AN ABSTRACT OF THE THESIS OF

Chih-Hao Niu for the degree of Master of Science in Forest Science presented on March 17, 1987.

Title: Association of Nitrogen-fixing Bacteria with Ectomycorrhizae in Douglas-fir

Abstract approved Signature redacted for privacy.
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Many higher plants have mycorrhizae associated with their roots. These structures are often essential for survival and growth (Harley & Smith 1983). Mycorrhizae are known for the abilities to enhance nutrient absorption. While nitrogen-fixing organisms are found as components of the mycorrhizosphere, mycorrhizal fungi are not presently known to fix nitrogen (Trappe & Fogel 1977).

The role of mycorrhizal fungi in nitrogen fixation in the rhizosphere was explored in this study. The objectives were (1) to determine if nitrogen fixation is occurring in the rhizosphere of the Douglas-fir ectomycorrhizae formed with Hysterangium setchelli Fischer and Gautieria monticola Harkness in the Woods Creek area of Mary's Peak, Oregon; (2) to determine seasonal changes in nitrogen fixation and in populations of nitrogen-fixing bacteria; (3) to isolate and identify nitrogen-fixing bacteria associated with ectomycorrhizae.

The samples were collected each month from October 1985 to September 1986. Nitrogen fixation (acetylene reduction activity) was significantly higher each month in the mycorrhizosphere of the Douglas-fir than in the non-mat soil controls. Change in acetylene reduction rate with log-transformed incubation time was adequately expressed by a straight-line relationship. The average nitrogenase activities associated with H. setchelli and G. monticola were 1.36 and 1.44 nmoles/g/day, whereas the activity recorded for the non-mat soil controls was 0.38 nmoles/g/day. Acetylene reduction activity was significantly higher in spring, fall, and winter than in summer, but the activity during spring, fall, and winter did not differ from each other.

Nitrogen-fixing bacteria associated with mycorrhizae were also estimated by the most probable number methods. Nitrogen-fixing populations showed considerable variability from month to month. However, some of them (those in March, August, September) showed a similar trend to acetylene reduction activities.

Three different nitrogen-fixing bacteria were isolated from the fungal sheaths of H. setchelli, G. monticola, and within the sporocarps of H. setchelli. The three different strains showed a characteristic spiral movement when observed in H_2O using light microscopy. Cells are straight to curved, plump, and slightly pointed with phase-dense granules. They grew well on nutrient agar and could reduce C_2H_2 on Dobereiner's N-free medium under micro-aerophilic conditions. On the basis of the findings of Tarrand et al. (1978), these strains probably are strains of

Azospirillum brasilense.

The potential of nitrogen-fixing bacteria associated with coniferous ectomycorrhizal roots and their associated fungal sporocarps is of considerable importance to foresters. The N_2-fixer could be selected for inoculation so that it utilized the protective and nutritional advantage of the mycorrhizae. These discoveries open exciting possibilities for understanding the role of mycorrhizae in nitrogen cycling and developing information on how management practices might be modified to optimize biological nitrogen fixation in the rhizosphere of young-growth Douglas-fir.

Association of Nitrogen-fixing Bacteria with Ectomycorrhizae in Douglas-fir

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I. INTRODUCTION

Nitrogen is a major nutrient required for plant growth and low nitrogen availability is frequently the predominant factor limiting production in plant communities (Lee et al. 1982) or governing the rate of ecosystem development (Marrs et al. 1982). Nitrogen conservation in forest ecosystems is seldom sufficient for optimum tree growth. Although conventional nitrogen fertilization improves tree growth on most forest sites, the cost has increased with the cost of fuels needed for its manufacture, transportation, and application. As a result, nonconventional approaches should be developed to supply nitrogen to nonleguminous plants.

Many higher plants have mycorrhizae associated with their roots which are often essential for survival and growth (Harley 1975; Harley & Smith 1983). Mycorrhizal hyphae can be abundant in forest humus and soil (Went & Stark 1968) where they absorb and translocate nutrients and serve as reservoirs for nitrogen and phosphate (Bowen 1973).

The term "rhizosphere" was first introduced by Hiltner (1904) to designate the region of soil influenced by plant roots. There is evidence that nitrogen fixation is enhanced in the rhizospheres of trees. Data from $^{15}N_2$ studies with detached root systems of conifers have provided positive evidence for rhizosphere nitrogen fixation (Stevenson 1959; Richards and Voigt

1964).

The role of mycorrhizal fungi in rhizosphere nitrogen fixation remains ill defined. Richards and Voigt (1964) reported that the ectomycorrhizal fungus Rhizopogon rosealus Dodge stimulated nitrogen fixation in the rhizosphere of Pinus radiata D.Don. Mycorrhizae have been shown to influence populations of rhizosphere bacteria and fungi in yellow birch (Katznelson et al. 1962) and pine (Tribunskaya 1955). Populations of bacteria, fungi, and streptomycetes have been shown to vary with the type of mycorrhizal fungus on roots of the same Douglas-fir tree (Neal and Bollen 1964). While it appears that mycorrhizal fungi could influence populations of N_2 fixers in the rhizosphere, there has been no clear demonstration of any such influence.

The potential of nitrogen-fixing bacteria associated with coniferous ectomycorrhizal roots and their associated fungal sporocarps is of considerable importance to foresters. Silvester and Bennett (1973) presented evidence that apparent nitrogen fixation in mycorrhizae is probably due to free-living diazotrophic bacteria in the rhizosphere of the plant. Li and Castellano (1984, 1987) have isolated nitrogenase-producing bacteria from within sporocarps of ectomycorrhizal fungi, and Li and Hung (1987) have found similar bacteria to be active in the fungal sheaths of mycorrhizae of Douglas-fir. These discoveries open exciting possibilities for understanding the role of mycorrhizae in nitrogen cycling and developing information on how management practices might be modified to optimize biological nitrogen fixation in the rhizosphere of young-growth Douglas-fir.

The objectives of this study are:

(1) to determine if nitrogen fixation is occurring in the rhizosphere of the Douglas-fir ectomycorrhizae formed with Hysterangium setchelli Fischer and Gautieria monticola Harkness;

(2) to determine seasonal changes in nitrogen fixation and in populations of N_2-fixing bacteria;

(3) to isolate and identify N_2-fixing bacteria associated with ectomycorrhizae.

II. MATERIALS AND METHODS

Study area

The study area, as used by Fogel (1976), is a 50-75 yr old, second-growth Douglas-fir stand with a sparse understory located at Mary's peak, Oregon about 16 km west of the town of Philomath, at an elevation of 460 m. The soil is primarily of the Slickrock series, derived from weathered sedimentary rock (Knezevich 1975). Rainfall averages 191 cm a year but little occurs in the summer. The climatic information was obtained from a Water Bureau weather station, about 3 km from the site.

Determination of Nitrogen-fixation Activity

Mat-forming mycorrhizae of Hysterangium setchelli and Gautieria monticola were collected from beneath Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) at Mary's Peak, Oregon. These two fungi were common throughout the stands. Samples of soil 10-15 cm away from the edges of colonized areas were also collected to the same depth as mycorrhizae. Sporocarps were collected at the same time if found. Fresh mat and uncolonized soil samples were placed in 480 ml wide-mouth jars within one hour of collection.

Five fungal-mat samples and three non-mat soil samples were collected each month during October, 1985 to September, 1986 to observe the effect of seasonal change.

Nitrogen-fixation activity was estimated by the acetylene reduction assay (Hardy et al. 1968; Hardy et al. 1973). The jars were sealed with a lid previously fitted with a rubber serum

stopper. Acetylene generated from calcium carbide and water was injected into each jar to 10% (v/v), and the jars were immediately swirled and left to stand at 20 C. To test for endogenous C_2H_4 production in the absence of C_2H_2, a sample jar was prepared with soil and fungi which was not injected with C_2H_2. After 6, 12, and 24 hr, 0.1 ml gaseous samples from each jar were removed and analyzed for C_2H_2 and C_2H_4 with a Hewlett-Packard 5830A gas chromatograph fitted with a 2 m x 2.1 mm 80-100 mesh Porapak R column. Oven temperature was adjusted to 70°C. Injection and flame ionization detector temperature were each adjusted to 100°C. Nitrogen carrier gas flow rate was adjusted to 40 ml per min.

After the assay, each sample was oven-dried and weighed. Acetylene reduction rate was measured as nmoles C_2H_4/g dry wt. of samples for each incubation time (6, 12, 24 hr).

Methods of analysis

In the context of the first objective, it is hypothesized that nitrogen fixation is occurring in the rhizosphere of the Douglas-fir ectomycorrhizae. Data obtained from field samples were treated as a split-plot design with five replications for fungal-mat samples and three replications for non-mat soil samples. The two factors are (1) the presence of mycorrhizae H. setchelli or G. monticola in the sample; (2) incubation time (6, 12, 24 hr). Analyses of variance (ANOVA) were performed to determine whether there are significant differences between acetylene reduction rates according to the treatment and

incubation time (Joyner 1985). The data were based on means of acetylene reduction rates of samples.

For the data analysis, incubation time was differentiated into single degree of freedom contrasts. The contrasts were used to test for specific differences between the incubation time factor.

To observe the effect of seasonal change, the data were then grouped by season (January-March, April-June, July-September, October-December), thus a third factor was incorporated into the ANOVA. By doing so, the seasonal changes of acetylene reduction activity were determined. Contrasts were used to compare the effects of each factors.

Missing values for these samples were computed using the equations given in Steel and Torrie (1980).

Enumeration of Nitrogen-fixing Bacteria

Adhering soil was washed from the mycorrhizae, which were then rinsed several times in sterile distilled water. One gram of mycorrhizae and 49 ml of sterile tap water were mixed for 15 min in a beaker before making dilutions.

Numbers of heterotrophic, nitrogen-fixing bacteria associated with mycorrhizae were estimated by the most probable number (MPN) methods (Patriquin and Knowles 1972). Using the technique of Dobereiner (1980), 0.1 ml amounts of the dilutions 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} were spread over the surface of nitrogen-free malate (NFb) medium Petri dishes with a sterile bent-glass spreader. The NFb medium contained KH_2PO_4, 0.4 g; K_2HPO_4, 0.1 g; $MgSO_4.7H_2O$,

0.2 g; NaCl, 0.1 g; $CaCl_2$, 0.1 g; $FeCl_3$, 0.01 g; $NaMoO_4.2H_2O$, 0.002 g; Na malate, 5.0 g; H_2O, 1000 ml. Each dilution was replicated three times. The plates were incubated at 30 C in the dark and population counts were made after 2, 4, and 6 days of incubation.

Isolation and Identification of Nitrogen-fixing Bacteria

For isolating nitrogen-fixing bacteria from the fungal sheaths of mycorrhizae, 1 ml amounts of the dilution, as previously described for enumerating nitrogen-fixing bacteria, were inoculated into NFb liquid or semi-solid (0.05% agar) culture media in bottles supplemented with 0.002% yeast extract. For isolating nitrogen-fixing bacteria from within sporocarps of the mycorrhizal fungi, three to five pieces of interior tissue (0.2 - 0.4 cm) were aseptically removed and placed in NFb liquid medium as mentioned before. The liquid cultures were incubated both aerobically and also under 99% N_2 + 1% O_2 at 30°C.

Cultures showing good growth (medium became turbid) after 3-6 days were assayed for acetylene reduction activity. The bottles were injected with 0.1 atmospheres of acetylene. Gas samples were removed after 24 hours and the ethylene produced was measured by flame ionization chromatography as previously described.

Cultures in bottles that show acetylene reduction were purified by several transfers onto fresh NFb semi-solid agar or liquid media and streaked for isolation on low nitrogen agar media. Isolates were transferred back to new NFb semi-solid agar or liquid media and again assayed for acetylene reduction

activity. Nitrogen-fixing isolates were maintained on low nitrogen agar media. Identification of isolated nitrogen-fixers was based on morphological and physiological characterization following the methods outlined by Tarrand et al. (1978).

III. RESULTS

Data analysis for each month

The presence of ethylene was found in a few samples which had not been injected with acetylene (Table 1). Data were calibrated in these cases by subtracting the initial amount of ethylene from the final products (Fitter et al. 1985).

Samples from the two ectomycorrhizal funfi H. setchelli and G. monticola, and from the non-mat soil controls showed detectable levels of ethylene production in every month (Table 2). Differences among treatments (presence/absence of mycorrhizae), incubation times (6, 12, 24 hr) were significant ($P < .05$) for analyses of each month.

Mycorrhizal fungi H. setchelli and G. monticola showed significantly higher acetylene reduction activity than did the non-mat soil controls, but did not significantly differ from each other except in March and April. The change in acetylene reduction rate with incubation time can be adequately explained by a straight-line relationship. That is, the linear trend of acetylene reduction rate over log-transformed incubation time was highly significant in the contrast test (Tables 3-14).

