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Effect of Altered pO_2 in the Aerial Part of Soybean on Symbiotic N_2 Fixation[1]

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ABSTRACTS

Dry matter accumulation, nitrogen content and N_2 fixation rates of soybean (*Glycine max* [L.] Merr. cv. Wye) plants grown in chambers in which the aerial portion was exposed to a pO_2 of 5, 10, 21, or 30% and a pCO_2 of 300 μl CO_2/l or a pO_2 of 21% and a pCO_2 of 1200 μl CO_2/l during the complete growth cycle were measured. Total $N_2[C_2H_2]$ fixed was increased by CO_2/O_2 ratios greater than those in air and was decreased by ratios smaller than those in air; the effects on N_2 fixation of decreased pO_2 or elevated pCO_2 were quantitatively similar during the period of vegetative growth. Decreased pO_2 produced a smaller increase then elevated pCO_2 during the reproductive period, presumably because of the decreased sink activity of the arrested reproductive growth under subambient pO_2. At a pO_2 of 5% and a pCO_2 of 300 μl CO_2/l total N_2 fixed was increased 125% and per cent nitrogen content in the vegetative parts was increased relative to air while that in the seed was decreased. Dry matter production was increased and reproductive growth was arrested as previously reported for plants receiving only fertilizer nitrogen. At a pO_2 of 30% and a pCO_2 of 300 μl CO_2/l total N_2 fixed was decreased 50% and per cent nitrogen content in the vegetative part was increased relative to air while that in the reproductive structures was unaffected. Dry matter production was similarly decreased in both vegetative and reproductive structures. These effects of altered pO_2 in the aerial part on N_2 fixation are consistent with the hypothesis that the amount of photosynthate available to the nodule may be the most significant primary factor limiting N_2 fixation while sink activity of the reproductive structures may be a secondary factor.

A direct relationship appears to exist between source production of photosynthate or photosynthate available to the nodule and symbiotic N_2 fixation by legumes such as soybeans. Several factors that decrease photosynthate available to the nodule have been demonstrated to decrease N_2 fixation, *e.g.*, decreased light intensity from night and shading (5, 10, 16), decreased source size by partial defoliation (5, 12), high plant density and lodging (3), increased demand of competitive sinks during late seed development and interruption of translocation to nodule by girdling (9, 10, 12). Factors that increase photosynthate available to the nodule increase N_2 fixation, *e.g.*, increased light intensity during the day and supplemental light (5, 10, 16), increased source size by grafting additional foliage and low planting density (3, 17), decreased demand of competitive sinks by pod removal and increased rates of photosynthesis by CO_2 enrichment of the foliar canopy (3, 6, 9, 16). The most dramatic demonstration that production of photosynthate is a major limiting factor for N_2 fixation in field-grown soybeans was obtained from a 3-fold CO_2 enrichment of the soybean canopy during the period of reproductive growth. The amount of N_2 fixed was increased from 75 to 425 kg/ha while the amount of N obtained from the soil was decreased from 220 to 85 kg/ha (3, 6). We have attributed the major effect of CO_2 enrichment to an increase in net production of photosynthate made possible by a decrease in photorespiration produced by an elevated CO_2/O_2 ratio.

Lowering the O_2 content of the atmosphere will also greatly decrease photorespiration and the direct O_2 inhibition of photosynthesis, and thereby increase photosynthetic CO_2 fixation as shown by our recent long term studies (13, 14). These studies with intact plants grown to maturity under low O_2 concentrations showed that the growth rate of C_3 species like soybean is increased while the growth rate of C_4 species like sorghum is not affected markedly. In the N_2 fixation experiments we have altered the CO_2/O_2 ratio by manipulation of pO_2 and compared the effects with those produced by manipulation of pCO_2 in order to evaluate further the relationship between N_2 fixation and production of photosynthate.

