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Nitrogen Fixation, Nodule Development, and Vegetative Regrowth of Alfalfa (*Medicago sativa* L.) following Harvest[1]

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ABSTRACT

Nitrogenase-dependent acetylene reduction, nodule function, and nodule regrowth were studied during vegetative regrowth of harvested (detopped) alfalfa (*Medicago sativa* L.) seedlings grown in the glasshouse. Compared with controls, harvesting caused an 88% decline in acetylene reduction capacity of detached root systems within 24 hours. Acetylene reduction in harvested plants remained low for 13 days, then increased to a level comparable to the controls by day 18.

Protease activity increased in nodules from harvested plants, reached a maximum at day 7 after harvest, and then declined to a level almost equal to the control by day 22 after harvest. Soluble protein and leghemoglobin decreased in nodules from harvested plants in an inverse relationship to protease activity.

Nitrate reductase activity of nodules from harvested plants increased significantly within 24 hours and was inversely associated with acetylene reduction. The difference in nitrate reductase between nodules from harvested plants and control plants became less evident as shoot regrowth occurred and as acetylene reduction increased in the harvested plants.

No massive loss of nodules occurred after harvest as evidenced by little net change in nodule fresh weight. There was, however, a rapid localized senescence which occurred in nodules of harvested plants. Histology of nodules from harvested plants showed that they degenerated at the proximal end after harvest. Starch in the nodule was depleted by 10 days after harvest. The meristem and vascular bundles of nodules from harvested plants remained intact. The senescent nodules began to regrow and fix nitrogen after shoot growth resumed.

Harvesting the shoots of legumes, such as is periodically done in alfalfa and other forages, removes a primary source of energy for maintaining N_2 fixation (15), nodule structure and function (3, 25), and for the initiation of new nodules. The ability of nodules to remain functional after harvesting of the shoot may depend upon plant species, microsymbiont, nodule morphology, rate of shoot regrowth, nutrient availability, and competition between plant parts for energy. The interrelationships among some of these factors have been reported for several annual grain legumes (7, 8). Only scant information is available for perennial forage legumes.

It has been suggested that perennial legumes often lose their nodules after shoot harvest or grazing and that new ones form during shoot regrowth. Wilson (25) reported that white clover lost about a third of its nodules after harvest. Butler *et al.* (3) found that defoliation and shading of red clover and birdsfoot trefoil caused a severe reduction in nodule number, while nodules of white clover apparently were not affected. Whiteman (24) reported that defoliation and grazing of *Desmodium intortum* DC. and *Phaseolus atropurpureus* (Jacq.) DC. decreased nodule weight and nodule number. Moustafa *et al.* (15) reported that defoliation of white clover caused a sharp decrease in $N_2(C_2H_2)$ reduction activity within 24 hr as compared to nondefoliated controls.

The mechanisms controlling nodule degeneration, nodule loss, and nodule replenishment after harvest are not understood for any forage legume. Since alfalfa is frequently subjected to shoot removal by animal or by mechanical harvesting and since the rates of symbiotic nitrogen fixation for alfalfa are greater than for most other legume species (23) we thought that it was important to understand the effects of harvest on nitrogen fixation. The objectives of this study were to establish the patterns of nitrogen fixation, nodule activity, and nodule development associated with forage harvest and subsequent recovery during a cycle of vegetative regrowth.

MATERIALS AND METHODS

Plant Material. Alfalfa (*Medicago sativa* L. var. Agate) seedlings were grown in a glasshouse in plastic sleeves (5 × 5 × 20 cm) embedded in a sand bench with supplemental fluorescent light at a quantum flux density of 350 $\mu E\ m^{-2}\ sec^{-1}$ during a 16/8-hr light/dark cycle at 24/19 C. At the time of seeding, the sand was inoculated with a commercial preparation of *Rhizobium meliloti* (Nitragin Co., Milwaukee, Wis.)[2] and macro- and micronutrients except for N were incorporated into the sand. Additional P and K were added after the second harvest. Plants were maintained in the sand bench for the duration of the experiment with no supplemental N added. The available N in the sand was 1 $\mu g/g$ or less during the experiment. The plants were grown from seed until flowering, harvested, and allowed to regrow for 28 days until approximately 1% flowering before the experiment commenced. The seedings were sampled prior to harvesting of shoots (day zero). The plants were either harvested, *i.e.*, subjected to 70 to 80% shoot removal, or left unharvested as controls, and both treatments were sampled at 8:30 AM 1, 4, 7, 10, 13, 18, 22, and 26 days later. Roots from a portion of each replicate were shaken gently to remove sand and then washed in buckets containing cold water. Nodules were removed and placed in 20-ml beakers on ice. On each sampling day there were three replicates. At least two subsamples from each replicate were assayed for $N_2(C_2H_2)$ reduction[3]

[1] Contribution No. 10422 from the Minnesota Agricultural Experiment Station.

