ways that will eventually establish the relations between these events and the better known electrical sequence.

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8. The electric fields of the reference (R) and target (T) beams at the photodiode are given by

$$\bar{E}_R\cos[(\omega - 2\pi f_0)t + \phi_R]$$

and

$$\bar{E}_T\cos[\omega t + \phi(t) + \phi_T]$$

where ω is the optical frequency and f_0 is the Bragg cell frequency (40 Mhz). The photocurrent is proportional to the intensity

$$i \sim E_R^2 + E_T^2 + 2\,\bar{E}_R \cdot \bar{E}_T$$
$$\cos[(\omega - 2\pi f_0)t + \phi_R]\cos[\omega t + \phi(t) + \phi_T]$$

Neglecting the d-c terms $E^2_R + E^2_T$ and the terms at 2ω, we have

$$i \sim \bar{E}_R \cdot \bar{E}_T\cos[2\pi f_0 t + \phi(t) + \phi_T - \phi_R]$$

Defining $\phi_0 = \phi_T = \phi_R$ yields the expression given in the text.
9. The reference sinusoid is given by

$$V_{ref}\,(\text{volts}) = 10\cos(2\pi f_1 t + \phi_0 + \pi/2)$$

The signal is

$$V_{sig} = 10\cos[2\pi f_1 t + \phi(t) + \phi_0]$$

and $f_1 = 100$ khz. The multiplier gives an output

$$V_{out} = V_{ref}V_{sig}/10$$

Hence

$$V_{out} = 10\,\{\cos[\phi(t) - \pi/2]$$
$$+ \cos[4\pi f_1 t + 2\phi_0 + \phi(t) + \pi/2]\}/2$$

The term at $2f_1$ (200 khz) is removed by the 50-khz low-pass filter shown in Fig. 1b, leaving

$$V_{out} = 5\cos[\phi(t) - \pi/2]$$
$$= 5\sin[\phi(t)]$$
$$\simeq 5\phi(t)$$

For amplitudes in water ≤ 100 Å, V_{out} is proportional to the amplitude within 1.2 percent; at 200 Å the error is 5 percent. If $x(t) = x_0\sin(\omega_s t)$, the spectrum of the output is given by

$$V_{out} = 10 \sum_{\substack{k=1 \\ k\ \text{odd}}}^{\infty} J_k\left(\frac{4\pi n x_0}{\lambda}\right)\sin(k\omega_s t)$$

where J_k is the Bessel function of order k. This expression is analogous to the Bessel function expansion for sinusoids [H. A. Deferrari, R. A. Darby, F. A. Andrews, *J. Acoust. Soc. Am.* **42**, 982 (1967); S. M. Khanna, J. Tonndorf, W. W. Walcott, *ibid.* **44**, 1555 (1968); P. R. Dragsten, J. A. Paton, R. R. Capranica, W. W. Webb, *ibid.* **60**, 665 (1976)].
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23 August 1976; revised 12 November 1976

Elemental Sulfur: Accumulation in Different Species of Fungi

Abstract. *Sulfur, in elemental form, is present in several fungi especially in self-inhibited and resting structures such as dormant spores and sclerotia. The possible importance of sulfur in fungal spore dormancy is discussed.*

Pezet and Pont have shown that elemental sulfur (S_8) is responsible for self-inhibition in *Phomopsis viticola* Sacc., that is, the failure of the alpha spores to germinate (*1*). Recently, elemental sulfur was discovered in sporocarps of the ectomycorrhizal fungus *Pisolithus tinctorius* (*2*). Several self-inhibitory mechanisms have been described for different fungi (*3*) and the problem of dormancy of fungal structures has been discussed by several investigators (*4*, *5*), but free sulfur has never been implicated in self-inhibition or spore dormancy. Dormancy of fungal spores is widespread and presumably plays an important role in evolution (*6*). Dormant spores have a reduced metabolic activity. They are thus more resistant to external unfavorable factors such as drying, high temperatures, and other deleterious environmental influences. If no dormant stage were present, germination would take place and the potential of the fungus for multiplication or dispersal would be reduced (*7*). We report here on an examination of several dormant or self-inhibited structures of different fungi, from Myxomycetes to Basidiomycetes, for the presence of elemental sulfur.