Figure 1-12 show data of acetylene reduction assays from October, 1985 to September, 1986. Relative linearity was observed in most of the time-course curves. For some cases, there may be some increase in the rate of acetylene reduction between 12 and 24 hour, indicating the possibility of some depression of the nitrogenase enzyme.

All the samples from two ectomycorrhizae showed significant

acetylene reduction activity after 6 hr incubation for every month, but only some months of non-mat soil controls showed significant acetylene reduction activity (Table 15).

Data analysis for the whole year

The results of assays conducted between October, 1985 and September, 1986 from the Douglas-fir stand are shown in Fig. 13 along with precipitation and temperature data. Marked fluctuations in the activities of mycorrhizal fungi occurred during this period. The average nitrogenase activities associated with H. setchelli and G. monticola were 1.36 and 1.44 nmoles/g/day, whereas the activity recorded for the non-mat soil controls was 0.38 nmoles/g/day.

Mycorrhizal fungi H. setchelli and G. monticola showed significantly higher acetylene reduction rates than the non-mat soil controls, but did not vary significantly from each other during the whole year (Table 16). The acetylene reduction activities in spring, fall and winter were significantly higher than those in summer, but were not different from each other (Table 16).

There is a relationship between the acetylene reduction activities and precipitation. But no such relationship was found between acetylene reduction activities and the mean monthly air temperature of the field samples (Fig. 13). However, this non-relationship was expected, since all assays were done at 20°C.

Enumeration of Nitrogen-fixing Bacteria

The number of nitrogen-fixing bacteria associated with

mycorrhizae were estimated by the most probable number methods (Patriquin and Knowles 1972) from March to September, 1986 (Table 17). N-fixing populations showed considerable variability between the months. However some of them (those in March, August, September) showed a similar trend to acetylene reduction activities.

Isolation and Identification of Nitrogen-fixing Bacteria

Three different N_2-fixing bacteria were isolated from the fungal sheaths of H. setchelli, G. monticola, and within the sporocarp of H. setchelli. The three different strains showed a characteristic spiral movement when observed in H_2O using light microscopy (Photograph 1-3). Cells are straight to curved, plump, slightly pointed rods with phase-dense granules. They grew well on nutrient agar and could reduce C_2H_2 on NFb medium under micro-aerophilic conditions. These characteristics are typical of Azospirillum (Krieg and Dobereiner 1984). These strains showed the characteristics of poor growth in NFb semi-solid media containing glucose and ∝-keto glutarate as sole sources of carbon, non requirement of biotin for growth, no acid formation in peptone-based glucose medium and also no gas production from glucose when used as a carbon source (Table 18). On the basis of the findings of Tarrand et al. (1978), these strains are concluded to be probable strains of A. brasilense.

These strains showed dense, heavy-undersurface growth in NFb semi-solid agar, and differ markedly from the cultures isolated by Li et al. (1986) which showed the distinctive white pellicle

below the surface of the medium. They may represent a different ecotype of N_2-fixing bacteria than those of Li et al. (1986).

IV. DISCUSSION

There is a great deal to be learned about the interaction of nitrogen-fixation with the soil biota, particularly in the mycorrhizal symbioses. A characteristic feature of mycorrhizal fungi is their ability to absorb minerals, nitrogen, and water from the soil and transfer them to the host via their mycorrhizal roots.

Cromack et al., (1979), in the same study area, presented evidence of calcium oxalate accumulation in the mantle of ectomycorrhizae Hysterangium setchelli Fischer. It is suggested that oxalate production may enhance nutrient uptake by ectomycornhizal roots of forest species growing in nutrient impoverished soils.

Nitrogen Fixation in the Mycorrhizosphere

In this study, significant high nitrogenase activity was found in the rhizosphere of Douglas-fir mycorrhizae H. setchelli and G. monticola. The potential of nitrogen fixation in association with conifers in the Pacific Northwest is high due to the active presence of N_2-fixing bacteria in coniferous ectomycorrhizal roots and their associated fungal sporocarps.

Rhizosphere associations may be of considerable significance in plant associations, where both sufficient carbon is available and root respiration maintains a low PO_2. It has been suggested that after carbon, nitrogen is the factor which most frequently limits microbial growth in the rhizosphere (Stotzky and Norman 1961). The determination of significant N_2-fixation in

these mycorrhizae may therefore be of considerable economic significance.

Environmental Effects on Nitrogen Fixation

The supply of energy in the form of suitable carbohydrates is considered to be the main restriction to high levels of N_2 fixation by free-living heterotrophs (Stewart 1969). In addition to energy supply, temperature, moisture, and acidity are primary regulating factors (Alexander 1977).

Soil moisture is one of the most important factors affecting nitrogenase activity in the rhizosphere of Douglas-fir. As soil moisture increases, the oxygen partial pressure in the soil is likely to decrease as soil pore continuity with the aerial atmosphere is broken, with a concomitant large reduction in the diffusion rate into discontinuous soil pores. Root and microorganism respiration uses the oxygen held in such soil pores at a faster rate than diffusion can replenish the oxygen. The resultant lower oxygen partial pressure should favor increasing nitrogenase activity, since the bacteria isolated in this study are microaerophilic.

The samples were too dry for growth and activity of N_2 fixers during the summer because of low precipitation, so the acetylene reduction activities in summer were significantly lower than that in spring, fall, and winter. The highest nitrogenase activity during spring and fall, and a low activity in summer due to dry conditions have sometimes been obtained (Jones *et al.* 1974; Granhall and Lindberg 1978).

Low acetylene reduction activities were found in March and April. Sustained periods of precipitation may have suppressed nitrogenase activity and reduced acetylene reduction rates.

In this study, no relationship was found between acetylene reduction activities and the mean monthly temperature, since all assays were done at a temperature of 20°C. In fact, seasonal trends may not be as pronounced as they would be in other locations because of the mild winters in which the soil never freezes to any extended depth. The mean winter soil temperature at 5 cm was 8°C during sample collection. Another explanation is that the N_2-fixing microflora persists on the mycorrhizal roots during cold and dormant periods.

We were unable to observe any correlation between the acetylene reduction activities and the population of nitrogen-fixing (diazotrophic) bacteria, thus indicating the effects of the total environment on heterotrophic nitrogen-fixing bacteria are highly complex because of interactions between the individual environmental factors.

Todd et al., (1975) suggested that the incorporation of gaseous N is a major input of nitrogen to forest ecosystems. In forests with N capital which can be drawn from and recycled, free-living and associative nitrogen-fixing microorganisms may play a major role in maintaining and building pools of nitrogen.

Nitrogen-fixing Bacteria

Nitrogen-fixing bacteria are widely distributed in nature. They have been reported to occur in a wide variety of habitats

ranging from the polar regions to the tropics. Most notable among these are members of the genera Azospirillum, Azotobacter, Beijerinckia, and Derxia.

The identification of N_2-fixation isolates of Azospirillum from the mycorrhizae of Douglas-fir in this study indicate that this is the major organism responsible for the nitrogenase activities occurring in the rhizosphere. The discoveries of nitrogen-fixing bacteria associated with ectomycorrhizae and fungus sporocarps open a great potential for better exploiting these organisms in forestry.

Cromack et al. (1979), found that soil oxalate concentration on the same site was significantly greater within fungal mats and soil pH (4.9) was significantly lower than in soil adjacent to mats (pH = 6.1). However, Li et al. (1987) found that Azospirillum spp., which were isolated from within sporocarps of ectomycorrhizal fungi, exhibited maximum nitrogen-fixing ability (acetylene-reducing activity) at pH's ranging from 6.5-8.5. in semi-solid sodium malate medium. Because many forest soils are acidic, and because roots of plants can release H^+ ions and acidify their own rhizospheres, it seems reasonable to expect that more rhizosphere acid-tolerant, N_2 fixers may be found in forest ecosystems.

Azospirillum spp. are largely ubiquitous being found in most soils and associated with the roots of most crop plants and tropical grasses investigated (Dobereiner et al. 1976). Besides nitrogen-fixation for the plants, Azospirillum can benefit the plant by providing phytohormones (Inbal and Feldman 1982), which

increase mineral uptake and drought resistance (Sarig et al. 1984). Tien et al. (1979) found that A. brasilense produced gibberelin and cytokinin-like substances, while Vlassak and Reynders (1978) also noted that Azospirillum strains produced auxins when given tryptophan. It may be worthwhile to determine in future research, whether the effect of these bacteria on plant growth is solely through nitrogen fixation or if there is also a hormonal effect.

The best confirmation of suspected beneficial effects of certain diazotrophs on host plants is the demonstration of enhanced plant growth and (or) nitrogen fixation following inoculation of field-grown plants. The N_2-fixers could be selected for inoculation so that the protective and nutritional advantage of the mycorrhizae was utilized to optimize nitrogen fixation.

Unlike nodular N_2-fixers such as Rhizobium and Frankia, free-living, N_2-fixing bacteria fix small amounts of N_2 per gram of carbon compound consumed because they must use a large percentage of energy for the synthesis of cellular material and the exclusion of oxygen from the nitrogenase system (Mulder, 1975). However, their potential contribution should not be ignored as they are active over large areas, and they may be found in a variety of soil and rhizosphere habitats. More extensive work, such as inoculation experiments, is needed to confirm the hypothesis that biological N_2 fixation by the Azospirillum-mycorrhizae association is an important factor contributing to the forest ecosystem.

V. CONCLUSION

There is extensive literature on the rhizosphere effect of plant roots. However, the interaction between micro-organisms in the rhizosphere and mycorrhizal fungi has been neglected. Balandreau (1986) suggested that the most important underinvestigated habitat is probably the 'hyphosphere' of mycorrhizae.

Mycorrhizal fungi are known to be able to take up phosphorus from mineral soil phosphates and help to supply phosphorus for the ATP needed for the associative nitrogen-fixing bacteria. In established forest ecosystems where considerable nitrogen has accumulated mycorrhizal associations may be most important in making phosphorus more available (Silvester 1977).

Where possible and economical, management practices should be selected to encourage high rates of nonsymbiotic N_2 fixation. For example, it is well known that slash will decompose more rapidly if it is left in close contact with soil as a consequence of higher and more permanent moisture conditions. Evidence presented in this review (Baines and Millbank 1978) suggests that this practice may also encourage N_2 fixation by bacteria in the decomposing wood. An increase in the rate of wood decomposition accompanied by increased N_2 fixation would result in a more rapid lowering of the C:N ratio in the decomposing wood and a more rapid increase in soil organic matter. As the C:N ratio in duff and litter decreases, the availability of nitrogen to plants increases.

The potential exists for nitrogen fixation in associated with

conifers in the Pacific Northwest due to the active presence of N_2-fixing bacteria in coniferous ectomycorrhizal roots and their associated fungal sporocarps. More extensive work, such as inoculation experiments, is needed to confirm the hypothesis that biological N_2 fixation by the Azospirillum-mycorrhizae association is an important factor contributing to the forest ecosystem.

Table 1. Testing of endogenous C_2H_4 production without injecting C_2H_2 into the samples (nmoles/g dry wt.)

