This paper illustrated how the reduction of acetylene can be employed as an index of N₂-fixation in situ, in aquatic environments, in soils, and by nodulated plants. Ethylene produced from acetylene can be measured gas chromatographically after 5 seconds to 30 minutes of exposure to N₂-fixing agents to act as a test. The equation is used in this paper has been cited in over 535 publications.

Acetylene Reduction to Measure Biological Nitrogen Fixation

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The nitrogenase enzyme system is versatile and reduces a number of substrates other than N₂. This was first recognized when M.M. Mozen and R.H. Mozen reported that N₂O was reduced by nitrogenase. Later, it became clear that protons were reduced to H₂ by nitrogenase. Robert Schülhorn was a postdoc from Bonn, Federal Republic of Germany, in my lab in 1965. He was intrigued by the N₂O reduction process, and his thoughts about possible reduction of other compounds with comparable bondings led him to test ethylene and azide. Both were reduced by nitrogenase. The ethylene formed from acetylene could be measured readily by gas chromatography. Schülhorn first recorded inhibition of nitrogenase by C₂H₂ on August 9, 1965.

We first reported these observations on October 1, 1965, at an informal meeting at the University of California-Davis Field Station. There a dozen or so avid "nitrogen-fixers" met and reported their latest observations. The possibilities of using C₂H₂ reduction as a measure of nitrogenase was discussed. The first investigators to report actual application of the method were R. Koch and H.J. Evans.1

An interesting aspect of the C₂H₂ reduction story is that it was discovered independently and more or less simultaneously by Mike Dilworth in Niederland, Western Australia. Mike had been a postdoc in my lab the year before, but I have no recollection that we ever discussed the possibility that C₂H₂ would inhibit or be reduced by nitrogenase. In December 1965, Mike had written on November 30, 1965, in which he stated: "I found about two months ago that acetylene is a very potent inhibitor of nitrogen fixation with Clostridial extracts using either pyruvate or H₂ as the substrate... To my great surprise, I found that clostridial extracts can reduce C₂H₂ with H₂-the product is ethylene... The exciting possibility that all requirements for C₂H₂ reduction are the same as for N₂ reduction." Mike recalled that Dr. Malcolm Winfield some years earlier had suggested acetylene as a possible inhibitor of N₂ fixation. A series of unsuccessful experiments finally led Mike to brush off this old suggestion and to try it as a new avenue of research.

Mike and Schülhorn never met until March 1982. Mike has continued as an active contributor to N₂ fixation research, but after leaving here, Schülhorn left the field, returned to the Federal Republic of Germany, and later moved to Berlin. He came to the 7th International Conference on N₂ Fixation in Cologne, and there I had the pleasure of introducing him to Mike.

Citation of our paper is not because it is a limnological landmark, but because it describes a simple and highly sensitive means for detecting N₂ fixation both in the laboratory and in the field. The Michaelis constant for C₂H₂ is about 0.01 atm, and for N₂, about 0.1 atm. Hence, in the field one can ignore the N₂ in the exposure vessel, inject 10 percent C₂H₂, expose the system for a few minutes, inactivate the system, return the vessel to the lab, inject a sample of the gas into a flame ionization gas chromatography unit, and have the separation completed in a couple of minutes. C₂H₂ can be detected in the picomole range. One should calibrate the method by exposing samples to 15N₂ under the same conditions as the C₂H₂ exposure. Many investigators have been careless about such calibration, and have used a facile but invalid conversion factor of 3 C₂H₂ to 1 N₂. Despite such a quantitative limitation, the acetylene reduction method has been very useful and still is widely accepted in studies of N₂ fixation.

Authors of the cited paper were Bill Stewart of the University of Dundee, Scotland, and George P. Fitzgerald. As indicated in the May 1, 1990, issue of Nature, "Professor Bill Stewart, Secretary of the Agricultural and Food Research Council, will be the government's next chief scientific advisor." Bill has been a major contributor to biological N₂ fixation, and as a visiting scientist in my lab he was easier to test the C₂H₂ method on algae and other systems. I believe he was a colleague on campus with a particular interest in following the seasonal blooms of algae on Lake Mendota. The history of the development of the C₂H₂ reduction method has been recorded in more detail.