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# Time Course of Acetylene Reduction in Nodules of Five Actinorhizal Genera[1]

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## ABSTRACT

**The rate of acetylene reduction was measured as a function of time after addition of 10% acetylene in *Alnus, Casuarina, Ceanothus, Datisca,* and *Myrica*. The maximum rate occurred after 45 to 60 seconds and was maintained for an additional 0.5 to 4 minutes before a decline in rate to 30 to 90% of the maximum. The rate then recovered to a value of 63 to 98% of the maximum. Removal of the shoot and lower roots did not affect nodule activity.**

The reduction of acetylene to ethylene has been widely used as an assay for nitrogenase activity in root nodules. However, in many legume nodules, there is a decline in the rate of ethylene formation after only a few minutes of exposure to acetylene. It appears that the predecline rate of ethylene formation gives the most accurate measurement of nitrogenase activity (3). Detopping of plants and removal of the rooting medium by shaking can also reduce activity (2).

We undertook the present studies to see if similar phenomena occur in actinorhizal nodules, which are structurally different from legume nodules and contain a different endophyte (4). We found that actinorhizal nodules differ greatly from legume nodules in their response to both disturbance and exposure to acetylene.

## MATERIALS AND METHODS

Plants were grown either in water culture (250 ml wide-mouth plastic bottles) or in tubes filled with vermiculite (30-ml plastic syringe barrels), using one-fourth strength, nitrogen-free Hoagland's solution. The water level in the water cultures was kept below the region of nodules, with a gas space of about 125 ml and liquid volume of 150 ml. *Myrica gale* was inoculated with Frankia strain LLR161101, *Alnus rubra* with strain HFPArI3, *Casuarina cunninghamiana* with strain HFPCcI3, while *Datisca gomerata* and *Ceanothus americanus* were inoculated with crushed nodules of Ceanothus. The plants grown on vermiculite were kept in a growth chamber at a constant temperature of 23°C with a photoperiod of 17 h and light intensity of 300 $\mu$mol $m^{-2}$ $s^{-1}$ (400–700 nm). The conditions for the water cultures were the same, except the day temperature was 22°C and the night temperature was 20°C.

For the cumulative acetylene reduction assay (Fig. 1), plants were grown in small pots (6.5 cm diameter, 5 cm height of vermiculite) which were sealed in 600 ml beakers with a plastic lid and modeling clay. After adding 10% acetylene, gas mixing was maintained by sliding and shaking of the potted plants within the beaker before gas sampling.

For flow-through measurements of acetylene reduction, gas mixtures of 10% acetylene, air, and $O_2$ were made in Saran bags. Sufficient $O_2$ was added to keep the $O_2$ concentration at the atmospheric level of about 20%. A peristaltic pump (Cole-Parmer No. J-7520-25) pumped the gas mixture from the bag through a humidifier and then past the root system. A flow rate of 210 ml $min^{-1}$ was used unless otherwise noted. Gas exiting the root system was sampled with a 3-ml plastic syringe and was analyzed for acetylene and ethylene after storage in the syringe for 0 to 30 min. Analysis was with a gas chromatograph equipped with a flame ionization detector and a sampling loop. Just before the introduction of acetylene, humidified air was pumped through the root system at 210 ml $min^{-1}$ for a period of about 10 min. Throughout the assay the plants were kept in the growth chamber in which they had grown, so the only perturbation to the system caused by the assay was the introduction of 10% acetylene.

The methodology required that experiments be done with one plant at a time. Time courses for typical plants that were intermediate between the extremes are shown in the figures.

Simultaneous measurements of $CO_2$ evolution and acetylene reduction were by gas chromatography as previously described (5). The nodulated zone of water-cultured or vermiculite-grown plants was excised by cutting off the shoot and all non-nodulated roots extending away from the nodules. This reduced the size of the tissue so that it could be placed in a plastic syringe barrel with an internal volume of 12 ml. A flow rate of 31 ml $min^{-1}$ was used.

## RESULTS

When acetylene reduction by intact plants of *M. gale* growing in vermiculite was measured in a closed container, there was a decrease in the rate of ethylene accumulation after about 5 min. This was followed by a gradual recovery of the rate to a value approaching the original rate (Fig. 1). Similar results were obtained with a flow-through system that allowed more rapid and accurate measurements of rate (Fig. 2).

Considerable variation was found within a given experiment, with some plants showing a much greater decline in activity than others (Fig. 2). This variability was reproducible: when the same plant was assayed on successive days the extent of the acetylene-induced decline was similar (data not shown). The reason for the variability between plants could not be found. All plants were of the same age and size, and there was no correlation with time of day or time after watering.