Month/Yr	H.setchelli	G. monticola	Non-mat Soil
10/'85	None	None	None
11	None	None	None
12	None	None	None
1/'86	None	None	None
2	None	None	None
3	None	0.17	None
4	None	None	None
5	None	None	None
6	None	None	None
7	None	None	None
8	None	None	None
9	0.02	0.05	None

Table 2. Acetylene reduction activity associated with ectomycorrhizae and non-mat soil samples

Month / Yr	*H. setchelli*			*G. monticola*			Non-mat soil		
	Acetylene reduction rate (nmoles C_2H_4/g dry wt./day)								
	Max.	Min.	Mean + SE	Max.	Min.	Mean + SE	Max.	Min.	Mean + SE
10/'85	1.75	0.60	1.28 ±0.39	1.94	0.69	1.23 ± 0.43	0.67	0.33	0.46 ± 0.18
11	2.90	0 48	2.16 ± 0.71	2.13	1.25	1.67 ± 0.39	1.26	0.45	0.77 ± 0.31
12	2.05	0.96	1.52 ± 0.41	1.74	0.86	1.19 ± 0.38	0.74	0.47	0.59 ± 0.14
1/'86	1.80	0.86	1.50 ± 0.37	2.15	1.20	1.71 ± 0.34	0.61	0.19	0.38 ± 0.21
2	2.14	0.87	1.52 ± 0.46	1.78	1.03	1.33 ± 0.34	0.95	0	0.49 ± 0.30
3	1.10	0.64	0.88 ± 0.17	1.56	1.13	1.29 ± 0.21	0.60	0.36	0.48 ± 0.12
4	1.23	0.53	0.88 ± 0.32	1.91	0.89	1.32 ± 0.41	0.39	0	0.26 ± 0.22
5	3.38	1.12	2.19 ± 0.72	5.03	0.82	2.03 ± 0.70	0.21	0	0.07 ± 0.12
6	2.57	0.83	1.62 ± 0.55	2.75	0.78	1.79 ± 0.65	0.52	0	0.29 ± 0.17
7	1.07	0.52	0.76 ± 0.21	2.52	0	1.26 ± 0.70	0.37	0.22	0.28 ± 0.08
8	1.72	0.99	1.31 ± 0.30	2.47	0.92	1.66 ± 0.64	0.20	0.08	0.12 ± 0.07
9	0.83	0.61	0.74 ± 0.09	1.09	0.55	0.74 ± 0.21	0.39	0.29	0.35 ± 0.05

Table 3. Analyses of variance and degree of freedom contrasts for acetylene reduction rates in October, 1985

Source	DF	Mean Square	F
TREATMENT	2	1.07	4.12*
H vs G	1	0.18	0.69
H vs C	1	2.11	8.12*
G vs C	1	1.20	4.96*
Error (A)	9	0.26	
TIME	2	0.81	18.90***
linear	1	1.52	35.63***
quadratic	1	0.08	1.31
TIME*TREATMENT	4	0.09	2.09
Error (B)	18	0.04	

H = H. setchelli ; G = G. monticola; C = Non-mat Soil

*p < .05; **p < .01; ***p < .001

NITROGEN FIXATION ASSAY (C_2H_4 nmoles/g dry wt.)
October 14, 1985

TIME	6HR	12HR	24HR
SP			
H1	0.55	0.97	1.38
H2	1.05	1.66	1.75
H3	0.37	0.60	0.60
H4	0.43	0.54	0.97
H5	0.68	1.02	1.71
TOTAL	3.08	4.79	6.41
MEAN	0.62	0.96	1.28
G1	0.58	0.73	1.02
G2	0.54	0.86	1.94
G3	0.42	0.55	0.69
G4	0.38	0.58	1.25
G5	.	.	.[1]
TOTAL	1.92	2.72	4.90
MEAN	0.48	0.68	1.23
C1	0.20	0.27	0.33
C2	0.27	0.34	0.67
C3	0.31	0.31	0.38
TOTAL	0.77	0.91	1.39
MEAN	0.26	0.30	0.46

[1] Missing value

Table 4. Analyses of variance and degree of freedom contrasts for acetylene reduction rates in November, 1985

Source	DF	Mean Square	F
TREATMENT	2	1.36	2.96
H vs G	1	0.33	0.72
H vs C	1	2.69	5.97*
G vs C	1	1.31	2.91*
Error (A)	10	0.45	
TIME	2	3.11	30.40***
linear	1	5.91	57.83***
quadratic	1	0.30	2.96
TIME*TREATMENT	4	0.32	3.03
Error (B)	20	0.10	

H = H. setchelli ; G = G. monticola; C = Non-mat Soil

*p < .05; **p < .01; ***p < .001

NITROGEN FIXATION ASSAY (C_2H_4 nmoles/g dry wt.)

November 9, 1985

TIME	6HR	12HR	24HR
SP			
H1	0.69	1.24	2.90
H2	0.70	1.40	2.89
H3	0.36	0.48	0.48
H4	0.76	1.41	2.81
H5	0.51	0.85	1.71
TOTAL	3.02	5.38	10.79
MEAN	0.60	1.08	2.16
G1	0.77	1.11	2.13
G2	0.44	0.73	1.31
G3	0.48	0.77	1.25
G4	0.68	1.16	1.93
G5	0.63	0.95	1.74
TOTAL	2.99	4.71	8.36
MEAN	0.60	0.94	1.67
C1	0.66	0.66	1.26
C2	0.27	0.54	0.60
C3	0.37	0.40	0.45
TOTAL	0.99	1.60	2.32
MEAN	0.33	0.53	0.77

Table 5. Analyses of variance and degree of freedom contrats for acetylene reduction rates in December, 1985

Source	DF	Mean Square	F
TREATMENT	2	1.16	4.64*
H vs G	1	0.38	1.52
H vs C	1	2.30	9.20*
G vs C	1	1.26	5.01*
Error (A)	8	0.25	
TIME	2	0.52	26.51***
linear	1	1.00	50.67***
quadratic	1	0.05	2.35
TIME*TREATMENT	4	0.05	2.68
Error (B)	16	0.02	

H = H. setchelli ; G = G. monticola; C = Non-mat Soil

*p < .05; **p < .01; ***p < .001

NITROGEN FIXATION ASSAY (C_2H_4 nmoles/g dry wt.)
December 28, 1985

TIME	6HR	12HR	24HR
SP			
H1	0.97	1.10	1.93
H2	1.29	1.54	2.05
H3	0.73	0.96	0.96
H4	.	.	.[1]
H5	0.61	0.79	1.13
TOTAL	3.51	4.39	6.08
MEAN	0.88	1.10	1.52
G1	1.02	1.31	1.47
G2	0.60	0.60	1.02
G3	0.58	0.67	0.86
G4	0.61	0.79	1.14
G5	.	.	.
TOTAL	2.80	3.36	4.77
MEAN	0.70	0.84	1.13
C1	0.40	0.46	0.46
C2	0.47	0.54	0.74
C3	0.42	0.42	0.55
TOTAL	1.28	1.42	1.76
MEAN	0.43	0.47	0.59

[1] Missing value

Table 6. Analyses of variance and degree of freedom contrasts for acetylene reduction rates in January, 1986

Source	DF	Mean Square	F
TREATMENT	2	2.08	8.00**
H vs G	1	0.12	0.46
H vs C	1	2.76	10.62**
G vs C	1	3.86	14.85**
Error (A)	10	0.26	
TIME	2	1.13	34.83***
linear	1	2.25	69.17***
quadratic	1	0.16	0.48
TIME*TREATMENT	4	0.17	5.87*
Error (B)	20	0.03	

H = *H. setchelli* ; G = *G. monticola*; C = Non-mat Soil

*p < .05; **p < .01; ***p < .001

NITROGEN FIXATION ASSAY (C_2H_4 nmoles/g dry wt.)

January 10, 1986

TIME	6HR	12HR	24HR
SP			
H1	0.80	0.97	1.61
H2	0.59	0.95	1.54
H3	0.54	0.86	0.86
H4	0.79	1.13	1.69
H5	0.77	1.16	1.80
TOTAL	3.50	5.06	7.51
MEAN	0.70	1.01	1.50
G1	0.37	0.46	1.20
G2	0.63	0.95	1.74
G3	0.94	2.15	2.15
G4	0.88	1.13	1.76
G5	0.79	1.13	1.69
TOTAL	3.61	5.83	8.55
MEAN	0.72	1.17	1.71
C1	0.61	0.61	0.61
C2	0.13	0.13	0.19
C3	0.32	0.39	0.39
TOTAL	1.07	1.13	1.14
MEAN	0.36	0.38	0.38

Table 7. Analyses of variance and degree of freedom contrasts for acetylene reduction rates in February, 1986

Source	DF	Mean Square	F
TREATMENT	2	1.50	6.82*
H vs G	1	0.03	0.14
H vs C	1	2.67	12.14**
G vs C	1	2.18	9.91**
Error (A)	10	0.22	
TIME	2	0.69	29.28***
linear	1	1.30	55.51***
quadratic	1	0.07	3.05
TIME*TREATMENT	4	0.10	4.19*
Error (B)	20	0.02	

H = H. setchelli ; G = G. monticola; C = Non-mat Soil

*p < .05; **p < .01; ***p < .001

NITROGEN FIXATION ASSAY (C_2H_4 nmoles/g dry wt.)

February 22, 1986

TIME	6HR	12HR	24HR
SP			
H1	0.80	1.03	1.38
H2	0.55	0.71	0.87
H3	1.02	1.24	2.14
H4	0.78	1.06	1.61
H5	0.75	0.97	1.61
TOTAL	3.91	5.02	7.62
MEAN	0.78	1.00	1.52
G1	0.95	1.19	1.78
G2	0.69	0.86	1.12
G3	0.92	1.03	1.03
G4	0.63	0.79	1.11
G5	0.78	1.06	1.61
TOTAL	3.97	4.92	6.64
MEAN	0.79	0.98	1.33
C1	0.69	0.69	0.95
C2	0.44	0.44	0.51
C3	0	0	0
TOTAL	1.13	1.13	1.46
MEAN	0.38	0.38	0.49

Table 8. Analyses of variance and degree of freedom contrasts for acetylene reduction rates in March, 1986

Source	DF	Mean Square	F
TREATMENT	2	0.77	17.91***
H vs G	1	0.48	11.16**
H vs C	1	0.39	7.91*
G vs C	1	1.50	34.88***
Error (A)	10	0.04	
TIME	2	0.37	37.23***
linear	1	0.74	74.22***
quadratic	1	0.002	0.23
TIME*TREATMENT	4	0.06	5.64*
Error (B)	20	0.01	

H = *H. setchelli* ; G = *G. monticola*; C = Non-mat Soil

*p < .05; **p < .01; ***p < .001

NITROGEN FIXATION ASSAY (C_2H_4 nmoles/g dry wt.)

March 15, 1986

TIME	6HR	12HR	24HR
SP			
H1	0.53	0.74	0.95
H2	0.54	0.81	0.81
H3	0.55	0.77	1.10
H4	0.55	0.71	0.87
H5	0.43	0.53	0.64
TOTAL	2.60	3.57	4.37
MEAN	0.52	0.71	0.88
G1	0.78	1.04	1.56
G2	0.86	1.19	1.19
G3	0.58	0.88	1.46
G4	0.56	0.75	1.13
G5	0.55	0.71	1.12
TOTAL	3.34	4.56	6.45
MEAN	0.67	0.91	1.29
C1	0.40	0.50	0.60
C2	0.46	0.46	0.46
C3	0.36	0.36	0.36
TOTAL	1.22	1.32	1.42
MEAN	0.41	0.44	0.47

Table 9. Analyses of variance and degree of freedom contrasts for acetylene reduction rates in April, 1986

Source	DF	Mean Square	F
TREATMENT	2	1.63	13.58**
H vs G	1	0.64	5.33*
H vs C	1	1.23	10.25**
G vs C	1	3.25	27.08***
Error (A)	10	0.12	
TIME	2	0.48	22.21***
linear	1	0.91	42.52***
quadratic	1	0.04	1.90
TIME*TREATMENT	4	0.08	3.53
Error (B)	20	0.02	

H = H. setchelli ; G = G. monticola; C = Non-mat Soil

*p < .05; **p < .01; ***p < .001

NITROGEN FIXATION ASSAY (C_2H_4 nmoles/g dry wt.)

April 12, 1986

TIME	6HR	12HR	24HR
SP			
H1	0.49	0.73	1.21
H2	0.42	0.52	0.62
H3	0.45	0.57	0.68
H4	0.47	0.57	0.76
H5	0.51	0.82	1.22
TOTAL	2.34	3.20	4.51
MEAN	0.47	0.64	0.90
G1	0.63	0.89	1.52
G2	0.88	1.18	1.91
G3	0.59	0.69	0.89
G4	0.64	0.82	1.28
G5	0.64	0.76	1.02
TOTAL	3.38	4.34	6.62
MEAN	0.68	0.87	1.32
C1	0.39	0.39	0.39
C2	0	0	0
C3	0.10	0.11	0.39
TOTAL	0.49	0.50	0.78
MEAN	0.16	0.17	0.26

Table 10. Analyses of variance and degree of freedom contrasts for acetylene reduction rates in May, 1986

Source	DF	Mean Square	F
TREATMENT	2	4.98	4.40*
H vs G	1	0.13	0.12
H vs C	1	8.88	7.86*
G vs C	1	7.10	6.28*
Error (A)	10	1.13	
TIME	2	3.16	11.06***
linear	1	5.86	20.55***
quadratic	1	0.45	1.58
TIME*TREATMENT	4	0.67	2.36
Error (B)	20	0.29	

H = H. setchelli ; G = G. monticola; C = Non-mat Soil

*p < .05; **p < .01; ***p < .001

NITROGEN FIXATION ASSAY (C_2H_4 nmoles/g dry wt.)

