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NITROGEN FIXATION IN ASSOCIATION WITH THE ROOT SYSTEMS OF GOLDENRODS (*SOLIDAGO* L.)

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Summary—The roots of seven species of goldenrod (Asteraceae: *Solidago*) were assayed for associative nitrogen fixation. We used the acetylene reduction method to measure N_2 fixation rates in sampling jars that contained roots of sampled plants and soil. The rate of C_2H_2 reduction in jars with *S. rigida* (12.9 ± 1.2 nmol h^{-1}, mean $\pm$ SE) and *S. canadensis* (6.0 ± 2.0 nmol h^{-1}) was significantly higher than in soil-only control jars. There was measurable C_2H_2 reduction in jars of the other five species, but the rates were not significantly different from controls. The rates of C_2H_2 reduction per root dry weight and per total plant dry weight also were higher for *S. rigida* and *S. canadensis* than for the other species.

INTRODUCTION

Dinitrogen is fixed in the rhizosphere of a variety of non-nodulated angiosperms, particularly tropical grasses (Dobereiner and Day, 1975; Neyra and Dobereiner, 1977; Woldendorp, 1978; Weir, 1980). Such associative fixation can be a significant source of nitrogen for plants (Boddey *et al.*, 1983; Rennie *et al.*, 1983; Biesboer, 1984).

A few dicotyledons have been sampled for associative nitrogen fixation (Harris and Dart, 1973; Conklin and Biswas, 1978), but the proportion of species sampled remains small. We report the result of assays for N_2 fixation in association with the roots of seven species of goldenrod (Asteraceae: *Solidago* L.), common perennial herbs of prairies and old-fields in North America.

METHODS

We sampled plants in Cedar Creek Natural History Area, approximately 50 km north of the St. Paul campus of the University of Minnesota. Plants were sampled from mid-July to early September, 1984, during flowering and early fruiting. We haphazardly selected plants from large populations.

Although soil cores are often used in studies of associative N_2 fixation, cores can contain roots of several species so that the precise source of N_2 fixation activity is unclear. Attempts by others to localize N_2 fixation activity have included the use of excised roots that were washed and incubated, but this excised root method is unreliable and can seriously overestimate fixation rates (van Berkum, 1980; van Berkum and Bohlool, 1980; Lethbridge *et al.*, 1982).

To avoid the lack of specificity of soil core assays and the errors of the excised root method, we extracted root systems from the soil and held them (without washing) in the soil from which they were dug. For each sample, we dug up the soil around the target plant and shook the plant until its roots were free of the soil and the roots of other species. The shoots were clipped off at soil level and saved for weighing. The roots were placed immediately in a 1 l canning jar fitted with a serum stopper for gas sampling. To prevent drying, the roots in the sampling jars were covered with some of the same soil that had been shaken from them earlier. Control jars contained only soil, taken from the same source as the soil in the experimental jars. Soil moisture and temperature were measured for each sample taken.

It was usually possible to prepare and seal the experimental jars within 5 min from the time the roots were taken from the ground. To maintain constant temperatures, we placed the jars as soon as they were filled into a plastic cooler.

We estimated N_2 fixation activity by the acetylene reduction assay (Hardy *et al.*, 1968; Hardy *et al.*, 1973). We removed 10% of the atmosphere of each jar and replaced it with C_2H_2 that had been generated from calcium carbide and water. C_2H_2 was injected and the first gas samples were taken 4 h after the roots were placed in sampling jars in the field. We sampled the jars periodically (usually 5 times over 2 days) to determine the rate of C_2H_4 production, an indirect measure of nitrogenase activity. To test for endogenous C_2H_4 production in the absence of C_2H_2, a sample jar was prepared with soil and *Solidago* roots but was not injected with C_2H_2.

We took gas samples directly from the sample jars, and analyzed them using a Hewlett–Packard 5840A

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Table 1. Acetylene reduction activity (mean ± SE) associated with *Solidago* root samples

Species and number of samples	Maximum acetylene reduction rate (ARR) per sample (nmol h^{-1})	ARR per plant (root plus shoot) dry wt (nmol $g^{-1} h^{-1}$)	ARR per root dry wt (nmol $g^{-1} h^{-1}$)
S. rigida L. (6)	12.93 ± 1.19a*	0.54 ± 0.05	0.89 ± 0.14
S. canadensis L. (6)	6.00 ± 2.00b	0.46 ± 0.16	1.12 ± 0.45
S. gigantea Ait. (6)	2.92 ± 1.13bc	0.18 ± 0.08	0.60 ± 0.34
S. speciosa Nutt. (4)	2.82 ± 0.63bc	0.25 ± 0.05	0.34 ± 0.16
S. missouriensis Nutt (3)	2.03 ± 0.43bc	0.16 ± 0.05	0.36 ± 0.12
S. nemoralis Ait. (4)	0.87 ± 0.31c	0.07 ± 0.02	0.25 ± 0.07
S. graminifolia (L.) Salisb. (6)	0.85 ± 0.18c	0.09 ± 0.03	0.22 ± 0.06
Soil-only control (9)	1.16 ± 0.26c	—	—

*Means that differ by less than Tukey's Honestly Significant Difference are followed by the same letter.

