Hydrogen cyanide was released in an appreciable amount only from the *M. sativa* filtrate. Lack of activity with the *M. falcata* filtrate suggests that this material may have a lower glycoside content or, alternatively, contains a β-glycosidase inhibitor or a detoxification mechanism for cyanide. It appears likely therefore that production of hydrogen cyanide in infected alfalfa plants in the field is due to β-glycosidase activity on the part of the fungus and the provision of cyanogenic substrates by the host. The difference in response of the two filtrates is of particular interest since *M. falcata* is comparatively resistant to winter crown rot in the field.

Further work is being carried out, and detailed results will be reported in due course.

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Ethylene Production by Cytoplasmic Particles from Apple and Tomato Fruits in the Presence of Thiomalic and Thioglycolic Acid

Despite several tracer studies[1,2] and the use of very sensitive techniques for the detection of ethylene[3,4], there has been no clear indication of a biosynthetic pathway that can evolve ethylene in fruits. Spencer[5] reported ethylene production by cytoplasmic particles from tomato fruit in the presence of malate and co-factors. This suggested that the biosynthesis of ethylene could be related to the metabolism of organic acids in the cells. Tracer experiments by Burg[2], however, showed no evidence linking ethylene evolution to Krebs cycle metabolism. Meigh *et al.*[4] could not detect ethylene in incubation mixtures containing apple mitochondria, malate and co-factors. The latter experiments are especially significant since they used apple mitochondria known to metabolize Krebs-cycle substrates, and the system for detecting ethylene was considerably more sensitive than that used in any of the other investigations. It therefore appears that ethylene synthesis in fruits must be associated with some other aspect of organic acid metabolism.

Recently we have isolated cytoplasmic particles, from apples and tomato fruits, that evolve ethylene in the presence of thiomalic and thioglycolic acid. Cysteine, glutathione, methionine, and other related compounds (thiodiglycolic acid, α-monothioglycerol, β-mercaptopropionic acid, carboxymethylmercaptosuccinic acid) did not replace the requirement for thiomalic or thioglycolic acid. Results of a number of experiments with particles from apples are summarized in Table 1. Similar results were obtained with particles from tomato fruit.

These ethylene-producing systems have many characteristics of an enzymatic process. The rate of ethylene production is proportional to the concentration of the particles. The rate, during a 45-min. period, is approximately proportional to the length

Table 1. Ethylene Production by Cytoplasmic Particles from Apples in the Presence of Thiomalic and Thioglycolic Acid

Flask content*		Ethylene† (μl./mgm. N/hr.)
Thiomalic	+ co-factors + no particles	0·0
Thioglycolic	+ ,, + no particles	0·0
Thiomalic	+ ,, + particles	0·019
Thioglycolic	+ ,, + particles	0·016
Thiomalic	+ ,, + denatured particles	0·0004
Thiomalic	+ ,, − PO_4 + particles	0·004
Thiomalic	+ ,, + DIECA + particles	0·003
Thiomalic	+ ,, + DIECA + 10^{-5} *M* copper sulphate + particles	0·006

* The flask contents consisted of the following: 50 μm. thiomalic or thioglycolic acid, 3 μm. adenosine triphosphate, 60 μm. phosphate, 0·08 μm. coenzyme-*A*, and 400 μm. sucrose. 1 ml. of particulate suspension containing approximately 1·5 mgm. nitrogen was added to each flask. The flasks were incubated 2 hr. at 30° C. in an automatic shaker. The *p*H of the reaction mixture was 6·2.

† Ethylene was determined by gas chromatography with a system previously described (ref. 4). The method was sensitive to 3×10^{-5} μl. of ethylene in a 1-ml. sample.

of the incubation, and the reaction is essentially inhibited by heat denaturation. The reaction appears to be stimulated considerably by phosphate. Furthermore, some chelating agents that inhibit enzymatic reactions, such as diethyldithiocarbamate (DIECA) and ethylenediamine tetraacetic acid (EDTA), markedly inhibited this reaction and the inhibition is partially reversed by the addition of copper to the system.

It is noteworthy that these preparations did not oxidize Krebs cycle substrates. Another important difference between mitochondria and these particles is in the *p*H of the isolating media. Whereas active mitochondrial particles are isolated from homogenates of neutral or slightly alkaline media, it was found necessary to isolate these particles from homogenates of acid media. Particles that cause the evolution of ethylene were obtained from fractions precipitated at 500*g*, 16,000*g* and 40,000*g*.

These findings offer a completely new approach to the study of ethylene biosynthesis. Work is now in progress on the purification of the enzyme or enzymes and the elucidation of the mechanism of the reaction by tracer techniques.

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ENTOMOLOGY

Ribonucleic Acids in the Moult Cycle of an Insect

It is well known that the cuticle in insects is shed at the end of each instar and a new one is formed by the underlying epithelium. Although much is known about the histological changes in the epithelium and the chemical changes in the cuticle during this complicated process of moulting[1,2], nothing is known about the role of the ribonucleic acids (RNA) during