MATERIALS AND METHODS

Plant Material. Soybeans (*Glycine max* [L.] Merr. cv. Wye) were grown in 18-cm plastic pots containing a Jiffy-mix (Jiffy Products of America, West Chicago, Ill.)-sand mixture (1:1, v/v) and watered daily with a N-free modified Hoagland nutrient solution (7). Plants were grown at various O_2 and CO_2 concentrations using techniques previously described (13, 4). Plants were grown to maturity in a controlled environmental growth room (6000 ft-c, 12-hr photoperiod, 75% relative humidity, 29 C day, 18 C night). The aerial portion was exposed to air or a modified gas mixture containing 5, 10, or 30% O_2 balanced with 300 μl CO_2/l and N_2 or 21% O_2 enriched to 1200 μl CO_2/l in chambers made of Lucite abrasion resistant sheet of 145-liter capacity with urethane foam gaskets around the stem entrance, while the roots were exposed to air. Gas mixtures were prepared in a continuous flow system and purged continuously with needle valves and flow meters at a rate of 15 l/min. The plants were grown in the chambers from 18 to 83 days, the period from early seedling growth to maturity. At the conclusion of the experiment the plants were removed from the chambers, and dry weights of stems, leaves, roots, nodules, pods, and seeds were determined.

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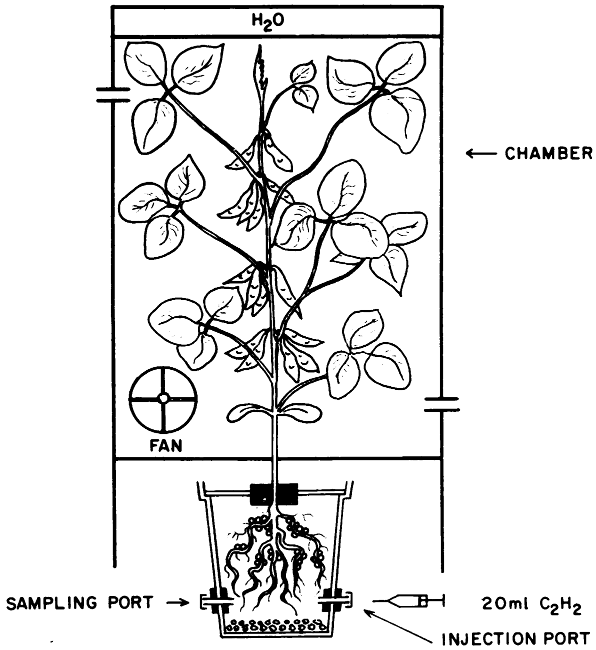

FIG. 1. Diagrammatic representation of the chamber for growing plants under modified atmospheres and the modified nondestructive intact plant-pot system for measuring $N_2[C_2H_2]$-fixing activity.

Measurement of Nitrogen Fixation. $N_2[C_2H_2]$-fixing activity of nodules was determined by the acetylene-ethylene assay (4, 5). This assay, as shown in Figure 1, provided a nondestructive method for measuring nitrogen fixation in intact plants. It was adapted from previously described techniques (2, 8, 15). This method allows repeated measurements of N_2 fixation on individual intact plants over the entire growth cycle thereby greatly reducing the number of plants and chambers required compared to destructive assay procedures utilizing excised nodulated roots. Other advantages of this method for the measurement of N_2 fixation by intact plants are the smaller gas phase and the ability to expose the root and aerial parts of the plant to different gas environments.

