The Effect of Source–Sink Manipulations on Nitrogen Fixation in Peas

By

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Abstract

The effect on nitrogen fixation of excising leaves or pods in pea (*Pisum sativum* L. cv. Alaska) was determined over a 60-day period. Flower buds or their subtending leaves were removed, and C_2H_2 reduction, H_2 evolution and N accumulation were measured at weekly intervals. Highest percentage nitrogen content in all treatments coincided with time of maximal C_2H_2-reduction rates. Nitrogen fixation, calculated from C_2H_2-reduction and H_2-evolution data, was significantly lower in the partially defoliated and generally higher in the depodded plants than in the controls. Total N accumulation was greatest in the depodded plants and least in the defoliated ones. Percentage nitrogen content and N_2-fixation rates in the depodded plants were maximized approximately 10 days later than in the defoliated or control plants. The absolute rates of C_2H_2 reduction and H_2 evolution were significantly altered by plant organ removal, but the relative rates were proportional. As a result the ratios of H_2/C_2H_4 production and the related relative efficiency of N_2 fixation in the treatments were not significantly different from the controls.

Introduction

The effects of partial defoliation or complete pod removal on vegetative growth and symbiotic N_2 fixation in legumes have been studied previously (Wilson 1942, Roponen and Virtanen 1968, Lawn and Brun 1974, Huxley and Summerfield 1976). In general, defoliation reduces N_2 fixation, and removal of reproductive structures promotes vegetative growth and accompanying N_2 fixation.

Recently Schubert and Evans (1976) demonstrated that legumes evolve significant amounts of H_2 from root nodules under ambient conditions. In the presence of C_2H_2 no detectable H_2 is evolved, as the nitrogenase complex apparently diverts electrons normally used in H_2 production to reduce C_2H_2 (Burns and Hardy 1975). Attempts to measure instantaneous N_2 fixation with the C_2H_2-reduction assay in root nodules therefore often overestimate N_2 reduction (Schubert and Evans 1976). These workers proposed a measure of the apparent relative efficiency of N_2 fixation (RE) in root nodules based on these facts (Schubert and Evans 1976).

Bethlenfalvay and Phillips (1977a,b) observed that the amounts of C_2H_2 reduced and H_2 evolved vary as a function of plant age and growth-light intensity in peas. Their data suggested that physiological stresses related to reproductive development or low light intensity correlated with preferential decreases in H_2 evolution relative to C_2H_2 reduction. The purpose of the present study was (1) to determine whether photosynthate availability, varied through partial defoliation or complete depodding of the shoot, exerted a differential influence upon the two measured components of N_2 fixation (C_2H_2 reduction and H_2 evolution) and (2) to test the hypothesis that competition for photosynthetic products affects the magnitude and apparent relative efficiency of N_2 fixation.

Abbreviations: μE, microeinstein $\cdot$ $m^{-2} \cdot s^{-1}$; RE, apparent relative efficiency of N_2 fixation = 1 – (H_2 evolved/C_2H_2 reduced).

Materials and Methods

Pea plants were grown in the absence of combined nitrogen as described previously (Bethlenfalvay and Phillips 1977a), but at a photosynthetic photon flux density of 1000 μE. Flower buds or the leaves and stipules subtending the flower buds were removed daily. As a result, the depodded plants lacked reproductive structures but had a leaf at every node and the partially defoliated plants had leaves only at the lower (vegetative) nodes. An average of 10 vegetative nodes were present on all plants; all nodes above the 10th node were initially reproductive. Excision of plant organs started 25 days after planting at the time of macroscopic flower initiation. Four replicates of treated and control plants were

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assayed for N_2 fixation by measuring H_2 evolution and C_2H_2-dependent C_2H_4 production (Bethlenfalvay and Phillips 1977a) at weekly intervals starting 18 days after planting. Total nitrogen was determined by Kjeldahl analysis (Burris 1972).

Results and Discussion

Depodding and partial defoliation significantly altered the rates and amounts of N_2 fixed and the absolute rates of C_2H_2 reduction and H_2 evolution at different stages of plant growth (Figures 1 and 2). The relative rates of C_2H_4 and H_2 production, or the H_2/C_2H_4 ratio, however, were essentially the same as in intact controls.

