

the angular anisotropies of the proton flux in the plane of the radiation belt. At the orbital altitude of 400 km the trajectory dispersion is $\leq 5^\circ$. Astronaut Pogue was oriented so that his visual axis was perpendicular to the plane of particle trajectories. Roughly one-fourth of the incident protons have angles of incidence such that they would have to penetrate a minimum of 11 g per square centimeter of shielding to reach the eye. These protons are ignored in our calculations. Because the remaining protons had to traverse a path length of between 3.2 and 3.8 g/cm² in the spacecraft shielding to reach the eye, we assumed that all protons with energies below 80 Mev were lost in the shielding.

The medium in the eye is assumed to have a stopping power equivalent to that of water. We used a Monte Carlo program to choose each incident proton based on the SAA energy spectrum and random positions and directions on the eyeball surface. As the particles travel through the eye, they can produce a star in the ocular mediums or directly in the retina. The evaporation prongs from the star then stop in the ocular mediums, pass through the retina, stop in the retina, or escape out of the front of the eye. When the Monte Carlo technique is used to cover all possibilities, a "pulse height" spectrum of energies deposited in the sensitive layer of the retina is generated. The number of protons and alpha particles emitted by each star and their energy distributions were estimated from the data of Powell *et al.* (5) and the Fermi evaporation model. In the work reported here, stars produced behind the retina are not included. This effect should increase the predicted flash rates shown in Fig. 1 by approximately a factor of 2.

The cross section for star production is closely approximated by the geometrical cross section, which leads to about 0.025 interaction per centimeter of proton trajectory. The detection efficiency for stars generated in the ocular mediums is quite low. Fewer than 15 percent of these stars emit particles that even reach the retina. The expected light flash frequency is plotted in Fig. 1 as a function of the threshold energy, E_{th} , that must be deposited within an area 300 μ m in diameter of the sensitive layer of the retina.

The direct contribution from trapped protons to the light flash rates in the SAA is a sharply decreasing function of the threshold requirement (Fig. 1). A threshold of 1.5 Mev would be in agreement with the flash rate as reported in (1).

The thresholds used in (1) are lower limits obtained by fitting the non-SAA

data while deliberately ignoring Cerenkov radiation in order to be conservative in describing the anomalously high rates. If one includes contributions from Cerenkov radiation to the Apollo and non-SAA Skylab data, it would raise the threshold and thereby lower the SAA flash rates calculated here considerably. Pinsky *et al.* (1) showed that spallation products do not contribute sufficiently, and such interactions in the shielding are not included here.

The solid curve in Fig. 1 represents the events in which one or more evaporation prongs from nuclear stars traverse the sensitive layer with the total energy deposited within 300 μ m exceeding E_{th} . There are obviously enough stars produced to explain the Skylab flash rates. If the peak flash rates of 20 per minute reported in the second Skylab session are used to obtain a threshold value from Fig. 1, a value of 1.4 Mev results. This value is in agreement with the limited accelerator data. Helium and nitrogen nuclei are detected near the end of their range (3). Even particles with a constant LET value as low as 10 keV/ μ m entering tangent to the retina may deposit as much as 3.0 Mev if they traverse an entire summation unit of 300 μ m diameter within the sensitive layer.

Although the available data do not assure that calculations based on threshold concepts are valid, the data presented here show that when the contribution from star production in and near the retina is included calculations of the type in-

troduced by Pinsky *et al.* (1) are brought into agreement with the experimental observations without the need to postulate trapped particles with $Z > 1$. Because of the large spatial fluctuations in the SAA flux, we made no attempt to achieve exact fits to the Skylab data. For this reason, the effects of ionization losses in the shielding, spallation, and star production in the sclera were not included in our calculations.

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Nitrogen Fixation in Grasses Inoculated with *Spirillum lipoferum*

Abstract. *Field-grown pearl millet (Pennisetum americanum) and guinea grass (Panicum maximum), lightly fertilized and inoculated with Spirillum lipoferum, produced significantly higher yields of dry matter than did uninoculated controls. Up to 42 and 39 kilograms of nitrogen per hectare were replaced by inoculation for pearl millet and guinea grass, respectively. The data demonstrate that nitrogen fixation by these grass-Spirillum systems is efficient and is achieved at a reasonable energy cost to the plant.*

Many important legume crops, such as soybeans and peas, have evolved symbiotic relationships with bacteria, which convert atmospheric nitrogen to a form available to the plants. This biological nitrogen fixation, mediated by the enzyme nitrogenase, requires about 15 molecules of adenosine triphosphate (ATP) for each molecule of N₂ reduced. It is highly wasteful of energy, but possibly only about one-half as wasteful as the commercial Haber-Bosch synthesis (1).