The 18-cm pot containing the individual plant root system grown in the Jiffy-mix-sand mixture (1:1, v/v) serves as the incubation chamber. In these experiments $N_2[C_2H_2]$ fixation of intact plants was measured every 7 to 10 days from early vegetative growth (26 days) to maturity (83 days). The top of the pot was fitted with a disc made of Lucite having a 5-cm diameter hole. Two 1-cm holes were made at the base of the pot to provide a port for injecting or flushing gases and removal of gas samples (Fig. 1). The closed pot system contained approximately 800 ml of gas space. No correction was made for water. The assay procedure was as follows. The pots were first flushed with air for 2 min to remove excess water and then purged for 2 min with an 80% Ar-20% O_2 gas mixture to replace the air. The pot system was then immediately sealed with a single holed 5-cm split stopper fitted around the base of the stem and sealed with Plasticine. Rubber stoppers fitted with a 6-cm glass tube covered by a stopall septum sealed the two 1-cm sampling ports at the base of the pot. Using a syringe and needle, 20 ml of acetylene were introduced into the closed system through one of the sampling ports (Fig. 1). Following 15- and 30-min incubation periods, 10-ml samples of gas were withdrawn and introduced into an evacuated vacutainer tube. Immediately following the 30-min incubation period the system was opened and flushed with air for 5 min. Analysis of acetylene and ethylene were determined by gas chromatography (5). After an initial lag period of 3 to 5 min, reduction rates of acetylene to ethylene were constant for at least 50 min (Fig. 2). From the individual measurements of C_2H_4 produced between 15- and 30-min incubations, N_2-fixing activities for the period from 26 to 83 days of age were calculated and values were converted to N_2-fixing activity utilizing a ratio of 1 mole of N_2 fixed for 3 moles of C_2H_2 reduced (5) and expressed as mg N_2 fixed/plant·day and integrated to obtain total mg N_2 fixed/plant. Each treatment consisted of one plant replicated twice with two pots per chamber. Similar results have been obtained in two separate experiments.

Nitrogen Determination. Nitrogen content of the vegetative portions of the plant consisting of leaves, stem, roots, and nodules and the reproductive portions consisting of pods and seeds harvested at maturity was measured by Kjeldahl analysis.

RESULTS AND DISCUSSION

Dry matter accumulation, nitrogen content and N_2 fixation rates of soybean plants grown in chambers in which the aerial portion was exposed to a pO_2 of 5, 10, 21, or 30% O_2 and a pCO_2 of 300 μl CO_2/l, or to a pO_2 of 21% and a pCO_2 of 1200 μl CO_2/l during the complete growth cycle are shown in Figures 3 and 4.

Exposures of 5% O_2 increased dry weight at maturity of vegetative plant parts consisting of stems, leaves, roots, and nodules by 212%, while reproductive growth consisting of pods and seeds was decreased by 66% compared to air (Fig. 3A). Intermediate effects on dry matter were produced by 10% O_2. In contrast to decreased pO_2, elevated pCO_2 increased dry matter accumulation in both vegetative (118%) and reproductive (188%) tissue. Exposure to elevated O_2 decreased dry weight of both vegetative and reproductive structures by 37%. These results of vegetative and reproductive growth where both fertilizer nitrogen and N_2 fixation are the source of nitrogen are in agreement with our previous reports (13, 14), where fertilizer N was the sole source of N, and indicate that the effects of O_2 concentration on the partitioning of dry matter between vegetative and reproductive growth is independent of nitrogen source. This inhibition of reproductive development by subambient O_2 levels was proposed (13, 14) to be due to a physical process altering O_2 diffusion or a chemical reaction associated with translocation to and accumulation of assimilates in reproductive structures.

The per cent nitrogen, based on Kjeldahl analysis, was increased in leaves, stems, pods, roots, and nodules of plants grown