May 17, 1986

TIME	6HR	12HR	24HR
SP			
H1	0.62	0.94	2.13
H2	0.42	0.70	1.12
H3	0.84	1.40	2.66
H4	0.54	0.91	1.63
H5	0.95	1.62	3.38
TOTAL	3.38	5.57	10.93
MEAN	0.67	1.11	2.18
G1	0.31	0.51	0.82
G2	0.59	0.71	1.31
G3	1.06	1.85	5.03
G4	0.55	0.68	1.37
G5	0.54	0.91	1.63
TOTAL	3.05	4.67	10.16
MEAN	0.61	0.93	2.03
C1	0	0	0
C2	0	0	0
C3	0.21	0.21	0.21
TOTAL	0.21	0.21	0.21
MEAN	0.07	0.07	0.07

Table 11. Analyses of variance and degree of freedom contrasts for acetylene reduction rates in June, 1986

Source	DF	Mean Square	F
TREATMENT	2	3.34	6.07*
H vs G	1	0.73	1.33
H vs C	1	3.13	5.69*
G vs C	1	6.54	11.89**
Error (A)	8	0.55	
TIME	2	1.13	14.03***
linear	1	2.19	27.28***
quadratic	1	0.06	0.79
TIME*TREATMENT	4	0.22	2.80
Error (B)	16	0.08	

H = H. setchelli ; G = G. monticola; C = Non-mat Soil

*p < .05; **p < .01; ***p < .001

NITROGEN FIXATION ASSAY (C_2H_4 nmoles/g dry wt.)
June 14, 1986

TIME	6HR	12HR	24HR
SP			
H1	0.54	0.91	1.63
H2	0.41	0.62	0.82
H3	.	.	.[1]
H4	0.77	1.16	2.57
H5	0.63	0.84	1.46
TOTAL	2.36	3.52	6.50
MEAN	0.59	0.88	1.62
G1	0.58	0.78	0.78
G2	1.30	1.74	2.75
G3	.	.	.
G4	1.21	1.67	2.44
G5	0.87	1.21	1.21
TOTAL	3.97	5.40	7.18
MEAN	0.99	1.35	1.79
C1	0	0	0
C2	0.29	0.36	0.36
C3	0.37	0.37	0.52
TOTAL	0.66	0.73	0.88
MEAN	0.22	0.24	0.29

[1] Missing value

Table 12. Analyses of variance and degree of freedom contrasts for acetylene reduction rates in July, 1986

Source	DF	Mean Square	F
TREATMENT	2	1.02	3.29
H vs G	1	0.40	1.29
H vs C	1	1.28	4.11*
G vs C	1	2.01	6.48*
Error (A)	10	0.31	
TIME	2	0.42	10.15***
linear	1	0.75	18.31***
quadratic	1	0.08	1.99
TIME*TREATMENT	4	0.12	2.95
Error (B)	20	0.04	

H = H. setchelli ; G = G. monticola; C = Non-mat Soil

*p < .05; **p < .01; ***p < .001

NITROGEN FIXATION ASSAY (C_2H_4 nmoles/g dry wt.)

July 30, 1986

TIME SP	6HR	12HR	24HR
H1	0.54	0.64	1.07
H2	0.41	0.52	0.73
H3	0.41	0.52	0.83
H4	0.51	0.51	0.64
H5	0.42	0.42	0.52
TOTAL	2.30	2.61	3.79
MEAN	0.46	0.52	0.76
G1	0.58	0.78	1.46
G2	0.58	0.72	1.30
G3	0.95	1.42	2.52
G4	0	0	0
G5	0.69	0.69	1.04
TOTAL	2.80	3.71	6.32
MEAN	0.56	0.72	1.26
C1	0.19	0.19	0.26
C2	0.22	0.22	0.22
C3	0.30	0.30	0.37
TOTAL	0.71	0.71	0.84
MEAN	0.24	0.24	0.28

Table 13. Analyses of variance and degree of freedom contrasts for acetylene reduction rates in August, 1986

Source	DF	Mean Square	F
TREATMENT	2	2.66	14.00**
H vs G	1	0.60	3.16
H vs C	1	2.63	13.84**
G vs C	1	5.26	27.68***
Error (A)	10	0.19	
TIME	2	1.47	37.95***
linear	1	2.79	72.32***
quadratic	1	0.14	3.58
TIME*TREATMENT	4	0.17	4.21*
Error (B)	20	0.04	

H = H. setchelli ; G = G. monticola; C = Non-mat Soil

*p < .05; **p < .01; ***p < .001

NITROGEN FIXATION ASSAY (C_2H_4 nmoles/g dry wt.)

August 26, 1986

TIME	6HR	12HR	24HR
SP			
H1	0.40	0.70	1.50
H2	0.37	0.61	1.11
H3	0.33	0.44	1.22
H4	0.36	0.63	0.99
H5	0.46	0.92	1.72
TOTAL	1.92	3.30	6.53
MEAN	0.38	0.66	1.31
G1	0.53	0.80	1.59
G2	0.48	0.60	1.21
G3	0.71	1.30	2.13
G4	0.72	1.34	2.47
G5	0.53	0.66	0.92
TOTAL	2.97	4.70	8.32
MEAN	0.59	0.94	1.66
C1	0.09	0.09	0.09
C2	0.08	0.08	0.08
C3	0.10	0.10	0.20
TOTAL	0.26	0.26	0.36
MEAN	0.09	0.09	0.12

Table14. Analyses of variance and degree of freedom contrasts for acetylene reduction rates in September, 1986

Source	DF	Mean Square	F
TREATMENT	2	0.26	6.50*
H vs G	1	0.01	0.25
H vs C	1	0.35	8.75*
G vs C	1	0.48	12.00**
Error (A)	9	0.04	
TIME	2	0.13	34.37***
linear	1	0.26	65.74***
quadratic	1	0.01	3.00
TIME*TREATMENT	4	0.01	2.85
Error (B)	18	0.004	

H = H. setchelli ; G = G. monticola; C = Non-mat Soil

*p < .05: **p < .01; ***p < .001

NITROGEN FIXATION ASSAY (C_2H_4 nmoles/g dry wt.)
September 30, 1986

TIME	6HR	12HR	24HR
SP			
H1	0.41	0.58	0.83
H2	0.38	0.48	0.76
H3	0.40	0.40	0.61
H4	0.61	0.61	0.76
H5	.	.	.[1]
TOTAL	1.81	2.07	2.96
MEAN	0.45	0.52	0.74
G1	0.49	0.61	0.73
G2	0.42	0.42	0.58
G3	0.37	0.55	0.55
G4	0.65	0.76	1.09
G5	0.48	0.57	0.77
TOTAL	2.40	2.91	3.72
MEAN	0.48	0.58	0.74
C1	0.22	0.22	0.29
C2	0.29	0.39	0.39
C3	0.31	0.31	0.38
TOTAL	0.82	0.91	1.06
MEAN	0.27	0.30	0.35

[1] Missing value

Table 15. Acetylene reduction activity associated with ectomycorrhizae and soil-only samples after 6 hours of incubation[1]

(nmoles C_2H_4/g dry wt.)

Month / Year	H. setchelli	G. monticola	Non-mat Soil
10/'85	0.62*	0.48*	0.26*
11	0.60*	0.60*	0.46*
12	0.88*	0.70*	0.43*
1/'86	0.70*	0.70*	0.35*
2	0.78*	0.79*	0.38
3	0.52*	0.66*	0.40*
4	0.47*	0.68*	0.16
5	0.68*	0.61*	0.07
6	0.59*	0.99*	0.22
7	0.46*	0.56*	0.24*
8	0.38*	0.59*	0.09
9	0.45*	0.48*	0.27*

[1] Data are mean of replicates
*$p < .05$

Table 16. Analysis of variance and degree of freedom contrasts for acetylene reduction rates from 1985-86

Source	DF	Mean Square	F
TREATMENT	2	17.58	86.10***
H vs G	1	0.41	1.76
H vs C	1	25.13	124.10***
G vs C	1	31.04	151.41***
Error (A)	10	0.20	
SEASON	3	1.88	11.28***
S vs U	1	3.35	20.00***
S vs F	1	0.07	0.42
S vs W	1	0.001	0.01
U vs F	1	4.32	25.85***
U vs W	1	3.47	20.76***
F vs W	1	0.07	0.41
TIME	2	11.46	68.46***
linear	1	21.99	131.45***
quadratic	1	0.87	4.91
TIME*TREATMENT	4	1.63	9.77***
TIME-L*TRT	2	2.59	15.51***
TIME-Q*TRT	2	0.10	0.56
Error	428	0.16	

S = Spring; U = Summer; F = Fall; W = Winter

H = *H. setchelli*; G = *G. monticola*; C = Non-mat Soil

*p < .1; **p < .05; ***p < .01

Table 17. Enumeration of nitrogen-fixing bacteria per gram fresh wt. of mycorrhizal fungi

	N_2-fixing bacterial population ($1x10^3$)	
Month/1986	H. setchelli	G. monticola
March	30 ± 11.5	80 ± 21.1
May	130 ± 38.7	75 ± 30.2
June	268 ± 81.2	60 ± 11.1
July	9 ± 0.9	14 ± 6.2
August	80 ± 3.5	150 ± 34.7
September	36 ± 9.1	24 ± 13.9

Table 18. Characteristics of different strains of Azospirillum isolated from the surface and within sporocarps of two ectomycorrhizal fungi

Characteristics	Azospirillum strains isolated from		Azospirillum strains isolated from[1]			Azospirillum[2] brasilense	Azospirillum[2] lipoferum
	Hysterangium setchelli	Gautieria monticola	Laccaria laccata	Hebeloma crustuliniforme	Rhizopogon vinicolor	(strain SL 33)	(ICM 1081)
Cell morphology in semi-solid malate medium for 72 hr at 30°C	Rods with round ends. 3-5 um in length. 0.5-0.7 um in width. Motile. PHB granules.	Rods with round ends 3-5 um in length. 0.5-0.7 um in width. Motile. PHB granules.	Medium sized. rods with round ends. PHB granules. conspicuous.	Dark colored. medium sized plumpy rods. Highly motile. PHB granules conspicuous.	Medium-sized thin rods. motile cells contain PHB granules.	Dark colored medium sized plumpy rods. Highly motile. PHB granules conspicuous.	Medium sized plumpy rods with smooth to pointed ends. PHB granules conspicuous
Requirement of biotin	Not required	Not required	Not required	Not required	Not required	Not required	Required
Growth in N-free semi-solid medium containing glucose and α-ketoglutarate as sole carbon sources	Poor	Poor	Poor	Poor	Poor	Poor	Good
Acidification of peptone-based glucose medium after 72 hr	Negative	Negative	Negative	Negative	Negative	Negative	Positive
Production of gas under anaerobic conditions	Negative	Negative	Negative	Negative	Negative	Negative	Variable
Urease activity	Good	Good	Good	Good	Good	Good	Good
Hydrolysis of gelatin	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Hydrolysis of starch	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Reduction of NO_3^- to NO_2^-	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Production of indole	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Growth in $(NH_4)_2SO_4$ semi-solid medium containing α-keto glutarate as sole carbon source	Negative	Negative	Negative	Negative	Negative	Negative	Positive

[1] Strains were isolated by Li et al. 1986.
[2] Standard strains were obtained from the International Crops Research Institute.

