A gas emitted by *Neurospora crassa*

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We investigated whether gases other than carbon dioxide are produced by *N. crassa*. A peak corresponding to ethylene has been detected using gas chromatography. Mass spectroscopy, however, indicated that the gas produced might be carbon monoxide which, surprisingly, migrated with the same chromatographic retention time as ethylene. Our results emphasize the need for caution when interpreting results based solely on gas chromatographic data.

Several observations support the idea that compound(s) are formed during sexual development in Neurospora which inhibit the subsequent fertilization and/or sexual development of other regions in a mating culture. Initially, Howe and Prakash (1969, Can. J. Genet. Cytol. **11**:689-705) demonstrated in *N. tetrasperma* and in *N. crassa* that when macroconidia of the opposite mating type were applied to one side of a mycelial lawn containing protoperithecia, perithecia failed to form on other parts of the same lawn. Howe and Prakash assumed that the effect was the result of a product diffusing through the hyphae and/or the medium. Later, Calhoun and Howe (1972, Planta **108**:289-302) attributed the inhibition primarily to exhaustion of nutrients by the first cross. In a recent study, Metzenberg (1993, Fungal Genet. Newsl. **40**:83) reported that an inhibitory effect across the plate is also seen when the agar medium on two sides of the plate is physically divided by cutting away a strip of agar. This result suggested that a gaseous compound may also play a role in inhibition of the mating process. However, transmission across an air-gap is not always seen. George Bistis did not find it (personal communication), and the effect has been somewhat erratic in our own hands. The effect probably depends on hard-to-standardize conditions like the tightness of the Petri dish lid.

We became interested in the nature of any gases produced by Neurospora. Several gaseous messengers, including ethylene, carbon monoxide and nitric oxide, have been previously implicated in various functions in different biological systems. We examined the gas profile in the headspace over a culture of *N. crassa*. The *fl* P-A strain was inoculated onto 5 ml of solidified Vogel's minimal salts medium supplemented with 1.5% sucrose or onto solidified Westergaard-Mitchell crossing medium in 50 ml flasks. Cultures were incubated for up to 5 days at room temperature (about 23 C) and sealed at intervals with a rubber stopper 2 h prior to withdrawing air samples. The samples (1 ml) were analyzed using a gas chromatograph equipped with an alumina column and a flame ionization detector. Several peaks were observed under both growth conditions. A standard of ethylene gas migrated to the same position as a prominent unknown peak, with a retention time of about 80 seconds.

To test whether ethylene had an effect on sexual development in the fungus, we applied increasing concentrations (up to 1,000 p.p.m.) in different stages of the mating process. The experiments were done in sealed chambers with a constant flow of air-ethylene mixtures. *fl* ^{P}-A was used for observation of pre-fertilization effects. Post-fertilization effects of ethylene were

monitored by challenging *fl* ^{P}-A lawn with conidia of ORS-a. Ethylene, even at a concentration as high as 1,000 p.p.m, had no effect on the production of protoperithecia or the development of perithecia or ascospores.

In light of these results, we wondered whether the characteristic chromatographic peak, assumed to be ethylene by us and by others, was correctly identified. Surprisingly, when we analyzed a standard of carbon monoxide, it migrated with exactly the same retention time as did an ethylene standard and as the unidentified gas produced by *N. crassa*. To further study the gas produced by the fungus, we used the more rigorous technique of mass spectroscopy. Analysis of gas mixtures at the level of one or a few p.p.m.can present complications. Ethylene and carbon monoxide both have a nominal molecular mass of 28, as does nitrogen (78% or 780,000 p.p.m. in air), and the huge nitrogen peak intefered with our ability to detect other gases in this molecular weight range. To reduce drastically the nitrogen levels in the headspace above the fungal cultures, we flushed the cultures with a mixture of argon (79%) and oxygen (21%) prior of sealing the flasks. This greatly reduced the nitrogen concentration and allowed separation of the different gases with molecular weights of about 28, i.e., residual $14N_2$ (MW = 28.01508), 1H212C=12C1H2 (MW = 28.04024), and 12C16O (MW = 28.00386). Our results suggest that no ethylene is being produced by *N. crassa* under the conditions tested (Vogel's minimal medium), whereas an increase in carbon monoxide above the basal air level was observed.

We must leave open the question whether carbon monoxide or other gases are involved in the mating process in *N. crassa*. Carbon monoxide is known to be produced by virtually all organisms during the breakdown of heme proteins. Our results point out the need for caution when interpreting results based on gas chromatographic data. Ethylene has been proposed to function in different fungal systems, but its identification has often been based solely on retention time in gas chromatography. At least in Neurospora, the conclusion that ethylene is produced would have been unwarranted.

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