Information on fungal origin, culture media, and the particular fungal struc-

Table 1. Results of tests for the presence of elemental sulfur in different species of fungi. Medium 1 consisted of 20g of Tryptone, 3 g of yeast extract, 20 g of glucose, 4 g of KH_2PO_4, 1.2 g of $CaCl_2 \cdot 2H_2O$, 1.2 g of $MgSO_4 \cdot 7H_2O$, 0.12 g of $FeCl_2 \cdot 4H_2O$, 0.17 g of $MnCl_2 \cdot 4H_2O$, 0.07 g of $ZnSO_4 \cdot 7H_2O$, 7 g of citric acid, 0.5 mg of hemin chloride per milliliter of medium in 1 percent NaOH, 2 percent agar, and 2000 ml of water (*p*H adjusted to 4.6). Medium 2 consisted of 2 percent agar in water. Medium 3 consisted of 2 percent malt extract and 2 percent agar in water. Reactions were as follows: +, positive; −, negative.

Genus or species	Medium or natural substrate	Structures tested	Method of analysis	
			TLC	H_2S
Physarum polycephalum	Medium 1	Sclerotia	+	+
Spongospora subterranea	Potato tubers	Spore balls	+	+
Albugo candida	Shepherd's purse	Sporangia	+	+
Pythium ultimum	Medium 2	Oogonia	+	+
Botrytis cinerea	Medium 3	Conidia	+	+
Botrytis cinerea	Medium 3	Sclerotia	+	+
Erysiphe convolvulii	Leaves of small bindweed	Perithecia	−	−
Podosphaera leucotricha	Leaves of apple trees	Oidia	+	+
Gnomonia comari	Medium 3	Sporulating pycnidia	+	+
Pestalozzia sp.	Medium 3	Sporulating acervuli	+	+
Nectria cinnabarina	Deadwood	Stromata	+	+
Cytospora sp.	Medium 3	Sporulating pycnidia	+	+
Sclerotinia sclerotiorum	Medium 3	Sclerotia	+	+
Phomopsis viticola	Medium 3	Sporulating pycnidia	+	+
Phomopsis sclerotioides	Medium 3	Sclerotia and pycnidia	+	+
Monilia fructigena	Medium 3	Microconidia	+	+
Diatrype sp.	Medium 3	Perithecia	+	+
Eutypa armeniacae	Medium 3	Sporulating pycnidia	+	+
Claviceps purpurea	Rye	Ergot (sclerotia)	+	+
Melanconis sp.	Shoots of grapevine	Perithecia	+	+
Coniella diplodiella	Medium 3	Sporulating pycnidia	+	+
Septoria nodorum	Sterilized wheat	Sporulating pycnidia	+	+
Neurospora crassa	Medium 3	Conidia	+	+
Neurospora crassa (fluffy)	Medium 3	Microconidia	−	−
Gliocladium roseum	Medium 3	Conidia	+	+
Trichoderma viride	Medium 3	Conidia	+	+
Rhizoctonia solani	Medium 3	Sclerotia	+	+
Typhula ishikariensis	Sterilized wheat	Sclerotia	+	+
Ustilago hordei	Barley	Chlamydospores	+	+
Tilletia caries	Wheat	Chlamydospores	+	+
Puccinia graminis	Barberry	Aeciospores	+	+
Gymnosporangium juniperi	Pine	Teliospores	−	−
Schizophyllum commune	Medium 3	Sporocarps and basidiospores	+	+
Agaricus bisporus	Commercial fungi	Young sporocarps	−	−

tures analyzed is summarized in Table 1. We extracted the sulfur by mixing the fungi in chloroform for 10 minutes. We removed the fungal structures by filtration and concentrated the chloroform extract under vacuum at 30°C.

Two analytical methods were used to test for elemental sulfur. In the first method the chloroform extract was analyzed by thin-layer chromatography (TLC) according to the following procedure: 10 to 50 μl of the extract was deposited on a KHF_{254} TLC plate. The solvent system was chloroform. In some extracts natural fungal pigments were mixed with sulfur, and cyclohexane-chloroform (99 : 1) was used to ensure a better separation. In each case a sulfur standard in chloroform was deposited with the fungal extract and was cochromatographed under the same conditions. The R_f of free sulfur varied between 0.85 and 0.90. Triphenyltetrazolium chloride (4 percent in methanol) mixed with an equal amount of sodium hydroxide solution (4 percent in methanol) was sprayed on the plates and developed for 10 minutes at 110°C. Sulfur, in the S_8 form, develops a red spot in the presence of this reagent.