Somewhat different results were obtained for plants growing in water culture. The acetylene-induced decline was greater and the time for recovery of activity was longer (compare Figs. 2 and 3). It also took a longer time to reach the initial maximum activity in the water-cultured plants, but this was because the rooting

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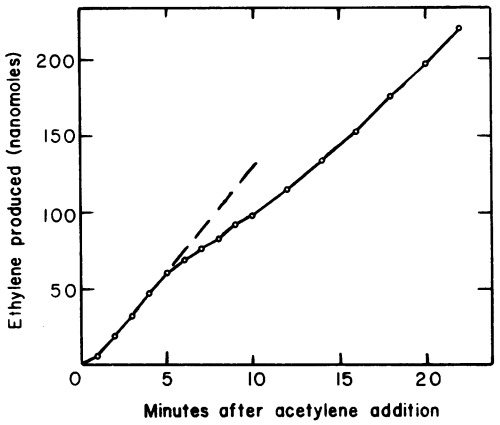


FIG. 1. Acetylene reduction by a potted plant of *M. gale* in a closed container. Typical result (see "Materials and Methods") (1 of 3 replicates).

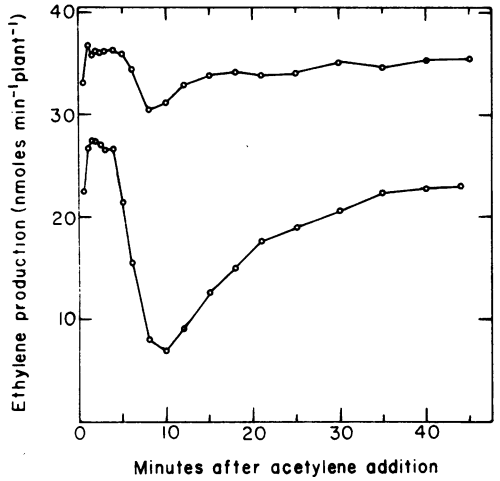


FIG. 2. Acetylene reduction by *M. gale* analyzed with a flow-through system. The results for two plants were selected from 5 replicates to show the greatest and least fluctuation in rate.

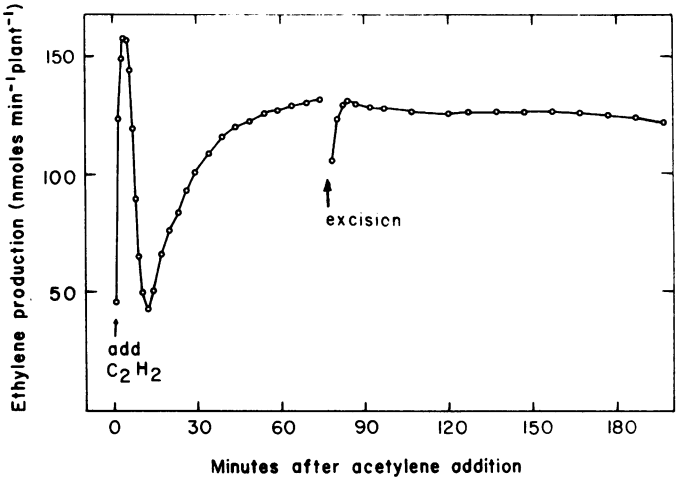


FIG. 3. Acetylene reduction by a *M. gale* plant growing in water culture. At the time marked "excision" the plant was detopped and the roots below the zone of nodules were excised. Typical result (1 of 4 replicates).

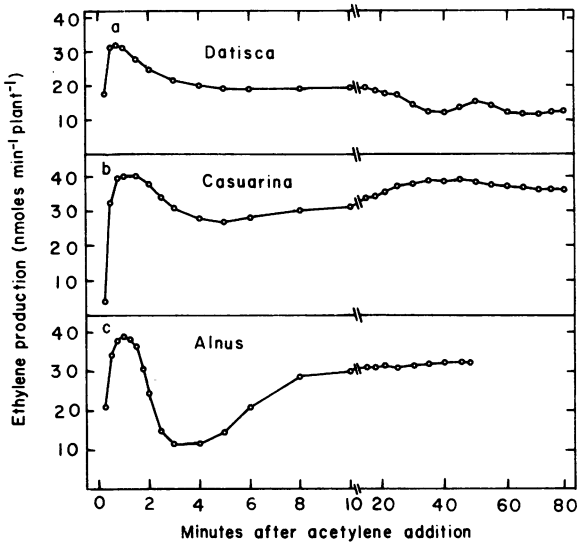


FIG. 4. Acetylene reduction by three genera of actinorhizal plants. Typical result (1 of 5 or 6 replicates per genus).

volume assayed was much larger relative to the gas flow rate. Thus it took about 15 s for the acetylene concentration in the gas outflow in Figure 2 to reach 90% of the inflow concentration, whereas in Figure 3 the 90% value was reached in about 2 min.

Other actinorhizal plants also displayed an acetylene-induced decline followed by a recovery (Fig. 4). However the initial period of maximal activity was much shorter than in *M. gale* (compare Figs. 2 and 4). Moreover, in *D. glomerata*, the recovery was slight and followed by periods of additional decline and recovery. These differences are summarized in Table I.

In some cases the acetylene-induced decline in activity was rather small. This was the case for all of a group of one-year-old plants of *C. americanus* (Fig. 5). Thus the magnitude of the acetylene-induced decline was highly variable, being almost negligible in some instances (Figs. 2 and 5), and very large in others (Figs. 2 and 3).

