NITROGEN FIXATION BY WOODY PLANT SPECIES AS MEASURED BY ACETYLENE REDUCTION ASSAY

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BY

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Technical Paper

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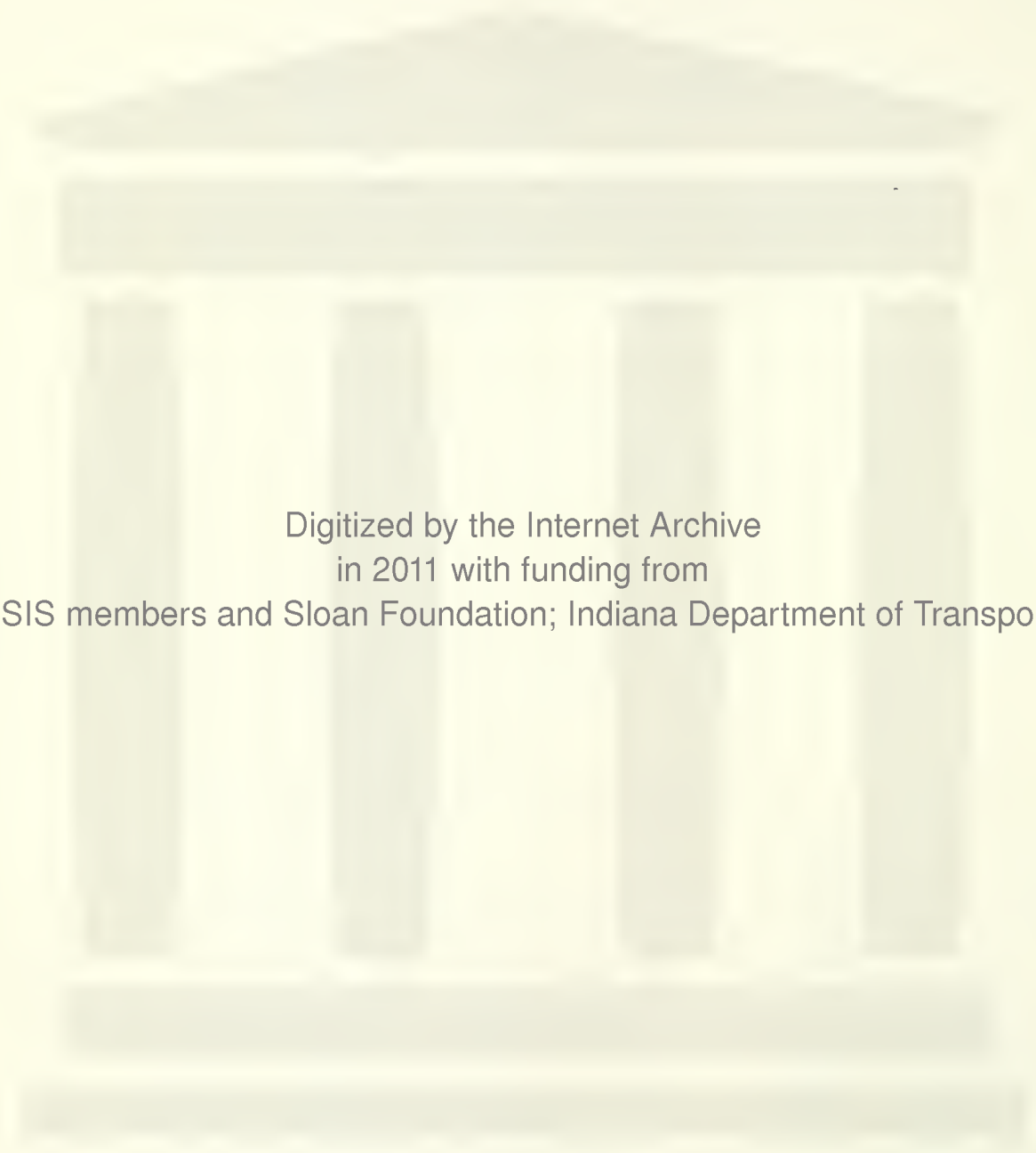