Fig. 1. Acetylene reduction assay in October 14, 1985

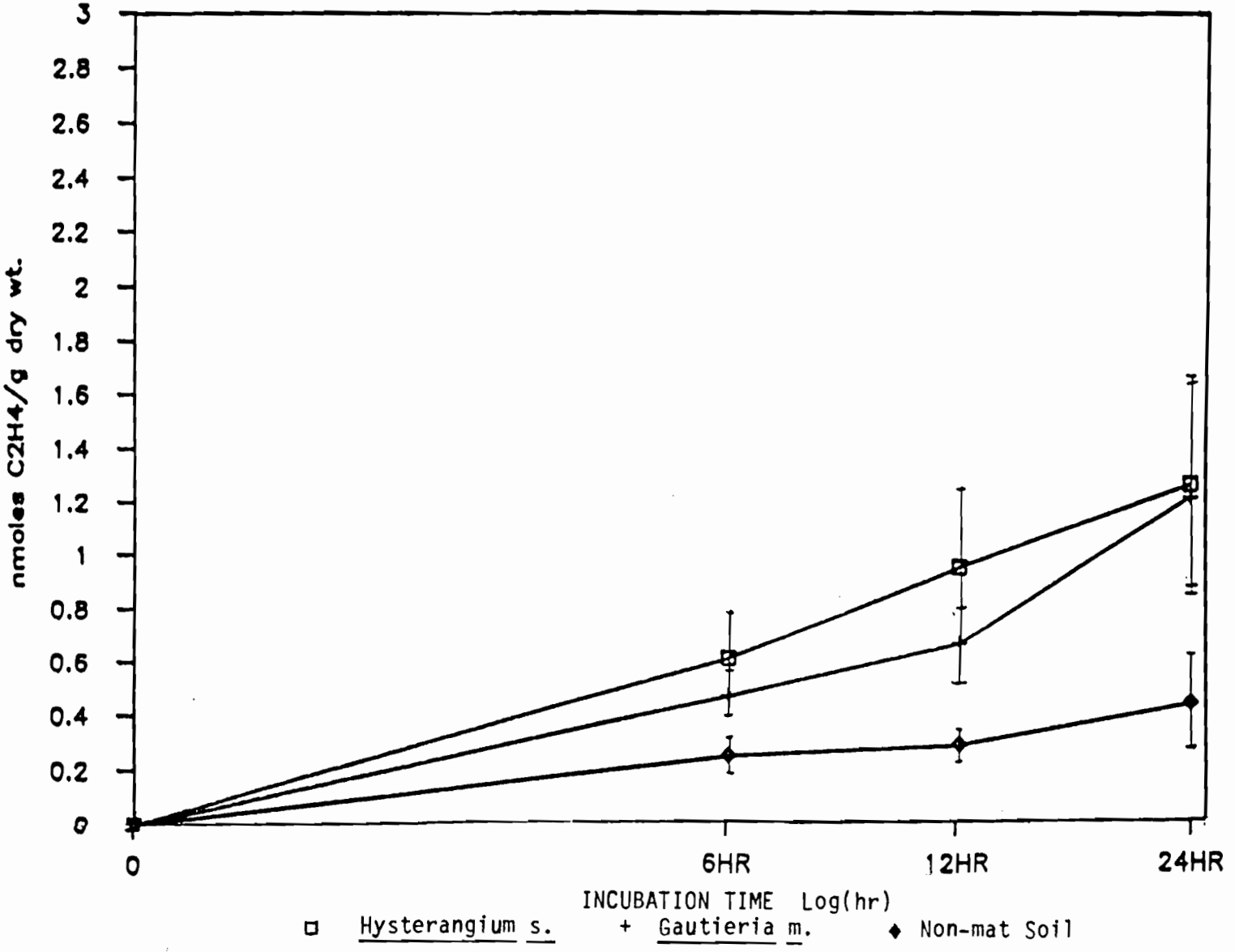


Fig. 2. Acetylene reduction assay in November 9, 1985

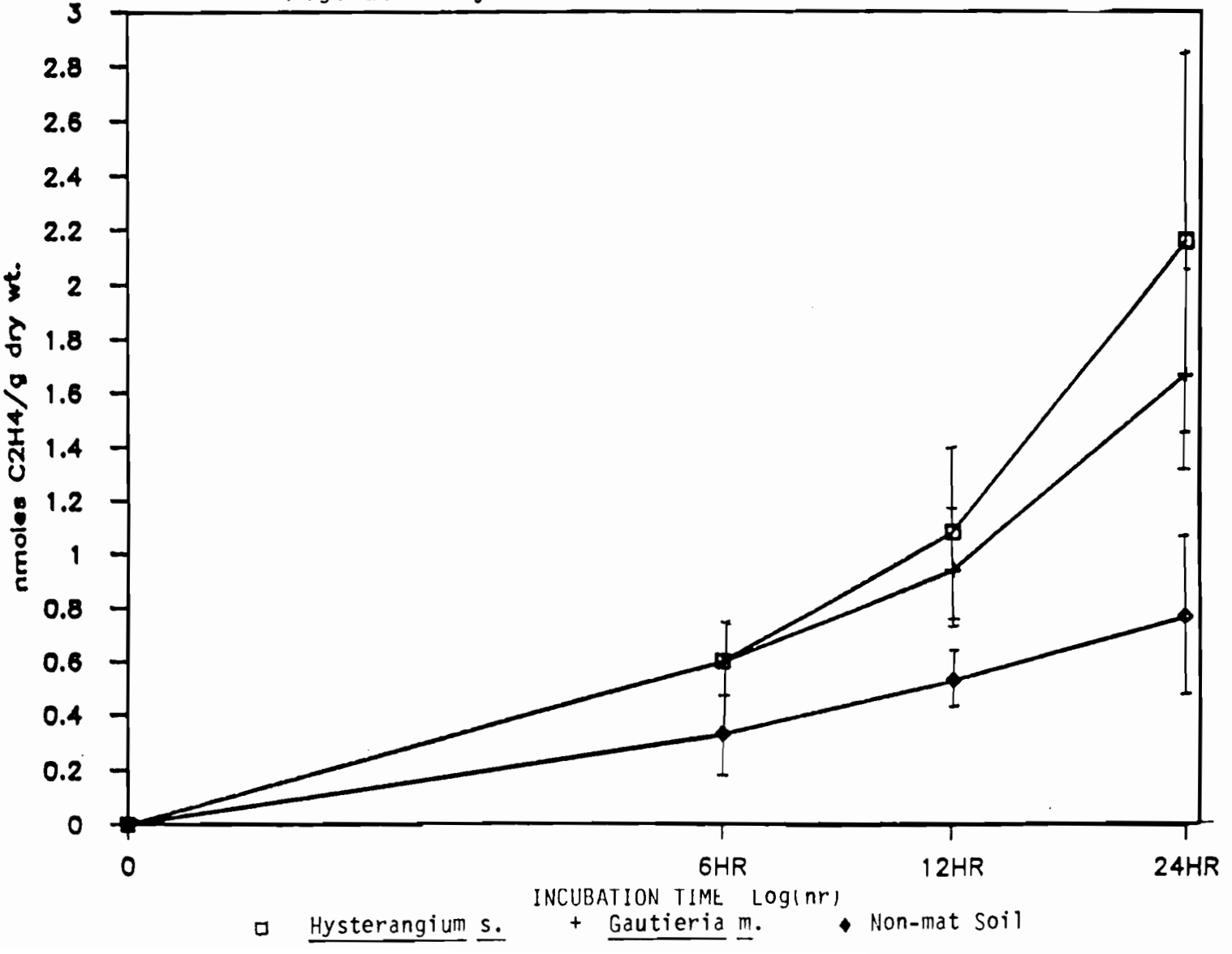


Fig. 3. Acetylene reduction assay in December 28, 1985

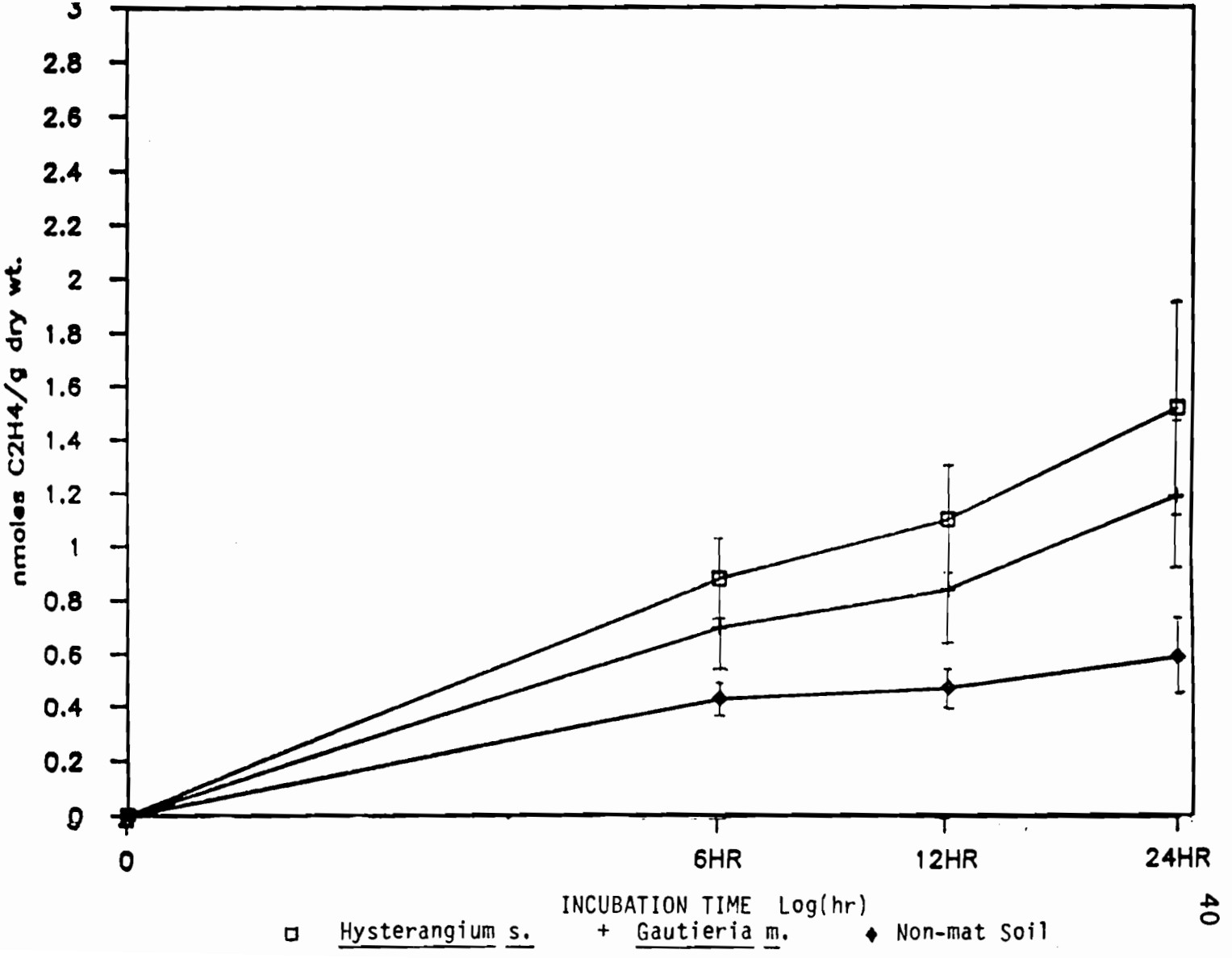


Fig. 4. Acetylene reduction assay in January 10, 1986

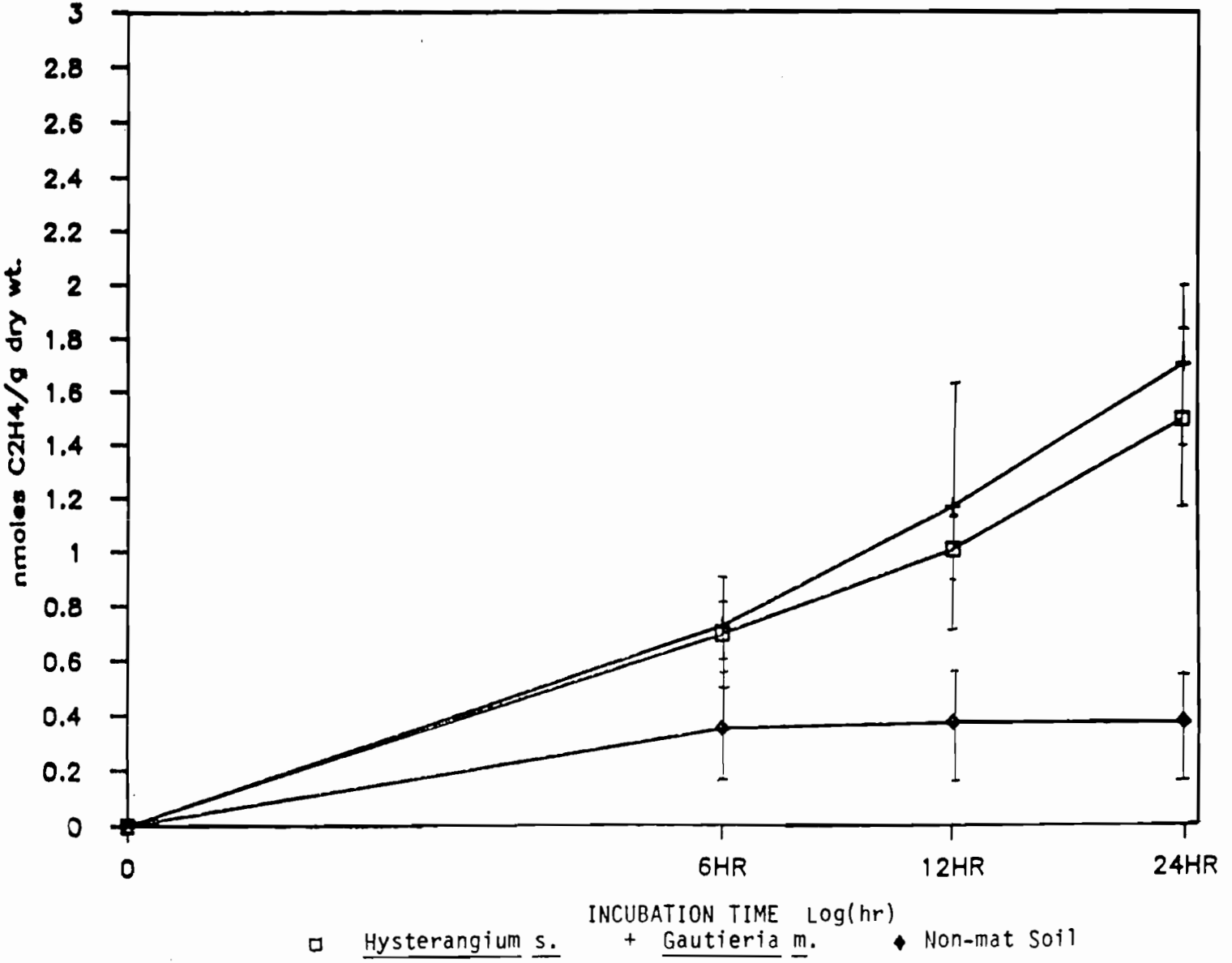