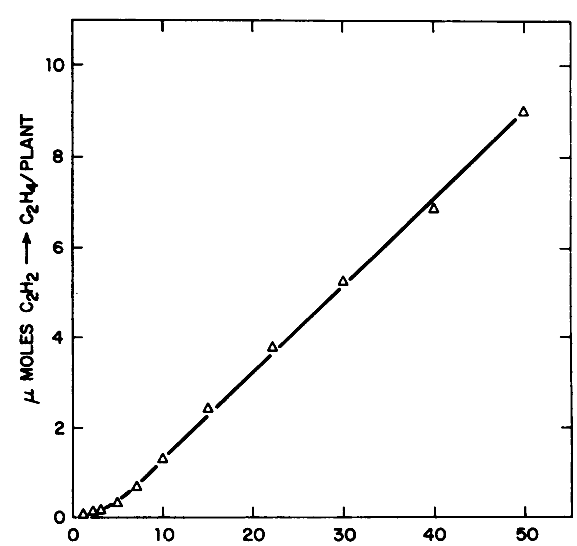

FIG. 2. Time course of $C_2H_2 \rightarrow C_2H_4$ reduction by intact 50-day-old soybean plants using the modified nondestructive intact plant-pot assay for measuring $N_2[C_2H_2]$-fixing activity.

in 5% and 10% O_2 relative to air but was decreased in the seed at 5% O_2 (Fig. 3B). In elevated CO_2 the percentage of nitrogen changed little except for the pods and leaves where it was somewhat decreased. The dissimilar responses of the nitrogen content to decreased pO_2 and CO_2 enrichment suggest that there is a difference between the interaction of O_2 and CO_2 with respect to partitioning of nitrogen assimilates as already observed for total dry matter. Total nitrogen content per plant was increased by both CO_2 enrichment and subambient O_2 while it was decreased by elevated O_2. The total nitrogen content was increased 150% by CO_2 enrichment with the greatest increase in the reproductive structures. Five per cent O_2 increased total nitrogen content by 74% with an even greater increase in the vegetative and a decrease in reproductive parts. The increase in nitrogen content of vegetative parts exceeded the increase in dry matter of vegetative parts in plants grown at 5% O_2. This indicated that the partitioning of nitrogen assimilates into reproductive structures in subambient O_2 environments is probably even less favorable than the partitioning of dry matter.