Most of the world's staple food crops (maize, wheat, rice, sorghum, and millet) and forages are grasses, which, until re-

cently, were believed to have no potential for biological nitrogen fixation. In 1973, however, rice rhizosphere associations were reported to fix appreciable amounts of nitrogen at the International Rice Research Institute, Los Banos, Philippines (2). In Africa, nitrogen fixation rates in flooded rice comparable to those in peanuts were reported (3). Researchers in Brazil, using acetylene reduction to measure nitrogenase activity, recently demonstrated nitrogen fixation in maize, wheat, and forage grasses (4, 5). They isolated *Spirillum lipoferum* from surface-disinfected grass roots and deter-

mined that most of the nitrogenase activity was associated with the roots and not the rhizosphere soil (5). On the basis of these results, together with cytological evidence of bacteria inside root cells (5, 6), we believe that *S. lipoferum* and grass roots form an associative symbiosis.

In 1974, we compared grasses inoculated with *S. lipoferum* (7) with their uninoculated counterparts. Inoculation was accomplished by diluting a bacterial culture, grown in a semisolid, malate-mineral salts medium (without fixed nitrogen), with five parts of water, homogenizing the mixture in a blender at slow speed, and pouring 100 ml directly on each plant. An equivalent amount of medium, without bacteria, was applied to controls. Additional water was used to wash the inoculum into the soil.

Forty genotypes were tested representing five tropical grass genera (3 *Digitaria*, 13 *Panicum*, 8 *Paspalum*, 2 *Cynodon*, and 13 *Cenchrus*). Two genotypes responded to inoculation with increased forage yields ($P = .05$). The yields from inoculated 'Transvala' digitgrass (*Digitaria decumbens*) and guinea grass (*Panicum maximum*) were 163 and 150 percent of those from their uninoculated controls, respectively. Inoculated *Panicum* also had a higher crude protein content than its uninoculated counterpart (see Table 1).

In 1975 we planted eight replicate samples of two grasses, pearl millet (*Pennisetum americanum*) and guinea grass, in a randomized block design in 3.2-m² plots in the field. We compared eight treatments, consisting of four rates of fer-

Table 1. Responses of two grasses to inoculation with *S. lipoferum*.

Grass	Mean dry matter yield (grams per plot)	Crude protein	
		Percentage of dry matter	Grams per plot
'Transvala'	Inoculated	300	6.4
	Control	180	6.6
<i>Panicum</i>	Inoculated	1228	10.2
	Control	800	8.6

tilizer N applied as ammonium nitrate over inoculated and uninoculated plots. The fertilizer rates were 0, 20, 40, and 80 kg of N per hectare applied after seedling emergence and 0, 10, 20, and 40 kg/ha after the first harvest.

Mass cultures of *S. lipoferum* strain 13t (7) were prepared in a malate-mineral salts medium without fixed nitrogen (5). The cultures were continuously sparged with a mixture of 95 percent N₂ and 5 percent O₂ in a 13-liter New Brunswick culture apparatus, and harvested near the terminus of logarithmic growth (24 to 48 hours). The medium containing bacteria was applied in aqueous solution to the experimental plots at approximately 8×10^7 cells per meter of row and irrigated into the soil by sprinkling. Control plots received malate medium without bacteria. In all plots the malate concentration is calculated to be 6.9 kg/ha. The millet was harvested once and the guinea grass twice. Forage nitro-

gen content was determined with a Technicon nitrogen analyzer and was found not to differ significantly over treatments.

Yield increases due to *Spirillum* inoculation in both of these grass species are shown in Fig. 1. With the millet, significant dry matter increases ($P = .05$) were obtained with inoculation in the presence of fertilizer N concentrations of 40 to 80 kg/ha (Fig. 1A). Yields with first-cut guinea grass were more variable, but significant increases were obtained with inoculation ($P = .10$) plus a fertilizer N concentration of 40 kg/ha (Fig. 1B). After refertilization, the available nitrogen for the second crop of guinea grass was uncertain, so we report total yields in Fig. 1C; significant yield increases with inoculation were obtained ($P = .05$) with fertilizer N concentrations of 30 to 60 kg/ha.