Technical Paper

NITROGEN FIXATION BY WOODY PLANT SPECIES AS MEASURED BY ACETYLENE REDUCTION ASSAY

TO: J. F. McLaughlin, Director
Joint Highway Research Project

June 13, 1973

Project: C-36-48C

FROM: H. L. Michael, Associate Director
Joint Highway Research Project

File: 9-5-3

The attached is a Technical Paper from Part II of the HPR Research Project "Research in Roadside Development and Maintenance". This Paper is titled "Nitrogen Fixation by Woody Plant Species as Measured by Acetylene Reduction Assay" and has been authored by Messrs. Robert E. McNiel and Philip L. Carpenter. It is from the Interim Report published in July 1972 as J.H.R.P. Report Number 13.

The Paper is submitted for approval of publication in the Journal of the American Society for Horticulture Science. It will also be submitted to the ISHC and the FHWA for comment and information.

Respectfully submitted,

Harold L. Michael
Associate Director

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Nitrogen Fixation by Woody Plant Species as Measured by Acetylene Reduction Assay [1]

Robert E. McNiel and Philip L. Carpenter [2]

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Abstract. Nodules from 13 woody plant species were analyzed by acetylene reduction assay to determine their nitrogen fixation activity. Activity was found with nodules from Caragana arborescens Lam., Robinia fertilis Ashe., R. hispida L., R. pseudoacacia L., Alnus glutinosa (L.) Graetn., Elaeagnus umbellata Thunb., E. angustifolia L., Shepherdia argentea Nutt., S. canadensis Nutt., Hippophae rhamnoides L., and Myrica pensylvanica Lois. In addition, nodules from A. glutinosa were found to exhibit nitrogen fixation activity for at least 9 hours after having been excised from the plant. No active nodules were found on Comptonia peregrina (L.) Coult. or Cercis canadensis L.

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[1] Received for publication . Journal paper no. Purdue University Agricultural Experiment Station, West Lafayette, Indiana. Prepared as part of an investigation conducted by Joint Highway Research Project, Engineering Experiment Station, Purdue University in cooperation with the Indiana State Highway Commission and the U. S. Dept. of Transportation Federal Highway Administration.

[2] Graduate Research Assistant and Associate Professor of Horticulture, respectively.

Symbiotic nitrogen fixation occurs most abundantly in the Leguminosae although not all species have nodules. Approximately one-tenth of the 12,000 species of Leguminosae are known to develop nodular structures (1).

Symbiotic nitrogen fixation also occurs with less known frequency among 8 additional families containing 13 genera and 342 species, 118 of which are known to develop root nodules (13). The 13 genera include: Alnus, Arctostaphylos, Casuarina, Ceanothus, Cercocarpus, Coriaria, Discaria, Dryas, Elaeagnus, Hippophae, Myrica, Purshia, and Shepherdia.

Nodular material on most legumes is spherical or cylindrical in shape with few or no lobes, while the individual nodule of nonlegumes are clusters of finger-like branches (6). Nodules of legumes are generally thought to be annual. The nonlegume nodules are perennial and with age may attain a diameter of several centimeters (6). However, in nodules from Myrica and Casuarina, the tip of the branch continues to produce a root of normal structure without nitrogen-fixing characteristics (7).

Nodules were identified on nonlegumes as early as 1892 (8). Early methods used to determine if nodule-bearing plants fixed nitrogen included determining the N-content of the foliage on nodulated versus nonnodulated plants (15). Also, N-content of the foliage was determined for plants grown in solution-culture lacking N (11). Later, a method using ^{15}N permitted the analysis of the quantity of nitrogen transformed by the following species: A. glutinosa (3,4), H. rhamnoides (3), S. canadensis (5), and Comptonia peregrina (17).