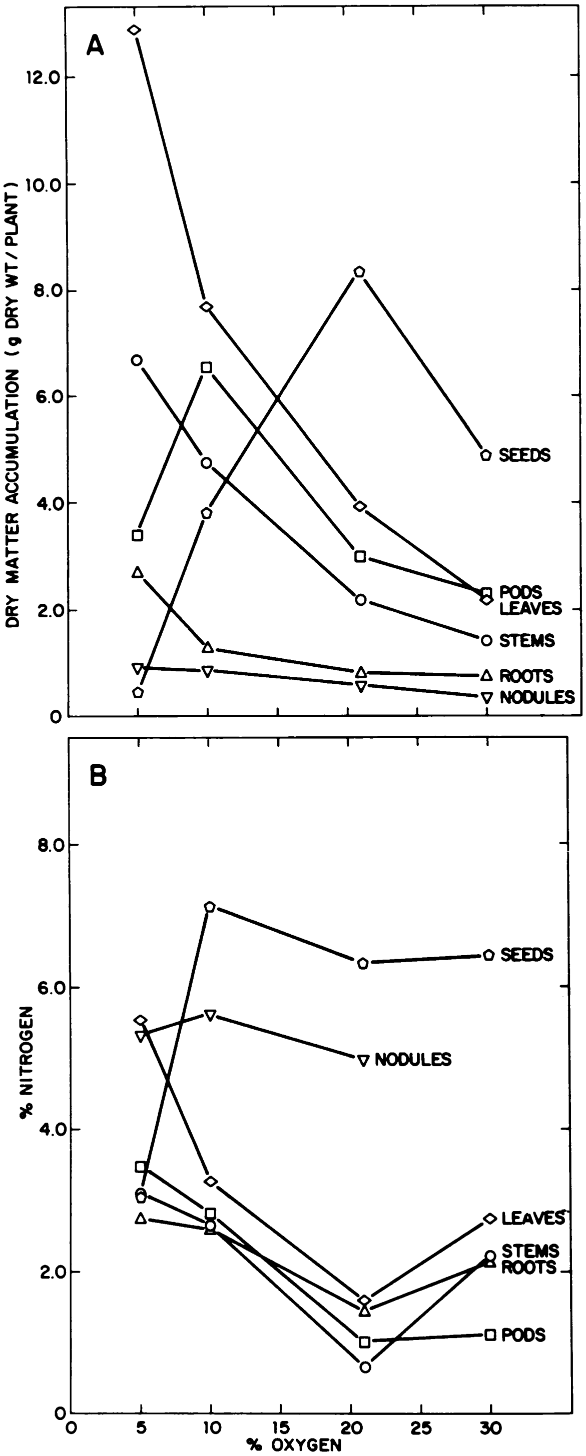

FIG. 3. A: Dry matter accumulation of vegetative and reproductive portions of soybean plants grown in chambers from 18 to 83 days, the period from early seeding growth to maturity with the aerial portion (Fig. 1) exposed to various pO_2 levels. The gram dry weights of plants grown at 21% O_2 and elevated CO_2 of 1200 μl CO_2/l are: seeds, 21.7; pods, 10.8; leaves, 6.8; stems, 6.1; roots, 1.9; nodules, 1.3. B: Nitrogen content by Kjeldahl analysis of vegetative and reproductive portions of soybean plants grown at various pO_2 levels. The per cent nitrogen content of plants grown at 21% O_2 and elevated CO_2 of 1200 μl CO_2/l are: seeds, 6.23; pods, 0.86; leaves, 1.36; stems, 0.68; roots, 1.93; nodules, 5.17.