In pea plants, the lower leaves are the major source of photosynthate for the root system (Pate 1966). In this experiment, defoliation at the reproductive nodes forced the remaining leaves at the lower, vegetative, nodes to supply photosynthate to the developing pods. As a result, the partially defoliated plants had markedly reduced nodule, shoot, and total dry weights (Table 1). Hydrogen evolution declined immediately following the start of defoliation and ceased approximately 50 days after planting (Figure 1). However, C_2H_2 reduction persisted throughout the assay period of 60 days and peaked one week after the start of defoliation. Both H_2-evolution and C_2H_2-reduction rates were significantly lower in partially defoliated than in control

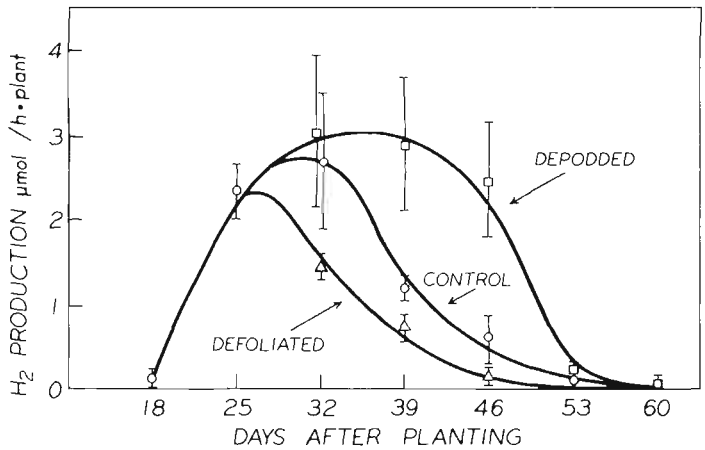

Figure 1. *Hydrogen evolution by root nodules of pea plants.* Data represent the mean ± SE of 4 replicates. Plants were depodded or defoliated at the flower-bearing nodes as these structures appeared, beginning on day 25.

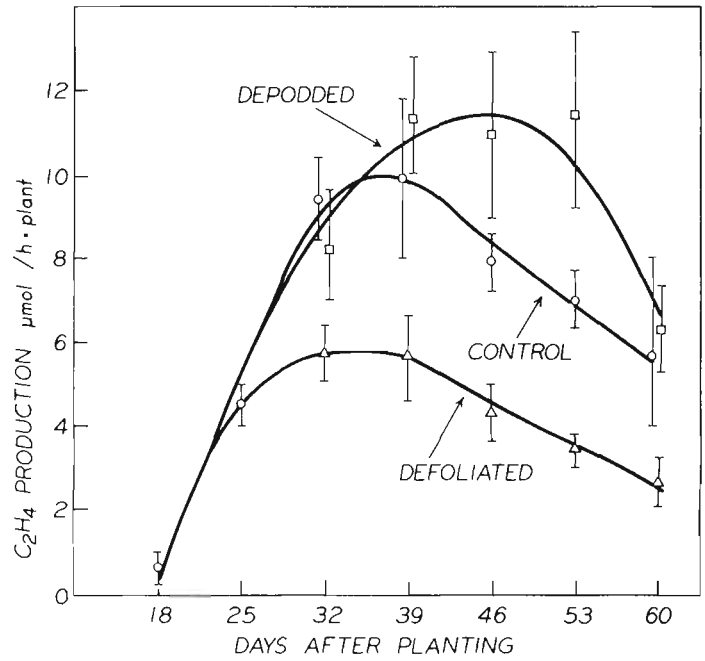

Figure 2. *Acetylene-dependent C_2H_4 production by root nodules of pea plants.* Data represent the mean ± SE of 4 replicates. Plants were depodded or defoliated at the flower-bearing nodes as these structures appeared.

Table 1. *Dry weights of pea root nodules, shoots and whole plants.* Means were calculated from 4 replicates. Means significantly different from the control in a t-test are indicated with * ($0.01 \leq p \leq 0.05$) or ** ($0.001 \leq p \leq 0.01$). Treatments were imposed on Day 25.