With the development of the acetylene reduction assay (12), a method became available which permits rapid measurement of nitrogen fixation by nodules. The technique involves the reduction of acetylene to ethylene. The reduction of N_2 to NH_3 is believed to occur in a similar manner since the nitrogenase involved with nitrogen fixation has a range of specificity. Nodules from Alnus rugosa (DuRoi) Spreng., A. glutinosa, Myrica gale L., and Comptonia peregrina have been determined to be active by this method (14, 16).

The present study, part of a search for N-fixing species for use in highway plantings, was to determine the nitrogen-fixing capabilities of several leguminous and nonleguminous woody plants.

Materials and Methods

Nitrogen-fixation capabilities were evaluated in 13 species. The species included 5 legumes (Robinia hispida, R. fertilis, R. pseudoacacia, Caragana arborescens, and Cercis canadensis) and 8 nonlegumes (Elaeagnus umbellata, E. angustifolia, Shepherdia argentea, S. canadensis, Hippophae rhamnoides, Alnus glutinosa, Myrica pensylvanica, and Comptonia peregrina). Fixation was determined by acetylene reduction assay as determined by Davidson [3] for those species that produced nodules.

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[3] Davidson, F. 1970. The influence of rhizobial strain and soybean variety on leghemoglobin, hematin, nodule weights, and nitrogen fixation. Ph. D. Thesis, Purdue University, West Lafayette, Indiana.

Plants were grown in the greenhouse in a sphagnum peat: horticultural grade perlite mixture (1:1 v/v) or in the field. In no case was there an attempt to inoculate a plant that did not bear nodules.

With nonlegumes, analyses were performed on detached nodules from plants 1 to 12 years old. Branched nodules were divided and only the tips were used; the base of the branch or center part of the cluster was discarded. Most legume nodules were left intact but if too large to enter an 18 ml culture tube, they were divided. All nodule tips were washed with tap water to remove soil particles and then blotted dry in a paper towel prior to weighing.

Samples of detached nodules were generally taken during mid-morning during the summer and fall of 1971. Of the various dates when samples were observed, Sept. 9 proved to be the most active for each species and only this date is reported.

Results and Discussion

Nitrogen fixation in legumes: Nodules obtained from 4 of the 5 species investigated (Caragana arborescens, Robinia fertilis, R. hispida, and R. pseudoacacia) reduced acetylene and therefore had N-fixing capability (Fig. 1). Each reduced acetylene over a period of several hours which is longer than reported by Aprison (2) who found 2 hr to be the maximum for excised soybean nodules. Cercis canadensis did not fix nitrogen since it does not form nodules (20).

Nitrogen fixation by nonlegumes: Of the 8 species investigated, 7 (A. glutinosa, E. umbellata, E. angustifolia, S. argentea, S. canadensis, H. rhamnoides, and M. pensylvanica) nodule-forming nonlegumes reduced acetylene and were classed as N-fixing species (Fig. 2). Comptonia peregrina had the remains of nodules which were no longer active. The length of activity of detached nodules was determined with A. glutinosa. Nodules detached for 8 hr still reduced acetylene during the next sampling hour (Fig. 3). This data agrees with data of Bond (3).

Nodule activity: There were color differences of nodules within and between species. Color ranged from white to orange and from dull to bright. Within a species, brightness of the nodule irrespective of color was observed to be associated with high acetylene reduction. An example using E. angustifolia is shown in Fig. 4.