[2] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by either the U.S. Department of Agriculture or University of Minnesota and does not imply its approval to the exclusion of other products that may also be suitable.

[3] Abbreviations: $N_2(C_2H_2)$ reduction; nitrogenase-dependent acetylene reduction.

activity, protease, nitrate reductase, leghemoglobin, and nodule soluble protein. Weights of plant parts were recorded after drying for 24 hr at 80 C.

$N_2(C_2H_2)$ Reduction Activity. $N_2(C_2H_2)$-dependent C_2H_4 production by intact roots (9) was used as a measure of N_2 fixation capacity. The plants remaining in each replicate were removed from the glasshouse, the tops were excised, and the sand gently shaken from the roots. Roots were then placed in liter jars sealed with a rubber-lined metal lid equipped with a serum stopper and transported to the laboratory. Sixty ml of air were withdrawn and replaced with an equal volume of C_2H_2. Root systems were incubated at 20 C and after 30 and 60 min 0.5 ml of the gas phase was withdrawn from the jar with a gas syringe. C_2H_2-C_2H_4 analyses were made with a Varian 3700 gas chromatograph equipped with an electronic integrator and with a column (0.4 × 180 cm) filled with Porpak N maintained at 80 C. The carrier gas was N_2 at a flow rate of 30 cc/min. Activity was expressed on a per plant basis or specific activity was expressed on the basis of nodule dry weight.

Enzyme Assays. Cell-free extracts were prepared by macerating 1-g aliquots of freshly harvested nodules in a cold mortar and pestle with Polyclar AT (0.1 g/g tissue) and 25 mM phosphate buffer (4 ml/g tissue) (pH 6.5) containing 10 mM mercaptoethanol. The resultant slurry was transferred to 15-ml tubes, centrifuged at 10,000*g* for 15 min, and the pellet discarded. The supernatant was then used to assay nodule protease, leghemoglobin, and soluble protein.

Leghemoglobin was determined in 1-ml aliquots as pyridine hemochromogen (12) using hemoglobin as a standard. Protein was determined in aliquots of the supernatant after precipitation with trichloroacetic acid by the method of Lowry (14) using BSA as a standard.

Protease was measured with azocasein as modified by Peterson and Huffaker (18). The reaction mixture contained 0.4 ml of 10 mg/ml azocasein, 0.4 ml of 0.05 M succinate buffer (pH 5.5) containing 10 mM mercaptoethanol, and 0.2 ml of nodule cell-free extract. After 1 hr at 35 C the reaction was stopped by adding 1 ml of 1 N perchloric acid. The precipitated protein was removed by centrifugation at 8,000*g* for 15 min and the *A* of the supernatant was read at 400 nm. Activity was expressed as ΔA 400 nm/mg protein/hr.

Nitrate reductase was measured with intact nodules according to a procedure similar to that described by Jaworski (11). Nodules (150 mg fresh weight) were washed with cold tap water and then suspended in test tubes (10 ml) containing 1.5 ml of a medium consisting of 0.1 M phosphate buffer (pH 7.0), 0.1 M KNO_3, and 0.07% tergitol. Nodules were then vacuum infiltrated three times (2 min/time period). The tubes were then placed in the dark at 30 C. Nitrite released into the medium was determined at zero time and at various intervals thereafter by treating 0.1-ml aliquots with 0.45 ml each of 1% sulfanilamide in 3 N HCl and 0.2% N-1 naphthyl-ethylenediamine-HCl. After 20 min the *A* at 540 nm was recorded and activity was expressed as nmol nitrite/hr·g fresh weight.

Histology. Nodules were fixed in 2.5% glutaraldehyde in 0.01 M phosphate buffer (pH 7.0) at 4 C for 2 to 4 days, postfixed with osmium tetroxide in the same buffer for 1 hr, dehydrated in a graded acetone series, and embedded in Spurr's medium (22). For light microscopy, 2-μm sections were cut with LKB MT-2 Ultratome from five nodules from each of five harvested and control plants on each collection date. Sections were placed on glass slides, stained with 1% aqueous solution of toluidine blue, counterstained with 0.5% aqueous solution of saffranin O, and examined with a Nikon binocular microscope.

RESULTS

Shoot Growth. Shoots of the plants regrew slowly for 12 days after harvest (Fig. 1A). From day 13 to 22 after harvest, shoot weight increased in the harvested plants so that total shoot weight reached the initial value by day 22. There was no change in shoot growth of the harvested plants from day 22 to 26.