Fig. 5. Acetylene reduction assay in February 22, 1986

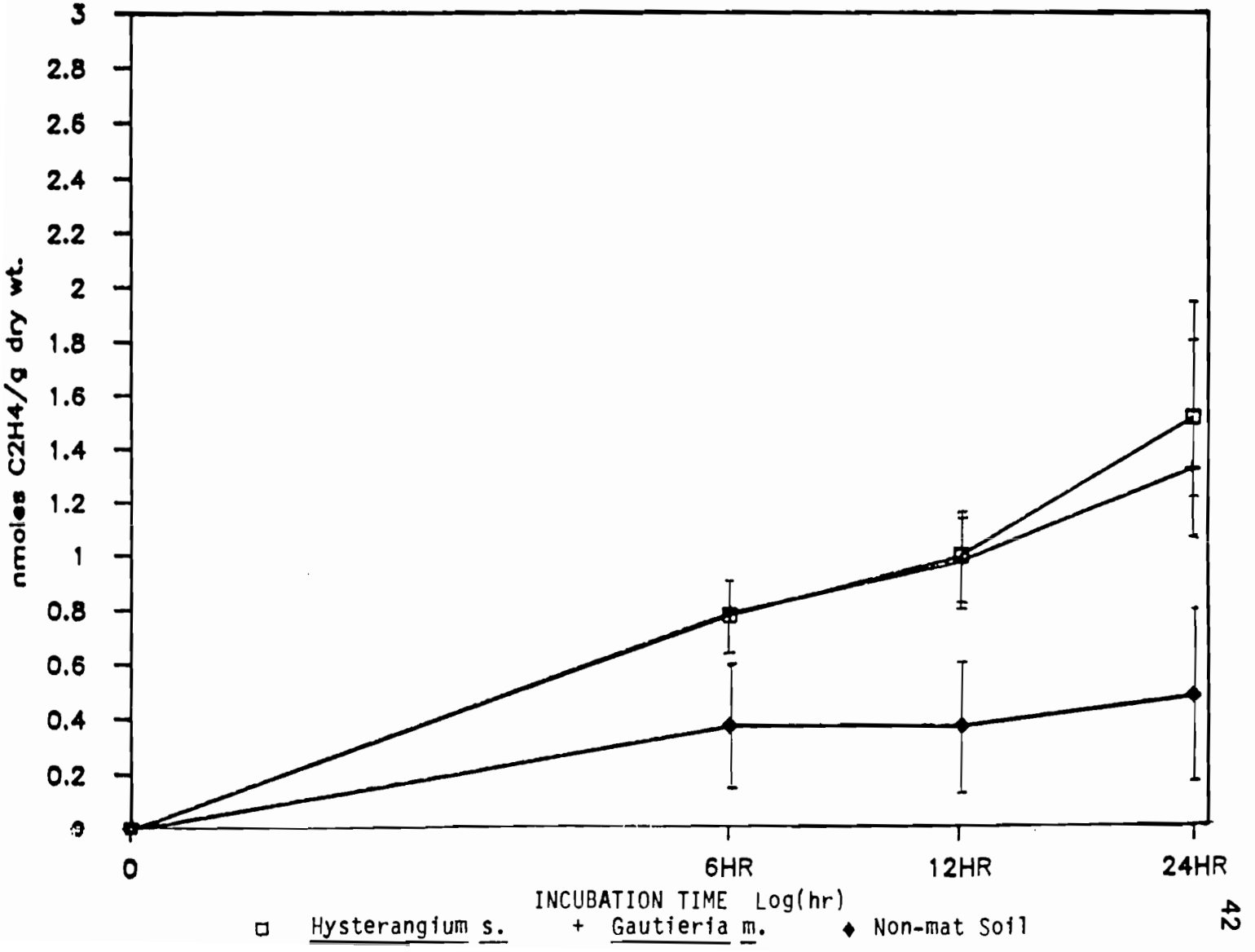


Fig. 6. Acetylene reduction assay in March 15, 1986

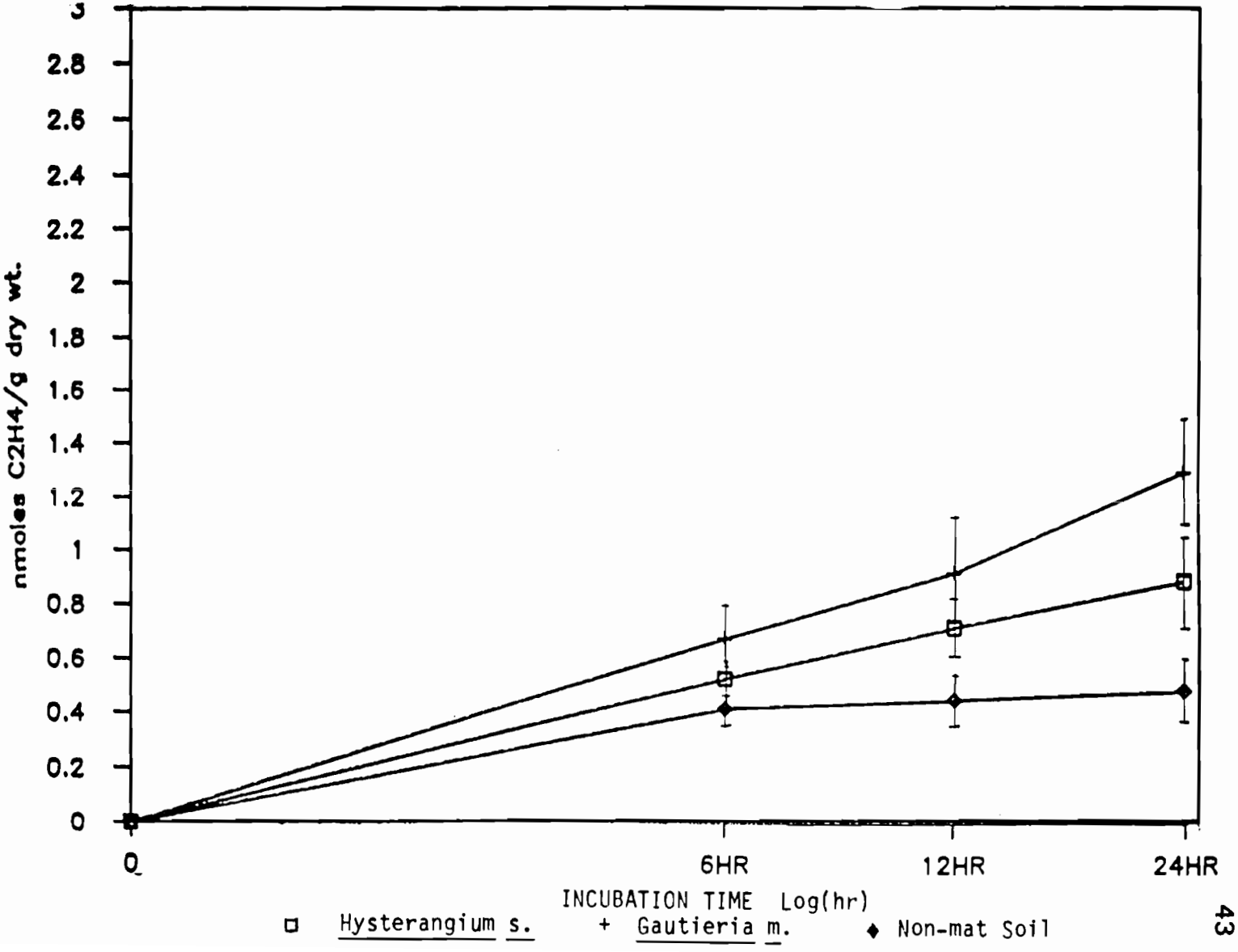


Fig. 7. Acetylene reduction assay in April 12, 1986

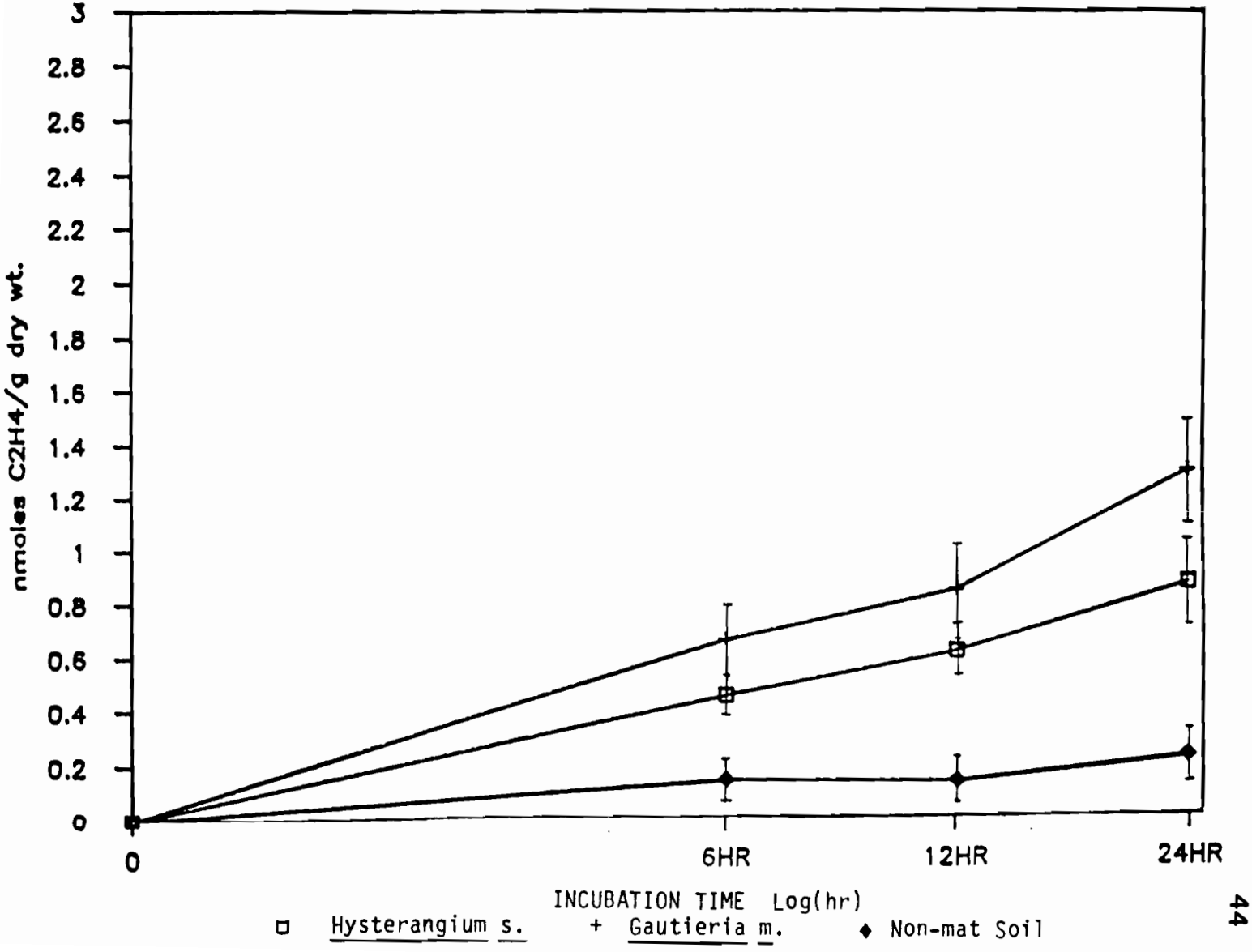


Fig. 8. Acetylene reduction assay in May 17, 1986

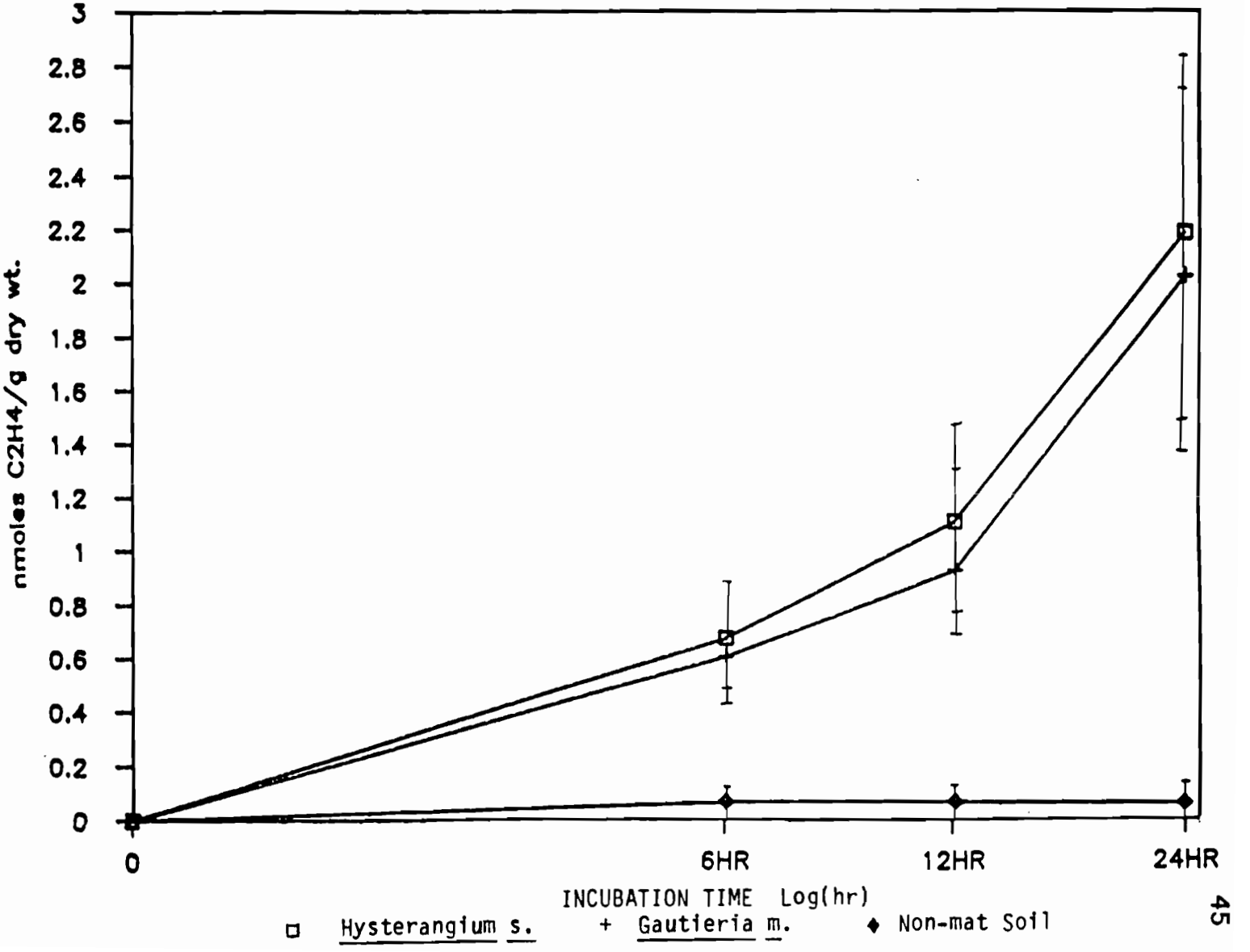


Fig. 9. Acetylene reduction assay in June 14, 1986