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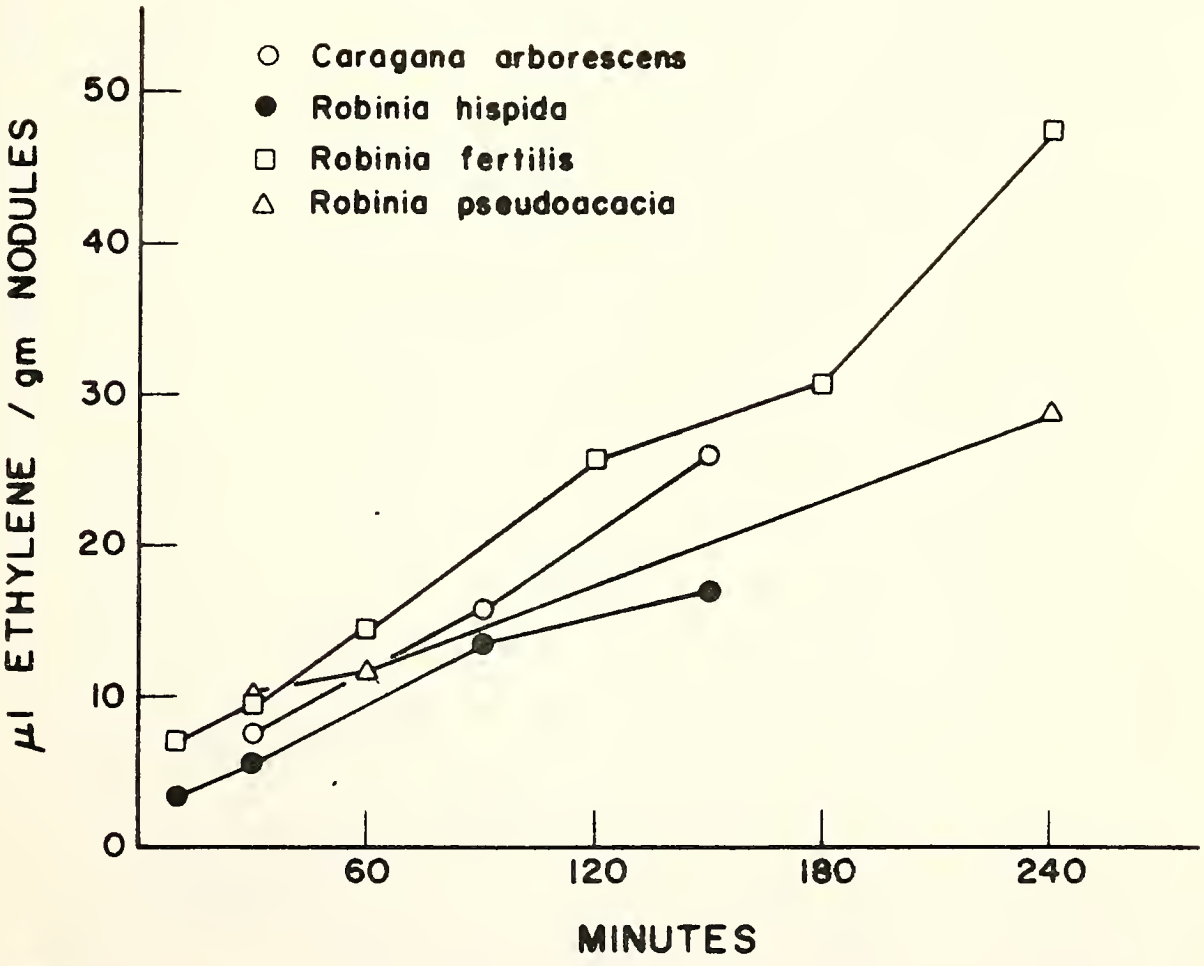

Figure 1. Reduction of acetylene by nodules from field grown Caragana arborescens and Robinia hispida and from greenhouse grown Robinia fertilis and R. pseudoacacia on September 9, 1971.