Treatment	Days after planting						
	18	25	32	39	46	53	60
			Whole plant dry weight, g				
Control	0.35	0.48	1.30	2.29	6.10	12.20	18.08
Depodded			1.20	2.49	7.17**	14.49	20.08
Defoliated			0.94**	2.08	4.17**	5.30**	9.92**
			Shoot dry weight, g				
Control	0.21	0.31	0.90	1.88	4.13	7.39	9.61
Depodded			0.82	1.95	5.30**	9.78**	14.03**
Defoliated			0.60*	1.31*	2.49**	2.57**	3.91**
			Root nodule dry weight, mg				
Control	4.9	25	78	136	170	242	298
Depodded			70	123	234**	287*	430*
Defoliated			41**	107*	133	142**	176*

Table 2. *Nitrogen fixation data in pea plants.* Apparent relative efficiency of N_2 fixation was calculated by the formula: RE = 1 − (H_2 evolved/C_2H_2 reduced). Nitrogen fixation activity was calculated as (C_2H_2 reduced − H_2 evolved)/3. Total nitrogen values reflect organic nitrogen and ammonia. Treatments were imposed after assaying on day 25. Means and standard errors were calculated from 4 replicates. Means significantly different from the control in a t-test are indicated with * ($0.01 \leq p \leq 0.05$), or ** ($0.001 \leq p \leq 0.01$). Relative efficiencies in treated plants were not significantly different from those of the controls.

	Days after planting						
Treatment	18	25	32	39	46	53	60
			N_2-fixation activity, μmol/h·plant				
Control	0.18	0.78	2.23	2.85	2.45	2.31	1.44
Depodded			1.74*	2.83	3.62*	3.73*	2.01
Defoliated			1.42**	1.69**	1.39**	1.14**	0.88
			Relative efficiency of N_2 fixation				
Control	0.92 ± 0.07	0.52 ± 0.04	0.73 ± 0.05	0.85 ± 0.01	0.93 ± 0.04	0.99 ± 0.01	0.99 ± 0.01
Depodded			0.65 ± 0.05	0.75 ± 0.08	0.84 ± 0.02	0.98 ± 0.01	0.98 ± 0.01
Defoliated			0.75 ± 0.03	0.87 ± 0.03	0.97 ± 0.02	1.00 ± 0.00	1.00 ± 0.00
			Total nitrogen, mg				
Control	8	14	43	99	205	378	475
Depodded			38	82	234*	432*	548*
Defoliated			32*	75*	133**	135**	266**

plants (Figures 1 and 2). This variation, however, did not significantly affect the RE values (Table 2), as the changes in H_2-evolution and C_2H_2-reduction rates were proportional. Total N content, on the other hand, was significantly less in partially defoliated than in control plants at each sampling date (Table 2). This decline in total N_2 reduction presumably can be attributed to the decrease in the amount of photosynthate available following defoliation. The increase in RE observed in controls was correlated in an earlier report with pod development and the senescence of the lower leaves (Bethlenfalvay and Phillips 1977a). The high RE observed 18 days after planting (Table 2) does not contradict the previous study. Rather, it represents a transitory stage in early pea root nodule development with a very low rate of H_2 evolution relative to C_2H_2 reduction. Whether this fact results from changes in the nitrogenase complex or in an uptake hydrogenase (Dixon 1967) cannot be determined from these data.

Leaves subtending fruits have been shown to supply most of their photosynthate to the developing pods and export negligible amounts of assimilates to the rest of the plant (Harvey 1971). Removal of reproductive structures therefore was expected to increase the flow of fixed carbon to other plant organs including root nodules. This was evidenced by significant increases in the dry weights of these organs (Table 1). In this study, additional energy available for N_2 fixation due to pod removal was reflected in higher rates of N_2 fixation and N assimilation in depodded plants than in controls (Table 2). The magnitudes of C_2H_2 reduction (Figure 2) and H_2 evolution (Figure 1) were significantly higher in depodded plants than in controls following the onset of rapid pod filling (Figure 4). These data support the hypothesis than N_2 fixation is stimulated by the removal of sinks competing for carbohydrate. As these increases in H_2 evolution and C_2H_2 reduction in the depodded plants were approximately proportional, their ratios, and the related RE (Table 2), did not differ significantly from those of the controls.

The increase with time in RE of partially defoliated plants and controls as well as depodded plants during plant ontogeny (Table 2) shows that the shift in RE, although correlated in time with pod development and the senescence of lower leaves on intact plants (Bethlenfalvay and Phillips 1977a), is not caused by these latter phenomena. An alternative cause for increase in RE as the host plant matures, may be changes in hormonal factors within the host plant, perhaps triggered by flowering, which affect hydrogenase (Dixon 1967) or nitrogenase functions within the bacteroids. Another factor, which cannot be eliminated in the present study, is the change in growth pattern due to depodding. Continued vegetative growth in depodded plants made pot volume severely limiting compared to defoliated plants and controls. Any resulting stress on water and nutrient balance in the depodded plants may have affected the observed RE.