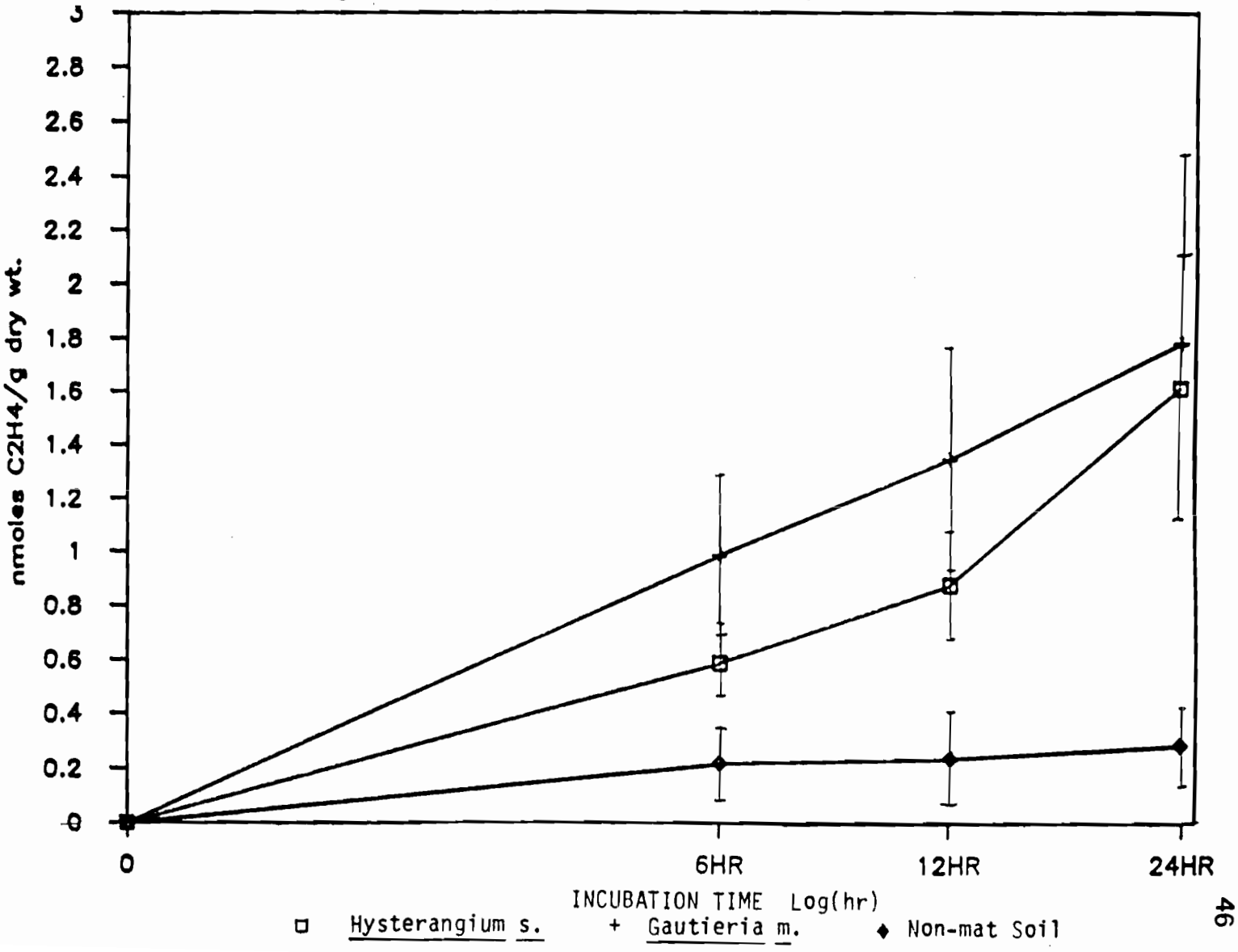


Fig. 10. Acetylene reduction assay in July 30, 1986

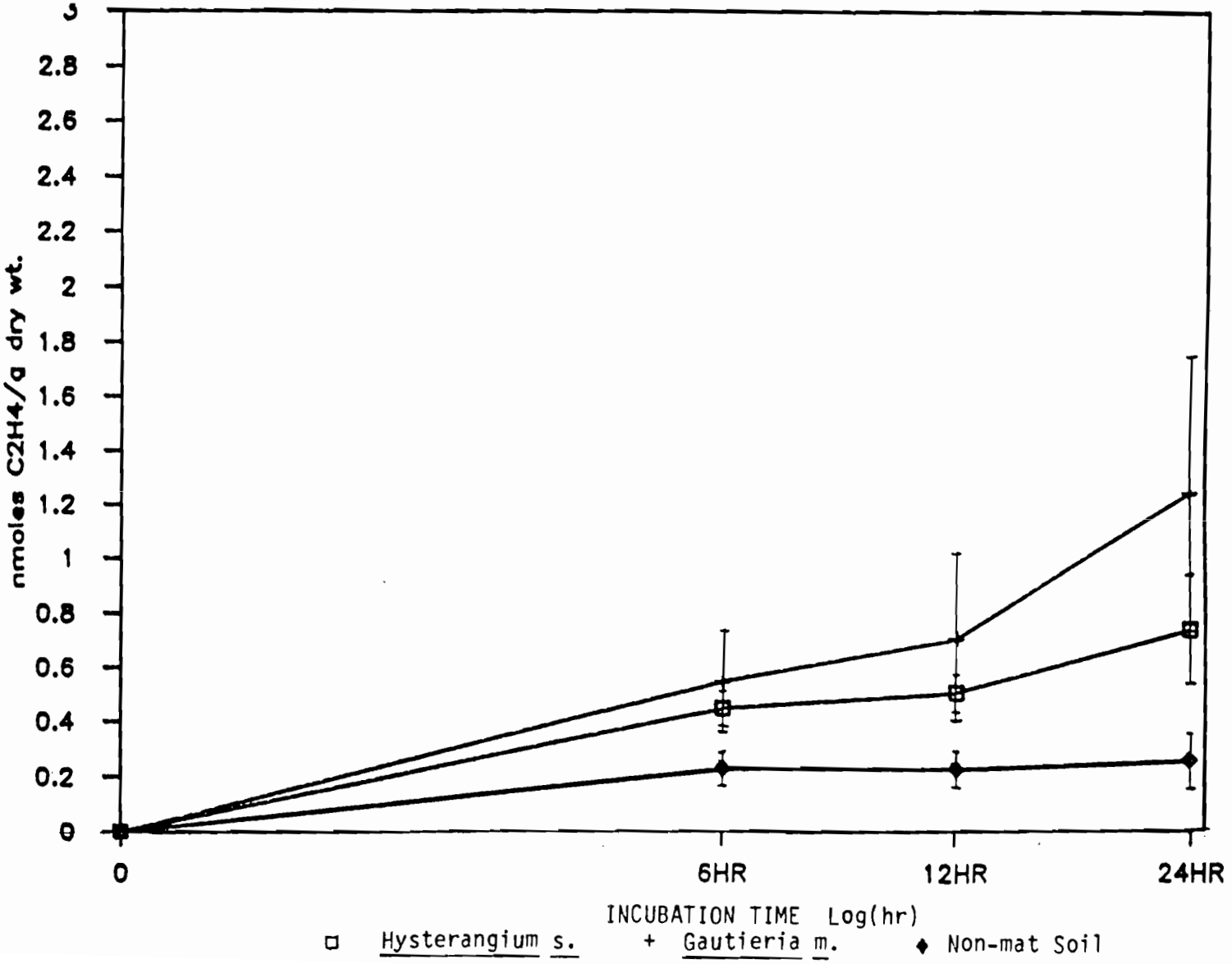


Fig. 11. Acetylene reduction assay in August 26, 1986

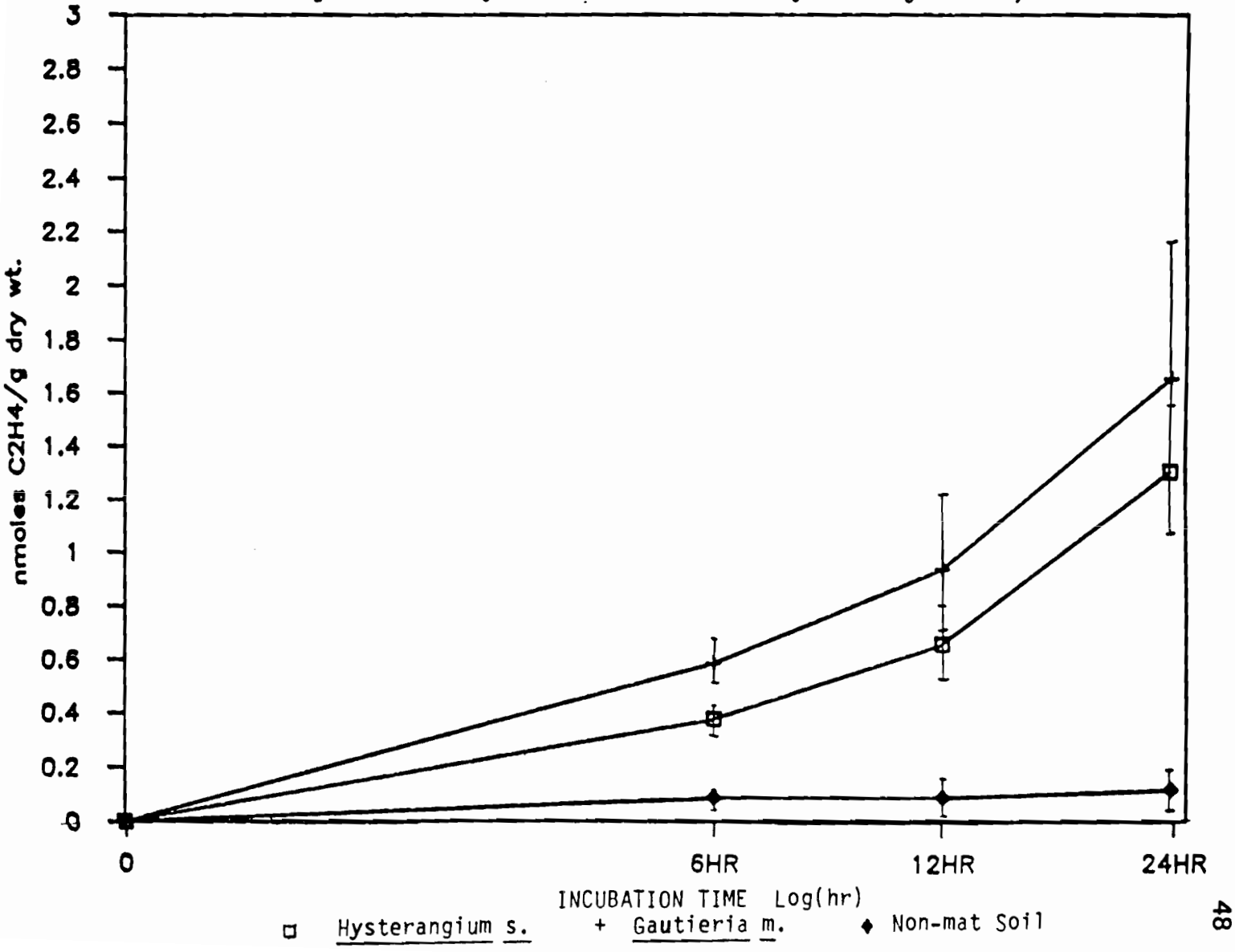


Fig. 12. Acetylene reduction assay in September 30, 1986

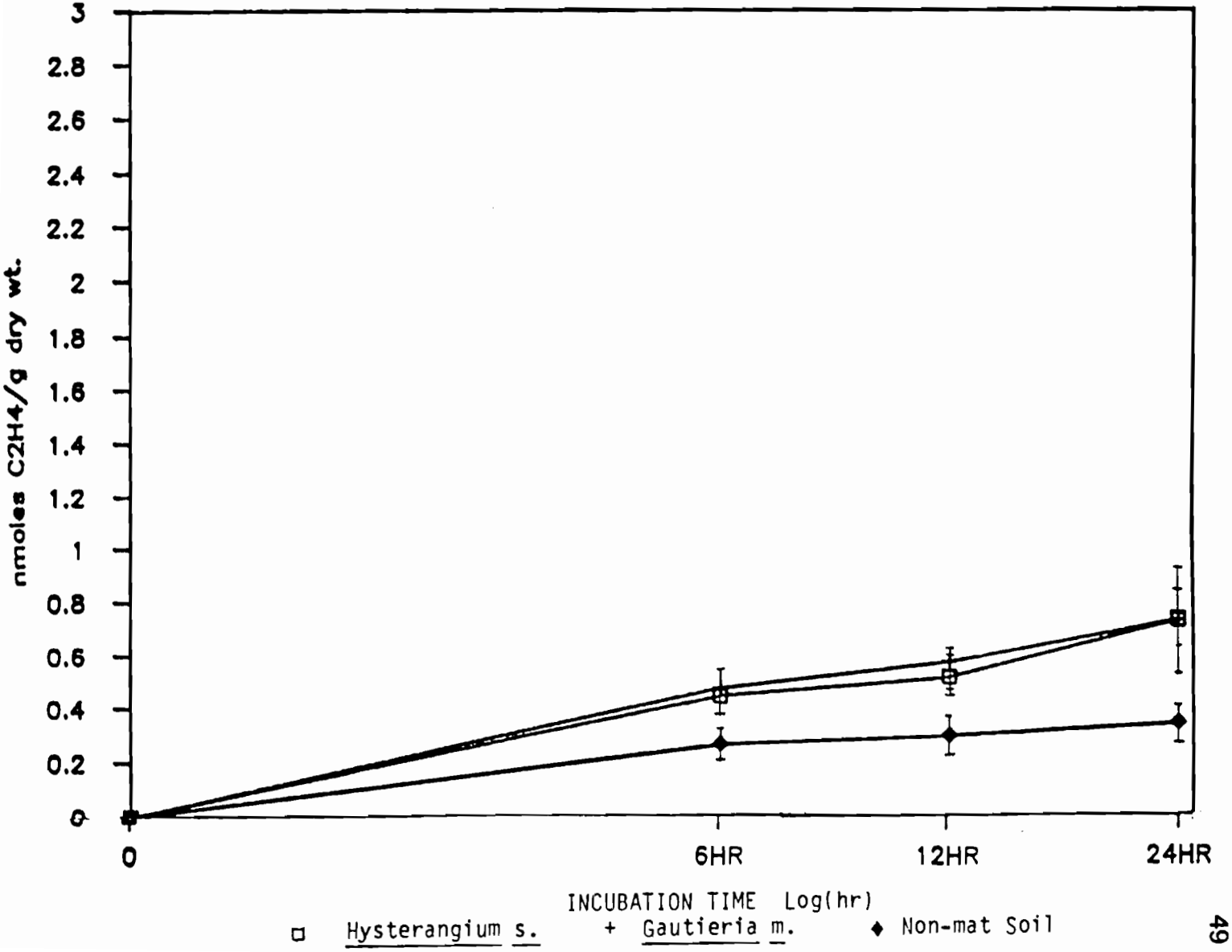
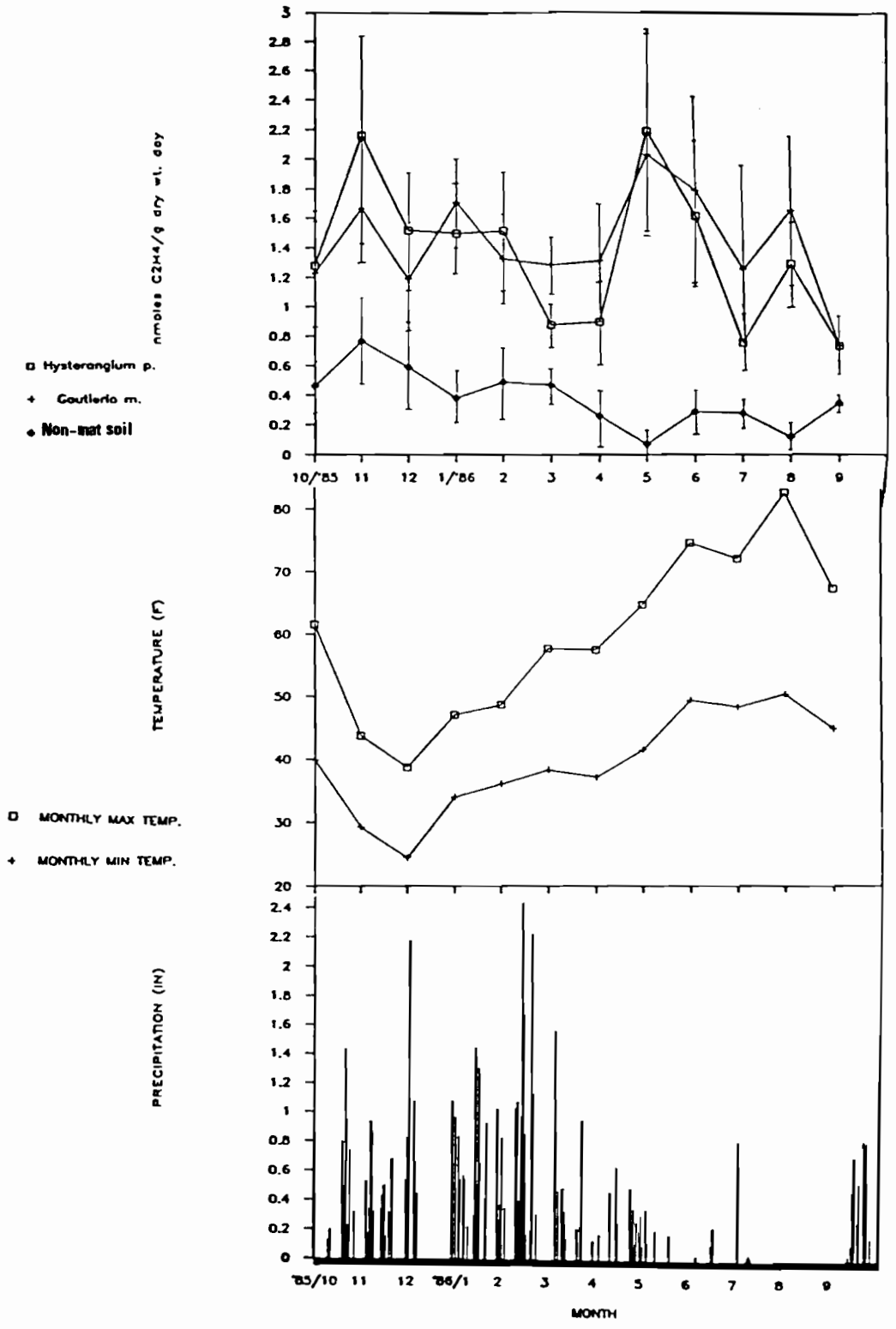
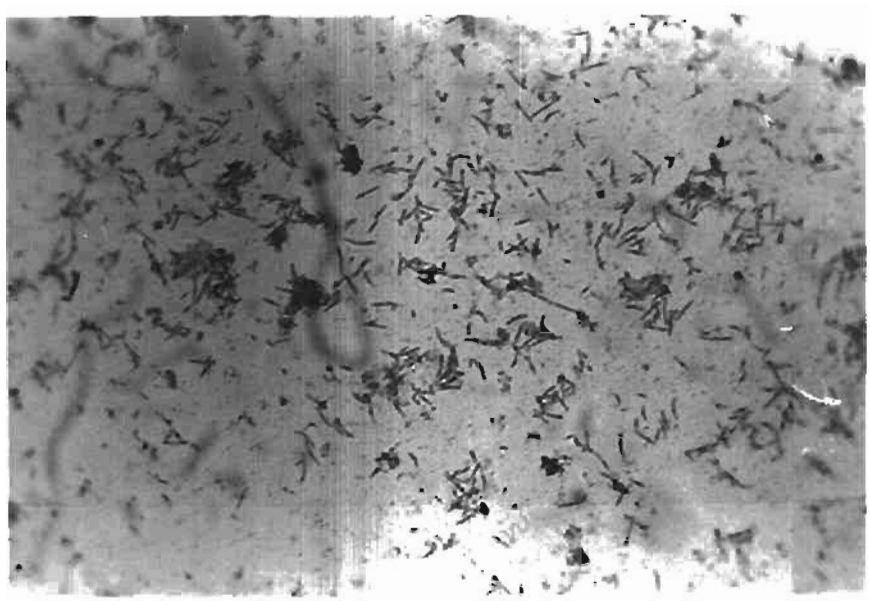