GC fitted with an f.i.d. and a 100/120 mesh Porapak R column (1.0 m × 2 mm i.d. stainless steel). Analyses were performed isothermally at 50°C; the carrier gas was N_2 at 40 ml min^{-1}. During the assay period the samples were at room temperature (*ca.* 20 ± 2°C).

After the assay, we washed the roots free of soil, then oven-dried and weighed roots and shoots from each experimental plant.

Species nomenclature follows Gleason and Cronquist (1963).

RESULTS AND DISCUSSION

Samples from all *Solidago* species and from the soil-only controls showed detectable levels of C_2H_4 production (Table 1). No C_2H_4 was produced when roots and soil were incubated without C_2H_2. The variance in acetylene reduction rate (ARR) was unequal among species (Bartlett–Box test, $P < 0.001$), so data were log-transformed.

The species differed significantly in ARR (analysis of variance, $P < 0.0001$). *Solidago rigida* and *S. canadensis* showed significantly higher ARR than the soil-only controls. *Solidago rigida* rates were approximately twice those of *S. canadensis*, and about 12 times greater than controls (Table 1). The other five *Solidago* species did not differ from the controls. There were no significant differences among species in soil temperature (overall mean ± SD = 22.8 ± 2.8°C) or soil moisture (5.1 ± 2.1% of oven-dry weight).

The controls used were conservative. Since the soil for controls was taken from the immediate vicinity of the plant roots, the C_2H_2 reduction activity of the soil could be due to the influence of the roots or other organic sources. Also, some fine roots unintentionally remained mixed with the soil in the control jars. If the C_2H_2 reduction in control soil was caused by association with *Solidago* roots, the rates for all of the *Solidago* species might be biologically significant.

ARR was highest for *S. rigida* and *S. canadensis* whether expressed per sample, per plant weight or per root weight. Overall, species differed significantly (Kruskal–Wallis test, $P < 0.05$) in their ARR when expressed per dry weight of plant (Table 1), though no pairwise differences were significant (Games–Howell test, $P > 0.05$). The ARR per dry weight of root differed among species only at a significance level of 0.057 (Kruskal–Wallis test).

van Berkum and Sloger (1979) reported C_2H_2 reduction from roots of a *Solidago* species (unfortunately unidentified) from an estuary. The ARR per root dry weight was 0.7 ± 0.4 nmol $g^{-1} h^{-1}$, similar to our values.

We never observed the 8–18 h lag commonly observed when the excised root method is used (van Berkum and Bohlool, 1980); our first estimate of C_2H_2 reduction was always at least as high as later estimates. Though C_2H_4 was not detected in the control without C_2H_2, the observed C_2H_4 production in jars with C_2H_2 conceivably could have resulted from the inhibition by C_2H_2 of bacterial C_2H_4 oxidation (de Bont, 1975, 1976; de Bont and Mulder, 1976). This is an unlikely explanation for the difference between the soil-only controls and jars with roots, since the only difference between control and experimental jars was the presence of roots. If the increase in C_2H_4 production in jars with roots was caused by inhibition of C_2H_4 oxidation, the responsible organisms must have been directly associated with roots. However, C_2H_4-oxidizing bacteria are commonly found in soil (Cornforth, 1975; de Bont, 1975, 1976).

Theoretically, nitrogenase can reduce C_2H_2 to C_2H_4 at 3 times the rate it can reduce N_2 to NH_3 (Hardy *et al.*, 1973), so rates of N_2 fixation could be calculated from Table 1 by dividing the rates of C_2H_4 production (equivalent to rates of C_2H_2 reduction) by 3. However N_2 fixation rates so derived must be treated as gross approximations, since the actual conversion factor varies widely in different soils (Hardy *et al.*, 1973; van Berkum and Bohlool, 1980; Nohrstedt, 1983).

The ARR in association with *Solidago* species is much lower than that for nodulated legumes. Using the same methods to measure ARR, we found rates per plant dry weight in four legume samples (*Vicia villosa* Roth., *Melilotus alba* Desr. and two of *Trifolium pratense* L.) to range from 20 to 51 nmol $g^{-1} h^{-1}$, about 40–100 times greater than the average rate we measured for *Solidago rigida*.

Although the low N_2 fixation rates we measured do not indicate an important community-wide input of nitrogen, it is unclear whether the N_2 fixed in association with *Solidago* roots is a biologically important source of nitrogen for individual plants. The soils of Cedar Creek Natural History Area are very nitrogen-poor (Grigal *et al.*, 1974) and plants there respond dramatically to nitrogen fertilization (Tilman, 1984). It is possible that even a small increment in nitrogen availability could affect the competitive ability, photosynthetic rate, or reproductive capacity of a plant.

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