Organ removal altered the time of maximum nitrogenase activity. A maximum rate of N_2 reduction was calculated for control and partially defoliated plants on day 39, while depodded plants had a maximum rate between days 46 and 53 after planting (Table 2). The time of highest percent N content in all treatments (Figure 3) coincided with the time of maximal C_2H_2-reduction rates (Figure 2). Controls and partially defoliated plants had maximum percentage N contents at the start of the logarithmic phase of pod filling 39 days after planting (Figure 3 and 4). The presence of developing fruits therefore may be regarded as an important

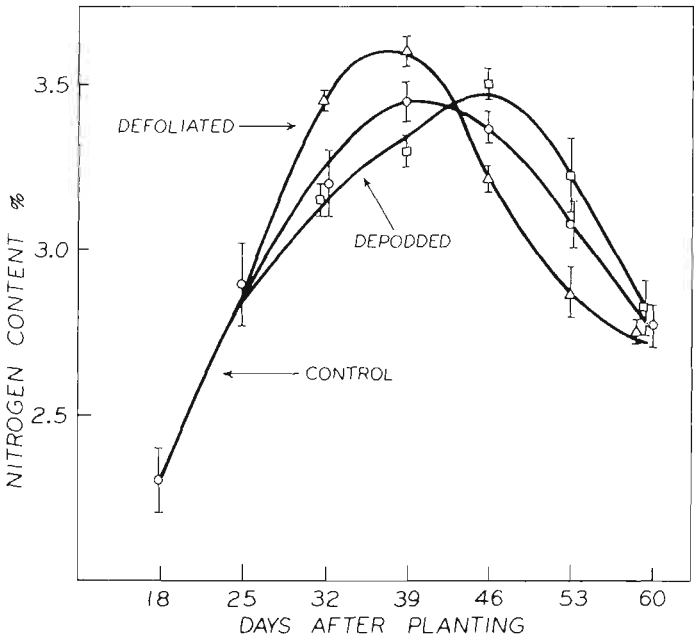

Figure 3. *Percentage nitrogen content of pea plants during ontogeny.* Data were derived from the same plants used to produce Figures 1 and 2.

factor in determining the N/C balance and the time at which this ratio is at a maximum. Since 46-day-old controls and defoliated plants were at the stage of rapid pod filling (Figure 4), the shift in maximal N_2-fixing activity in the depodded plants can be attributed to the removal of reproductive sinks which normally compete with root nodules for reduced carbon compounds.

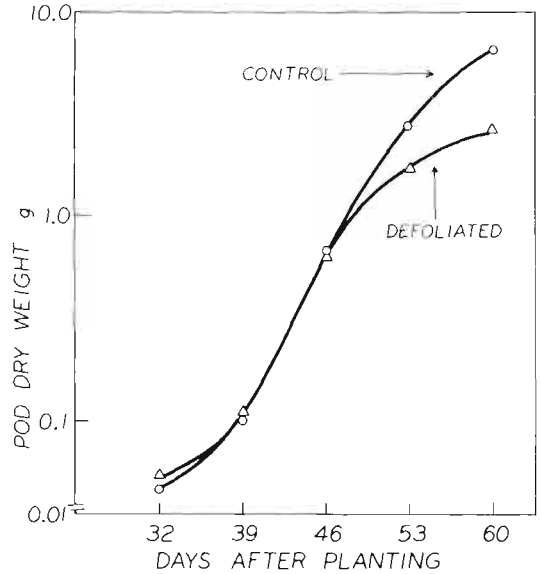

Figure 4. *Pod dry weight of control plants or plants defoliated at the fruit-bearing nodes.* Data were derived from the same plants used to produce Figures 1 and 2. Pod dry weights of treated plants and controls were significantly different ($p \leq 0.01$) 60 days after planting.

It is apparent from this study that pod removal and partial defoliation varied the magnitude of instantaneous N_2 fixation, total N assimilation, and the time at which maximum N content, C_2H_2 reduction and H_2 evolution occurred. Absolute rates of C_2H_2 reduction and H_2 evolution by partially defoliated plants differed significantly from those of controls immediately following defoliation, and depodded plants differed from controls after fruit set in the latter. Relative rates, however, varied proportionally. As a result the H_2/C_2H_4 production ratio and RE did not differ significantly between control and treatments. Competition for photosynthetic products was thus shown to affect only the magnitude and timing, but not the apparent relative efficiency of N_2 fixation.

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