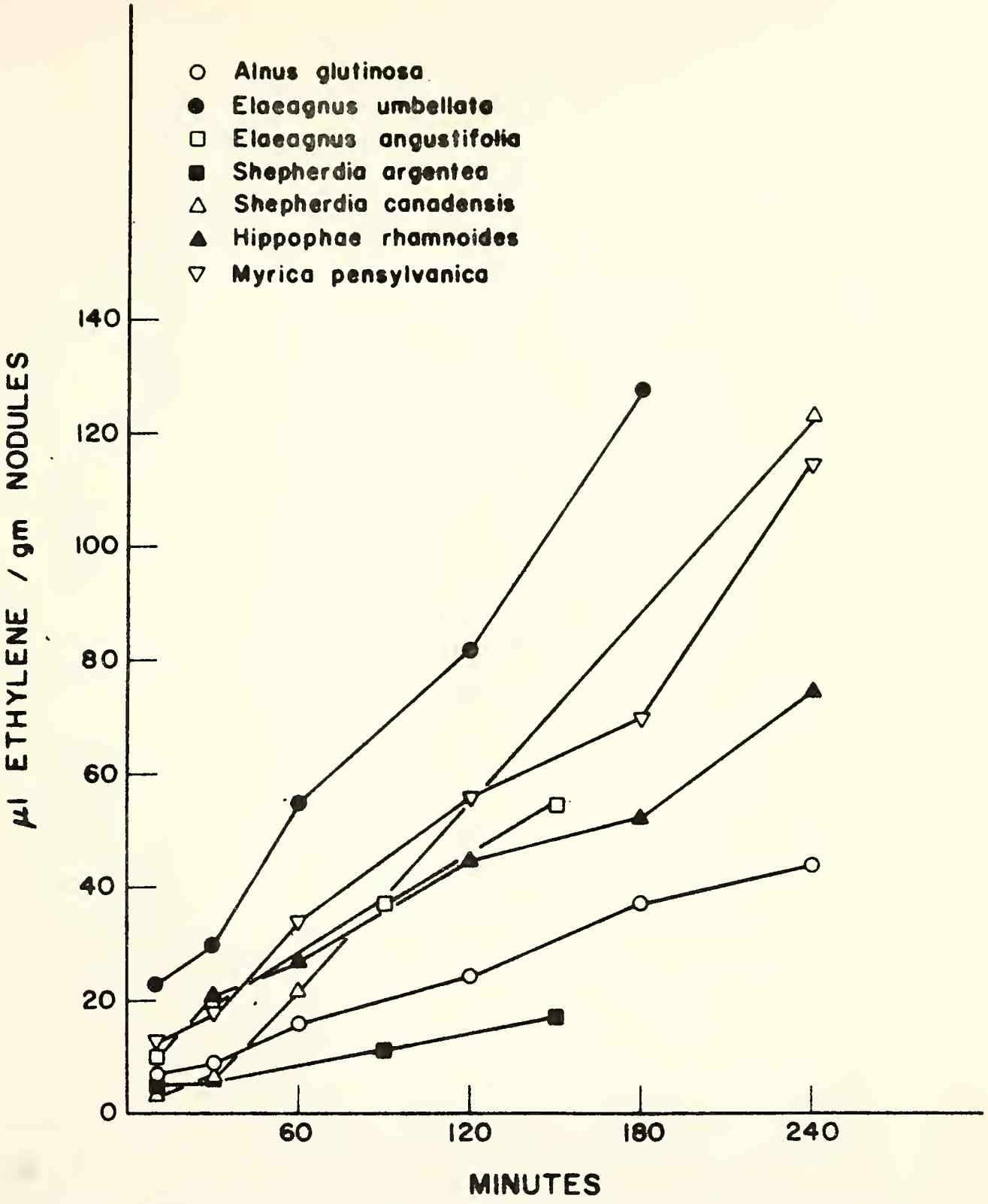

Figure 2. Reduction of acetylene by nodules from field grown Elaeagnus angustifolia, Myrica pensylvanica, and Shepherdia argentea and from greenhouse grown Alnus glutinosa, Elaeagnus umbellata, Hippophae rhamnoides, and Shepherdia canadensis on September 9, 1971.