Regression equations were calculated and it was projected that fertilizer N concentrations of 60 and 122 kg/ha would have been required in the uninoculated plots of pearl millet to produce yields equivalent to those obtained with 40 and 80 kg/ha in inoculated plots. That is, inoculation replaced up to 42 kg of fertilizer N per hectare. Since the growing period for the millet was 70 days, an average of 0.6 kg of N per hectare per day was replaced for the growing season.

In guinea grass it was estimated that fertilizer N concentrations of 55 to 99 kg/ha would have been needed in uninoculated plots to produce yields equivalent to those obtained with 30 to 60 kg/ha in uninoculated plots. In this case, in-

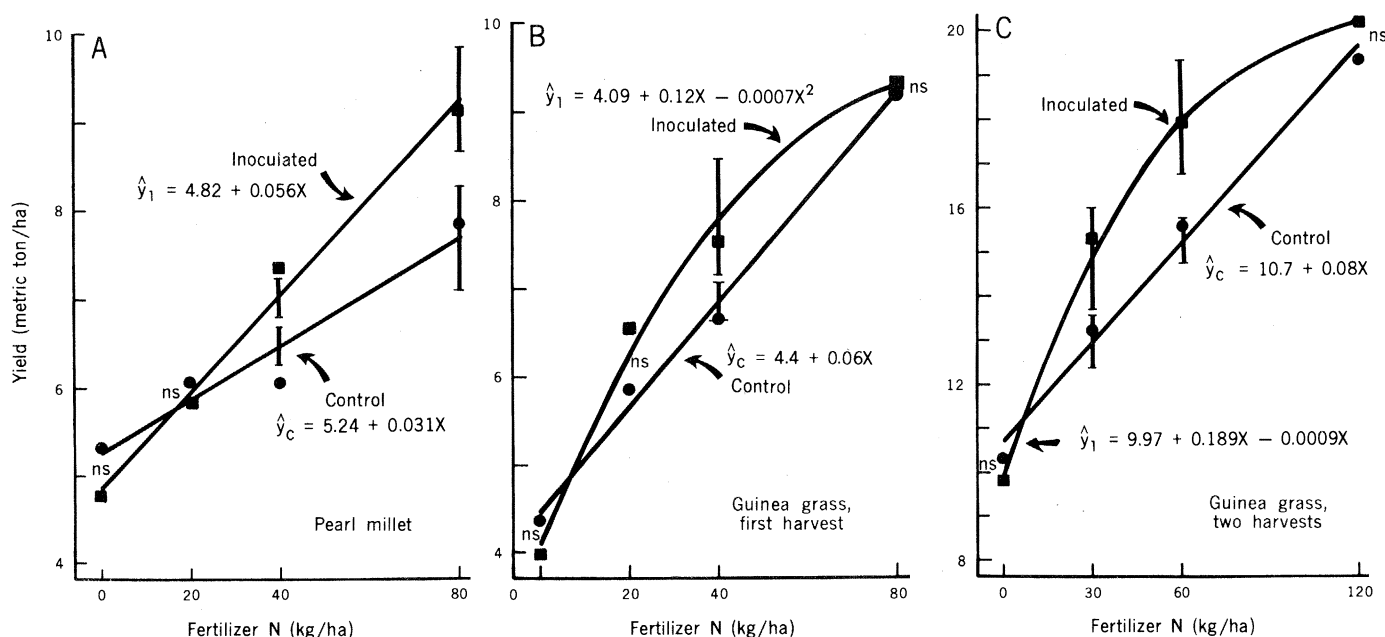


Fig. 1. Dry matter forage yields of grasses inoculated with *S. lipoferum* compared to uninoculated counterparts. Tests for significant differences were made for each fertilizer N concentration (9). Error bars represent standard errors (9). (A) Pearl millet. (B) Guinea grass, first harvest. (C) Guinea grass, total for two harvests. See text for discussion; ns, not significant.

oculation replaced up to 39 kg of fertilizer N per hectare, comparable to the 42 kg/ha replaced in the millet.

These results indicate that some fertilizer N was required to induce a response to inoculation. This might not be necessary where residual soil nitrogen is not extremely low, as it is in the sandy soil in which our experiments were conducted. We consider that this nitrogen may be required to stimulate plant growth and photosynthesis: the plant supplies energy to the bacteria for nitrogen fixation, and this requires a reasonable rate of plant metabolism at the outset.

In Fig. 1, B and C, the plots of dry matter yield against fertilizer N concentration are linear for uninoculated plots and curvilinear for inoculated plots. This may be because low concentrations of fertilizer N promote the establishment of nitrogen-fixing associations, giving a greater than normal growth response, while higher concentrations reduce the response by repressing nitrogenase (8).

