoxycinnamate isomers clearly showed that methyl 3,4-dimethoxycinnamate was the only possible candidate.

While the masses and relative abundance of the various fragments in the mass spectrum of synthetic methyl *trans*-3,4-dimethoxycinnamate were identical to those of the unknown, their infrared spectra were inconsistent at several points unless the synthetic compound was first dissolved in methanol and irradiated for 3 hours with ultraviolet light at 254 nm. After irradiation of the synthetic compound, the infrared spectrum was identical to that of the inhibitor. This phenomenon and the appearance of a double carbonyl peak in the infrared spectrum suggested that some of the pure synthetic compound was converted to a second form during irradiation. Consequently, a further study of the inhibitor was carried out by gas chromatography with columns of Convalex-10 (5 percent on Chromosorb W) and by thin-layer chromatography on plates of cellulose irrigated with water.

As originally demonstrated by Bell and Daly (*3*), it was found that the inhibitor was composed of two compounds. These compounds had gas-chromatographic retention times (3.2 and 6.0 minutes) and R_F values (0.3 and 0.6) on cellulose plates that were identical to the components in the mixture obtained by irradiation of the synthetic compound. Each of these compounds could be converted to the other by irradiation with ultraviolet light, and the mass spectra of the isolated compounds prepared by the gas chromatography from the native inhibitor were identical to each other and to the two isomers prepared from irradiated synthetic methyl 3,4-dimethoxycinnamate (*4*).

The infrared spectrum and the melting point of 68°C (*5*) showed that the original synthetic methyl 3,4-dimethoxycinnamate was the *trans* isomer. After irradiation of this compound in methanol with ultraviolet light, the new compound which was produced was resolved from the *trans* isomer by gas chromatography. This new compound had a melting point of 91°C, which was reported previously for the *cis* isomer to be 92° to 93°C (*5*). In addition, the infrared spectrum of this compound lacked the absorption peak at 985 cm^{-1} (*trans*) and had a single carbonyl peak at 1709 cm^{-1}. An equimolar mixture of these two isomers provided an infrared spectrum which was identical to that of the inhibitor, and it is concluded that the inhibitor from bean rust uredospores is a mixture of methyl *cis*- and *trans*-3,4-dimethoxycinnamate.

The extinction coefficient ($E_{320}^{1\%}$) of methyl *trans*-3,4-dimethoxycinnamate was 790 in absolute methanol. With this value, it was determined that approximately 4 μg of native inhibitor can be extracted from each gram of spores by our procedures. Each stereoisomer is equally toxic, and germination of 50 percent of the spores (ED_{50}) is inhibited by 5×10^{-3} μg/ml. The ED_{50} values were determined from a plot of the probit of percent inhibition against log concentration. The native inhibitor and the synthetic esters were equally toxic to spore germination, since their ED_{50} values were identical.

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3 August 1970; revised 10 September 1970

Spontaneous in vitro Neoplastic Transformation of Adult Human Prostatic Epithelium

Abstract. *The possible significance of spontaneous transformation of epithelium from a benign prostatic adenoma containing glandular hyperplasia is discussed.*

A variety of normal human tissues have been propagated in tissue culture, but most, with the exception of hematopoietic tissue, have a limited life span in vitro. Hayflick (*1*) postulates that this finite life span reflects a rapid "aging" process that culminates in cell death. However, it is well known that both animal and human cells will survive indefinitely in culture if they become "transformed." Transformation is a rather loosely defined, irreversible cellular alteration mainly characterized by rapid cell growth, neoplastic morphol-