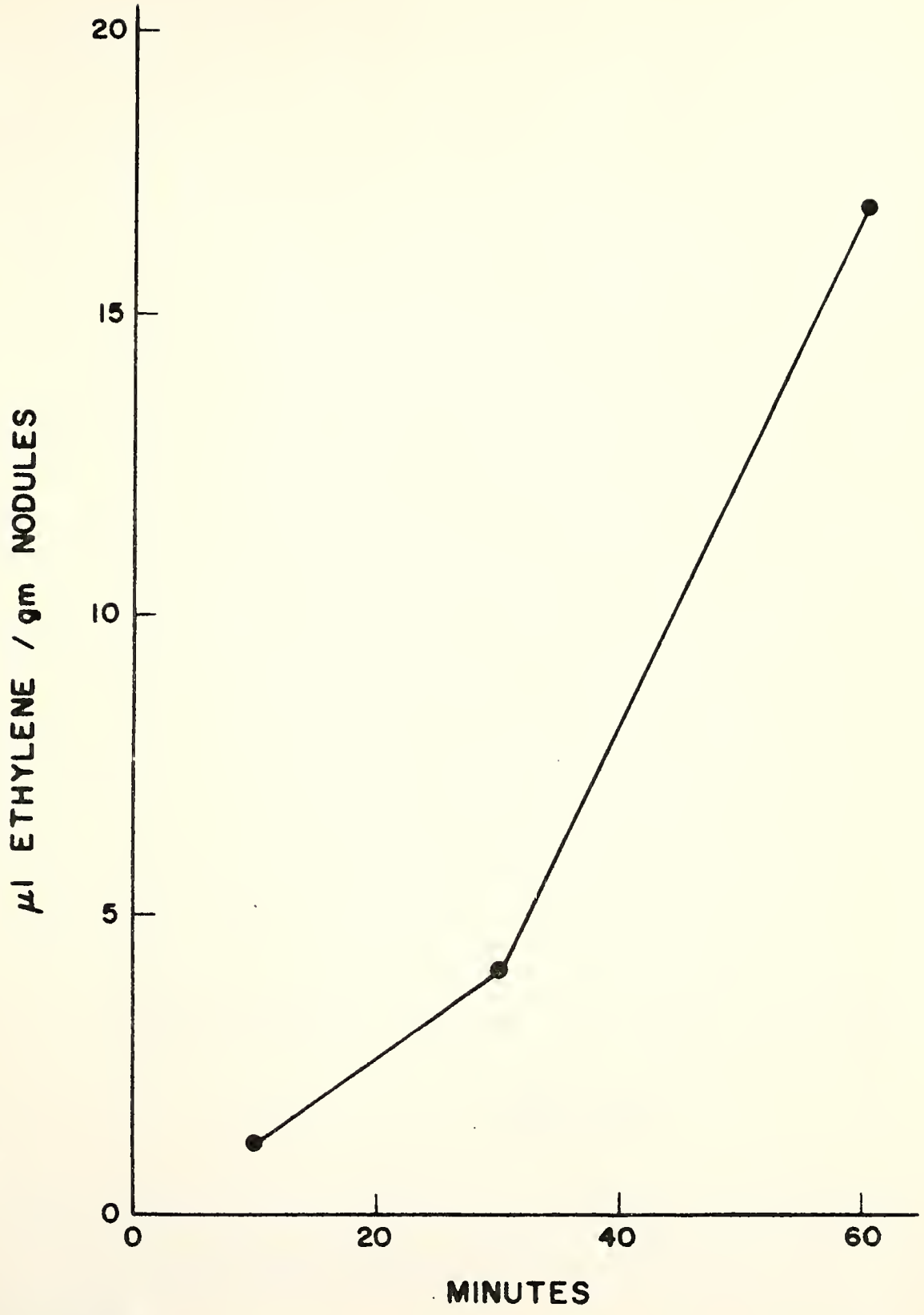

Figure 3. Reduction of acetylene by Alnus glutinosa nodules 8 hours after having been excised.