The data reported here show that increased dry matter yields or reduced fertilizer N requirements can be obtained with grass-*Spirillum* systems. Nitrogen fixation by these systems is efficient and is achieved at a reasonable energy cost to the plant.

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one-sided confidence interval, and $s(\hat{y}_1 - \hat{y}_c) = [s(\hat{y}_1)^2 + s(\hat{y}_c)^2]^{1/2}$ is the estimated standard error of $\hat{y}_1 - \hat{y}_c$ at the level of N . Error bars represent $\hat{y}_1 \pm s\hat{y}_1$ or $\hat{y}_c \pm s\hat{y}_c$.

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Acoustic Tracking of Ocean-Dumped Sewage Sludge

Abstract. With a modified 200-kilohertz acoustic echo sounder, it has been possible to detect and map sewage dumped into the ocean over several hours. The three-dimensional distribution of suspended material and its rate of diffusion are indicated after digital processing of the data.

Until now, the real-time tracking of sewage sludge dumped into the ocean has been difficult because in situ identification techniques were lacking. Chemical sampling is complicated by the inherent delay between the time the sample is taken and the time the analysis is complete, and also by the lack of reliable "tag" substances with which to distinguish samples inside the sludge "cloud"

from those outside it. Partial success in the chemical sampling of ocean-dumped sewage sludge has been reported by Due-dall *et al.* (1) and Callaway *et al.* (2). Shipboard measurements with a light beam transmissometer, whether towed or lowered, are made along a horizontal or vertical line and require extensive sampling of the sludge cloud to provide a reasonably complete three-dimensional

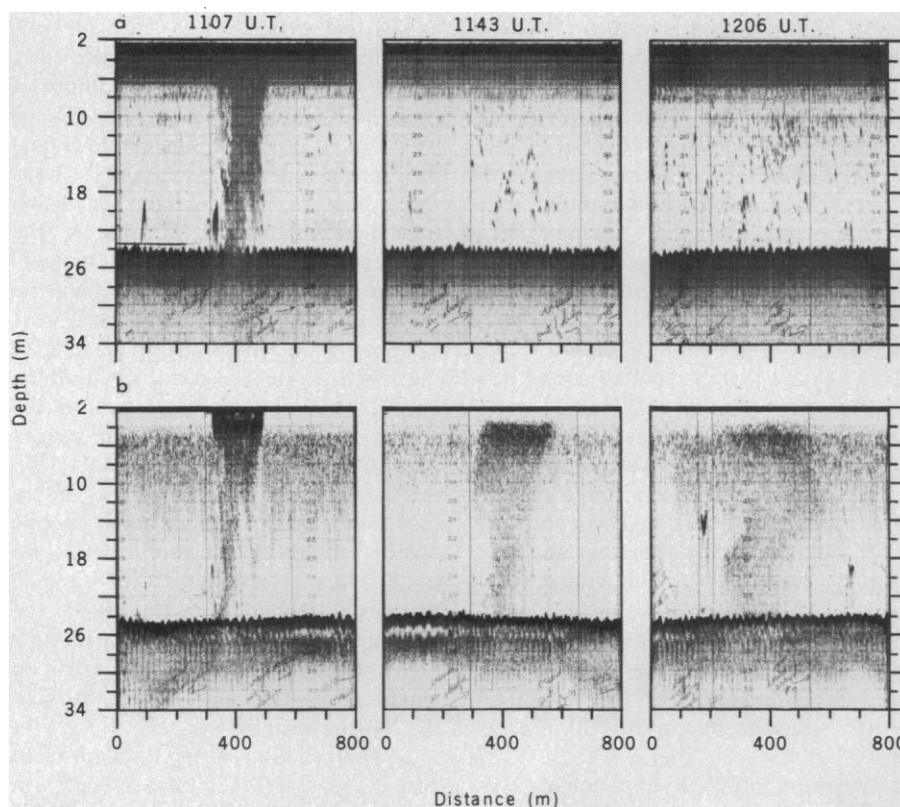


Fig. 1. Three segments of the (a) 20-kHz and (b) 200-kHz acoustic records for traverses of the 1045 E.D.T. line sewage sludge dump, 22 September 1975, during the sludge-tracking acoustical experiment. The distance indicated is along the ship track. The universal time (U.T.) of each traverse is given above the respective record. The first traverse shown (1107 U.T.) occurred approximately 9 minutes after the sewage sludge was dumped (that is, 9 minutes after that portion of the sewage sludge through which the traverse occurred was dumped).