In legume nodules, the rate of $CO_2$ evolution declines markedly along with the rate of acetylene reduction after 5 min of exposure to acetylene (3). This was not found in nodules of *M. gale*. In 6 experiments (1 plant each) the decline in the acetylene reduction rate was 61.0 ± 5.0% (± SE), while the decline in the $CO_2$ evolution rate was only 11.6 ± 2.8%.

*M. gale* nodules also differed from legume nodules in showing little or no response to plant disturbance (Fig. 3).

## DISCUSSION

While there is a decline in the rate of acetylene reduction within minutes of the addition of acetylene in both legume and actinorhizal nodules, actinorhizal nodules differ in that activity recovers after the decline to a rate that is generally between 79% and 98% of the predecline maximum (Table I). However in *D. glomerata* the activity recovered to only 63% of the maximum rate and this was followed by a further decline (Fig. 4a).

In legumes, the introduction of acetylene is thought to increase the resistance to oxygen diffusion into the nodule, thus decreasing the respiration rate (2). However, the introduction of acetylene had only a minor effect on the respiration rate of nodules of *M. gale*, so this is not a satisfactory explanation of our results. Since actinorhizal nodules differ from each other in the time course of their response to acetylene addition (Figs. 2–5), the explanation of the fluctuation in rate of acetylene reduction may be complex.

All actinorhizal nodules reached the initial maximum rate of acetylene reduction at 45 to 60 s under standard assay conditions

Table I. *Characteristics of the Time Course of Acetylene Reduction in Some Actinorhizal Plants*
Values are means ± SE. Plants are grown in vermiculite unless otherwise noted.

| Species | Maximum $C_2H_2$ Reduction Rate | Time of Maximum Rate | Time of Minimum Rate | Minimum Rate/ Maximum Rate | Recovered Rate[a]/ Maximum Rate | No. of Plants Assayed |
|---|---|---|---|---|---|---|
| | *μmol min⁻¹g⁻¹ dry wt* | *min after $C_2H_2$ addition* | | *ratio* | *ratio* | |
| *M. gale* (water culture) | 1.38 ± 0.13 | 4.38 ± 0.24 | 11.75 ± 0.25 | 0.30 ± 0.04 | 0.79 ± 0.03 | 4 |
| *M. gale* | 1.37 ± 0.10 | 2.65 ± 0.15 | 10.40 ± 1.21 | 0.67 ± 0.07 | 0.88 ± 0.03 | 5 |
| *C. cunninghamiana* | 1.95 ± 0.14 | 1.15 ± 0.10 | 6.00 ± 0.55 | 0.55 ± 0.11 | 0.98 ± 0.02 | 5 |
| *A. rubra* | 3.20 ± 0.26 | 1.13 ± 0.06 | 3.67 ± 0.33 | 0.47 ± 0.07 | 0.87 ± 0.03 | 6 |
| *D. glomerata* | 2.21 ± 0.39 | 0.70 ± 0.05 | 5.20 ± 0.86 | 0.51 ± 0.03 | 0.63 ± 0.02 | 5 |

[a] The maximum $C_2H_2$ reduction rate during the recovery phase that followed the minimum.

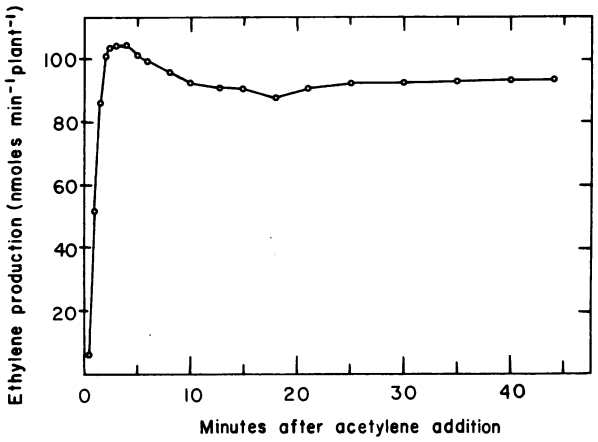


FIG. 5. Acetylene reduction by a 1-year-old plant of *C. americanus*. The total root chamber volume was 130 ml, rather than 30 ml as in Figure 2 and 4. Typical result (1 of 4 replicates).

(Figs. 2 and 4). Thus differences in the speed of acetylene diffusion into the nodule and ethylene diffusion out do not appear to explain the differences in nodule response to acetylene.

We find that the nodulated zone of *M. gale* roots can be excised from the plant without significant loss of nitrogenase activity by the nodules. Previous workers have sometimes noted large effects of disturbance on the activity of both legume and actinorhizal nodules (1, 2). At least part of the reason for the difference is that our plants were growing in water culture with the water level maintained below the nodule zone. Thus there was little possibility of injury to the nodule or changes in nodule aeration or extent of external water films when the root system was excised.

Overall these results demonstrate that the acetylene reduction assay must be used with caution in actinorhizal nodules, because of a decline in rate that begins from 1 to 5 min after the introduction of acetylene. However, in some nodules the decline is small and in many nodules activity recovers to rates that approach the predecline values. Such a recovery of activity has not been reported in legume nodules.

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