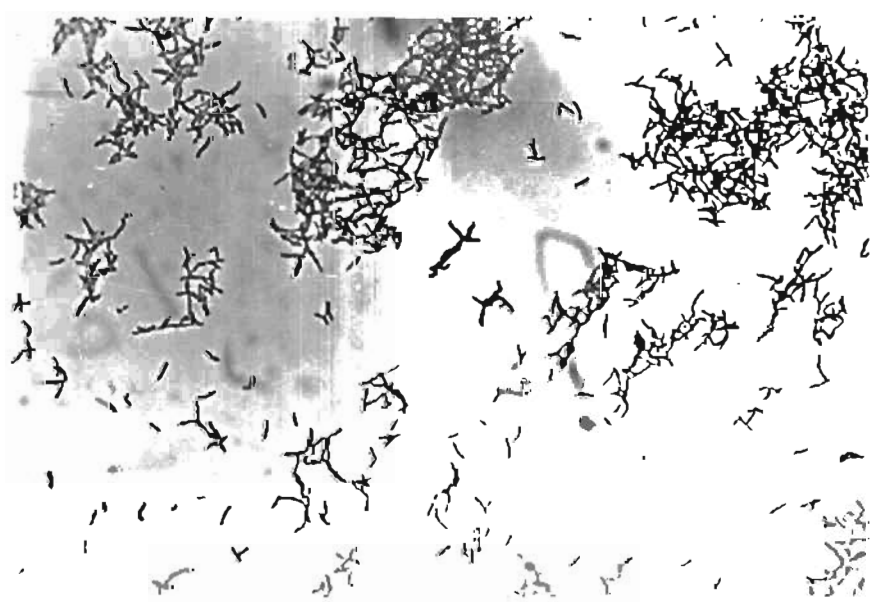


Fig. 13. Acetylene reduction activities from Oct. '85 - Sep. '86





Photograph 1. Strain isolated from the fungal sheaths of Hysterangium setchelli (160x)



Photograph 2. Strain isolated from the fungal sheaths of Gautieria monticola (160x)

Photograph 3. Strain isolated from the sporocarp of Hysterangium setchellii (160x)

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