The second method of analysis was based on the reaction of free sulfur with cysteine to produce H_2S (*8*). The chloroform extract was evaporated under vacuum to dryness, and the residue was dissolved in 1 ml of hot absolute ethanol. Then 1 ml of a cysteine solution (0.25*N*, *p*H 6.8) was added to the ethanol extract. The mixture was placed in a sulfide test tube (Fig. 1). This apparatus was placed in a thermostatically controlled water bath and gently shaken at 37°C for 1 hour. Then two drops of concentrated H_2SO_4 were added. The temperature of the bath was increased to 55°C and maintained at this temperature for ½ hour. The test was considered positive if the sulfide-sensitive paper became black. The reaction was more or less intense depending on the quantity of free sulfur present. In cases where both the TLC analysis and the sulfide test were positive, we concluded that the fungal structures contained free sulfur.

The presence of elemental sulfur in a great number of fungi, from Myxomycetes to Basidiomycetes (Table 1), especially in the self-inhibited and dormant structures, is important because these dormant fungal organs (spores, chlamydospores, sclerotia) characteristically have a reduced respiratory capacity (*5*). Miller *et al.* (*9*) and Tweedy and Turner (*10*) determined that, when free sulfur is added to fungal spores, it acts as a hydrogen acceptor in hydrogenation and dehydrogenation reactions, particularly in the terminal respiratory chain between cytochrome b and cytochrome c, with a concomitant production of H_2S. This production of H_2S could account for the low respiratory capacity. For example, Schmit and Brody have demonstrated that conidia of *Neurospora crassa* are dormant spores with a low metabolic rate and an endogenous respiratory capacity lower than that of mycelia (*11*). We found that these conidia contain free sulfur (Table 1). Elemental sulfur has never been detected in vegetative hyphae of *Phomopsis viticola*; it appears at the stage where pycnidia differentiate. Therefore, a relationship seems to exist between dormancy and the presence of elemental sulfur.

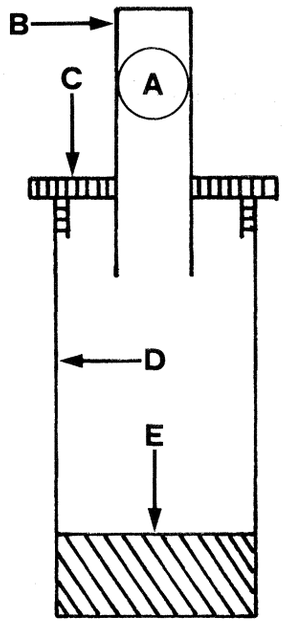

Fig. 1. Hydrogen sulfide test tube: sulfide-sensitive paper (*A*), small glass tube (*B*), polyethylene cap (*C*), large glass tube (*D*) containing the solution to be analyzed (*E*).

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12. We thank Drs. W. J. Moller, U. Ross, and D. Gindrat and Prof. G. Turian for critical reading of this manuscript and for constructive suggestions. We thank M. L. Chappuis for technical assistance.

19 August 1976; revised 8 October 1976

Cucumber Mosaic Virus Associated RNA 5: Causal Agent for Tomato Necrosis

Abstract. *A small replicating RNA, encapsidated with and dependent on, but not part of the viral genome, modifies disease expression depending on the host. In tomato plants, it causes a lethal necrotic disease which is probably the same as that which, in 1972, destroyed most of the field tomato crop in large regions of the French Alsace.*

In 1972, field tomato plants in large regions of the French Alsace were stricken with a severe necrotic disorder of epidemic proportions. As a result, almost the entire field tomato harvest in that part of France was annihilated (*1*). Prior to 1972, this "tomato necrosis" disorder had only been observed sporadically, as limited outbreaks, in different parts of France (*2*). In 1974, conclusive evidence was given that the disorder was of viral etiology and somehow associated with cucumber mosaic virus (CMV) infection. Several well-characterized cloned strains of CMV were shown capable of inducing tomato necrosis in greenhouse experiments (*3*). However, because the more characteristic CMV symptoms of chlorosis and "fern leaf" syndrome (*4*) were also observed in these experiments, and because the incidence of necrosis was variable, its precise relationship to CMV was unresolved. Several explanations were suggested to account for this variability: (i) variable growing conditions of the infected plants; (ii) the possibility of there still being mixtures of necrotic and non-necrotic CMV strains in the inoculums; (iii) instability of the inoculated CMV strains and their subsequent mutation; (iv) varying states of CMV's divided genome (*5*) in the inoculums or in the infected plants.