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Stewart W D P, Fitzgerald G P & Burris R H. In situ studies on N_2 fixation using the acetylene reduction technique. *Proc. Nat. Acad. Sci. USA* 58:2071-8, 1967.
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This paper illustrated how the reduction of acetylene can be employed as an index of N_2-fixation *in situ*, in aquatic environments, in soils, and by nodulated plants. Ethylene produced from acetylene could be measured gas chromatographically after 5 seconds to 30 minutes of exposure of N_2-fixing agents to acetylene. [The *SCI*® indicates that 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 N_2. This first was recognized when M.M. Mozen and I[1] reported that N_2O was reduced by nitrogenase. It later became clear that protons were reduced to H_2 by nitrogenase. Robert Schöllhorn was a postdoc from Bonn, Federal Republic of Germany, in my lab in 1965. He was intrigued by the N_2O reduction process, and his thoughts about possible reduction of other compounds with comparable bonding led him to test acetylene and azide. Both were reduced by nitrogenase.[2] The ethylene formed from acetylene could be measured readily by gas chromatography. Schöllhorn first recorded inhibition of nitrogenase by C_2H_2 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 C_2H_2 reduction as a measure of nitrogenase was discussed. The first investigators to report actual application of the method were B. Koch and H.J. Evans.[3]

An interesting aspect of the C_2H_2 reduction story is that it was discovered independently and more or less simultaneously by Mike Dilworth in Nedlands, 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 C_2H_2 would inhibit or be reduced by nitrogenase. In December I received a letter 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 H_2 as the substrate.... To my great surprise, I found that clostridial extracts can reduce C_2H_2 with H_2—the product is ethylene.... The exciting part is that all requirements for C_2H_2 reduction are the same as for N_2 reduction." Mike recalled that Dr. Malcolm Winfield some years earlier had suggested acetylene as a possible inhibitor of N_2 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.[4]

Mike and Schöllhorn never met until March 1988. Mike has continued as an active contributor in N_2 fixation, but after leaving here, Schöllhorn left the field, returned to the Federal Republic of Germany, and later moved to Berlin. He came to the 7th International Conference on N_2 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 N_2 fixation both in the laboratory and in the field. The Michaelis constant for C_2H_2 is about 0.01 atm.[5] and for N_2 about 0.1 atm. Hence, in the field one can ignore the N_2 in the exposure vessel, inject 10 percent C_2H_2, expose 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. C_2H_2 can be detected in the picomole range. One should calibrate fixation by exposing samples to $^{15}N_2$ under the same conditions as the C_2H_2 exposure. Many investigators have been careless about such calibration and have used a facile but invalid conversion factor of 3 C_2H_4 to 1 N_2. Despite such a quantitative limitation, the acetylene reduction method has been very useful and still is widely accepted in studies of N_2 fixation.

Authors of the cited paper were Bill Stewart of the University of Dundee, Scotland, and George P. Fitzgerald. As indicated in the May 3, 1990, issue of *Nature*,[6] "Professor Bill Stewart, Secretary of the Agricultural and Food Research Council, will be the government's next chief scientific adviser." Bill has been a major contributor in several areas of biological N_2 fixation, and as a visiting scientist in my lab he was eager to test the C_2H_2 method on algae and other systems. Fitzgerald 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 C_2H_2 reduction method has been recorded in more detail.[7]

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2. Schöllhorn R & Burris R H. Study of intermediates in nitrogen fixation. *Fed. Proc.* 25:710, 1966. (Cited 120 times.)
3. Koch B & Evans H J. Reduction of acetylene to ethylene by soybean root nodules. *Plant Physiol.* 41:1748-50, 1966. (Cited 135 times.)
4. Dilworth M J. Acetylene reduction by nitrogen-fixing preparations from *Clostridium pasteurianum*. *Biochim. Biophys. Acta* 127: 285-94, 1966. (Cited 350 times.)
5. Schöllhorn R & Burris R H. Acetylene as a competitive inhibitor of N_2 fixation. *Proc. Nat. Acad. Sci. USA* 58:213-6, 1967. (Cited 135 times.)
6. Aldhous P. UK chief scientist—greener advice? *Nature* 345:3, 1990.
7. Burris R H. The acetylene-reduction technique. (Stewart W D P, ed.) *Nitrogen fixation by free-living micro-organisms*. Cambridge, England: Cambridge University Press, 1975. Vol. 6. p. 249-57. (Cited 10 times.)