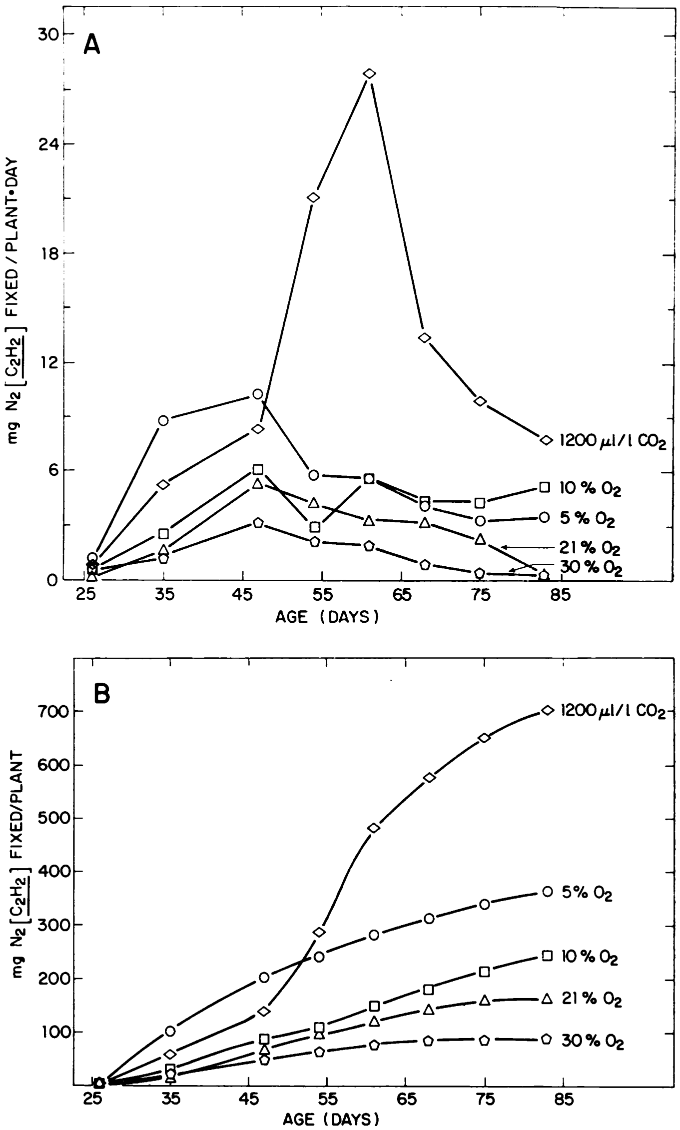

FIG. 4. $N_2[C_2H_2]$ fixation profiles measured every 7 to 10 days from early vegetative growth to maturity. Data expressed as mg $N_2[C_2H_2]$ fixed/plant·day (A) and integrated to obtain total mg $N_2[C_2H_2]$ fixed/plant (B). Soybean plants were grown in chambers with the aerial portion exposed to air or a modified gas mixture containing 5, 10 or 30% O_2 balanced with 300 μl CO_2/l and N_2 or 21% O_2 enriched to 1200 μl CO_2/l.

Total N_2 fixed was increased by CO_2/O_2 ratios greater than those in air and was decreased by ratios smaller than those in air (Fig. 4). The increase relative to air was 125% and 50% by 5% and 10% O_2, respectively, and 300 μl CO_2/l, and 339% by CO_2 enrichment of 1200 μl CO_2/l in 21% O_2. Total N_2 fixed was decreased 50% by 30% O_2 and 300 μl CO_2/l relative to air. The increase in total N_2 fixed and total growth by both subambient O_2 and CO_2 enrichment is attributed to a stimulation of net photosynthetic CO_2 fixation by reducing photorespiration, and the direct O_2 inhibition of photosynthesis. The decrease by elevated O_2 is attributed to decreased net CO_2 fixation by increasing these two processes. The observed competitive nature of the effect of altered O_2 and CO_2 concentrations on N_2 fixation is consistent with the primary effect at the site(s) of photorespiration and the direct O_2 inhibition of photosynthesis. These results, from the present study of the effects of O_2 concentration around the aerial part of the plant on N_2-fixing activity, support the hypothesis that the amount of photosynthate available to the nodule may be the primary factor limiting N_2 fixation for field-grown soybeans (3, 6).

A large proportion of the photosynthetic products are rapidly translocated to the nodule (11) where they are metabolized to provide ATP (1), electrons for reduction of N_2 to NH_3 as well as carbon skeletons used for incorporation of NH_3 and transport back to the aerial part of the plant. In contrast to reproductive structures the transfer of photosynthate from the leaves to the nodule and of reduced nitrogen from the nodules to aerial portions of the plant during vegetative growth is not O_2 limited. This suggests that the O_2 reactions in the aerial part of the plant involved in translocation and partitioning of photosynthate from leaves to nodules and vice versa is saturated at less than 5% O_2.

The effect of decreased pO_2 or elevated pCO_2 was quantitatively similar on N_2 fixation during the period of vegetative growth but different during the reproductive period (Fig. 4A). Up to 50 days of age the rate of N_2 fixation by plants exposed to 5% O_2 actually exceeded those exposed to 1200 μl CO_2/l. After 50 days the CO_2-enriched plants continued to increase their rate of N_2 fixation while the O_2-depleted plants actually decreased their rate. This lower rate of N_2 fixation under decreased O_2 *versus* CO_2 enrichment during the reproductive cycle is due presumably to decreased sink activity of the arrested reproductive growth toward the nodule source and suggests that reproductive source activity may be a secondary factor controlling N_2-fixing activity. Source photosynthate available to the nodule is certainly not limiting in the case of decreased O_2 *versus* increased CO_2 since the competition of the reproductive structures for photosynthate is greatly reduced under subambient O_2. The 5-fold increase in nitrogen content of stems and the 3-fold increase in leaf nitrogen accompanied by a decrease in nitrogen content of seeds under 5% O_2 suggests that the products of N_2 fixation accumulate in the vegetative parts of the plant because of the failure of transfer to the seed and thereby decrease the N_2-fixing activity of the nodule.

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