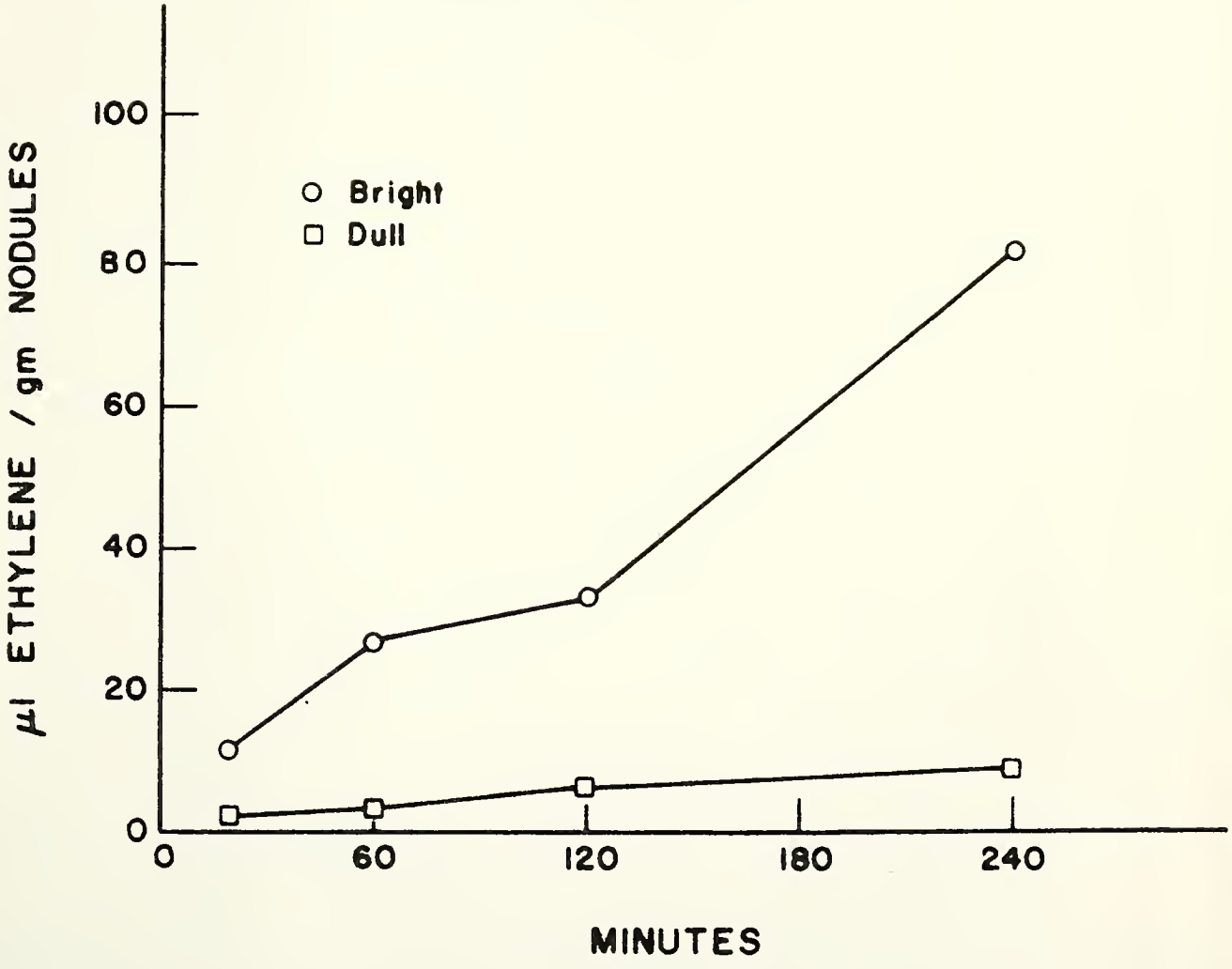

Figure 4. Reduction of acetylene by dull and bright nodules from field grown Elaeagnus angustifolia on July 14, 1971.