In comparison, shoot weight of the control plants increased through day 10 and then remained unchanged until the termination of the experiment (Fig. 1A). By day 10 the control plants were in full bloom.

Root Growth. Roots of the harvested plants grew little during the course of the experiment (Fig. 1B). In the control plants root

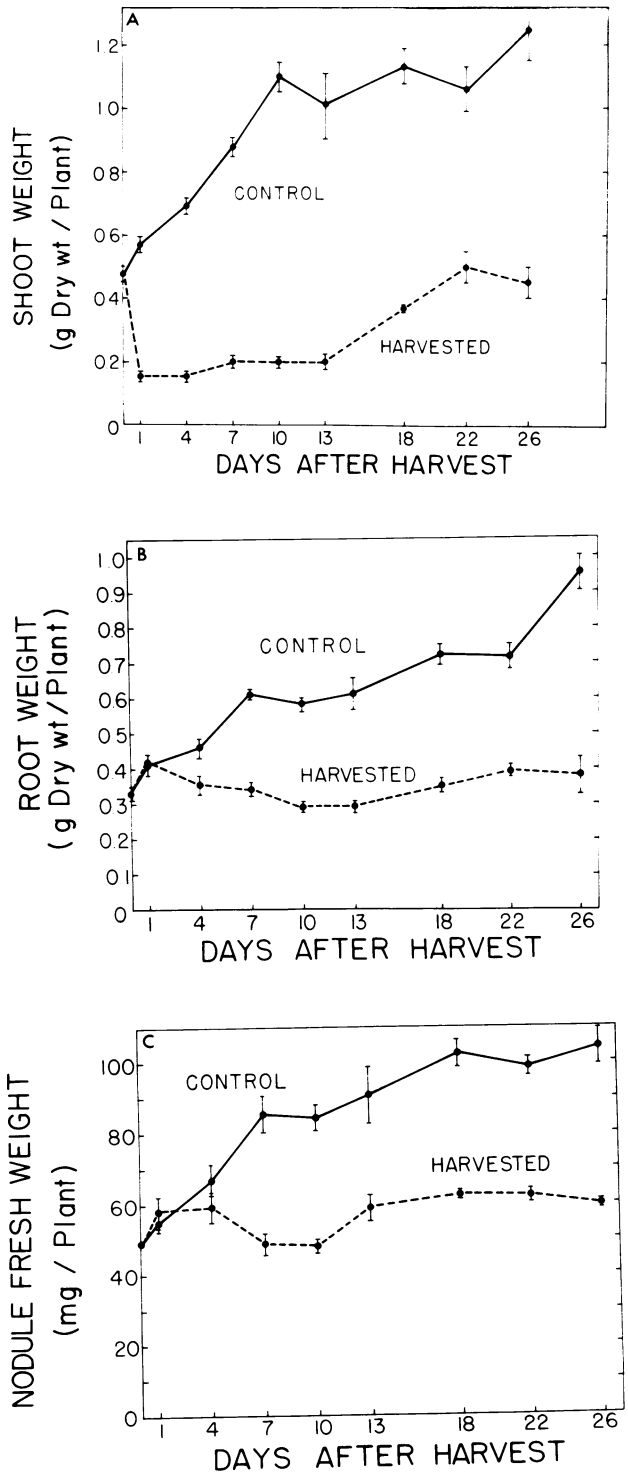

FIG. 1. Growth of (A) shoots, (B) roots, and (C) increase in nodule mass of harvested and control alfalfa plants. Each point is the mean of three replicates ± SE.

dry matter doubled throughout the course of the study (Fig. 1B).

Nodule Fresh Weight. Nodule fresh weight per plant showed a pattern similar to that observed for root growth (Fig. 1C). Nodule weight of harvested plants declined slightly between days 4 and 7, remained constant from day 7 to 10, and then increased slightly between days 10 and 18.

Nodule weight of control plants increased between days 1 and 13 and then remained unchanged through day 26. There was a doubling of nodule weight from the start until the termination of the experiment, which was similar to the increase in root weight observed in control plants.

$N_2(C_2H_2)$ Reduction Activity. Nitrogen fixation capacity estimated by $N_2(C_2H_2)$ reduction declined 88% within 24 hr after harvest as compared to controls (Fig. 2A). Between days 1 and 13, $N_2(C_2H_2)$ reduction activity remained constant at 20 to 30% of the control. From day 13 to 18 there was a slight recovery of $N_2(C_2H_2)$ reduction activity, and after 18 days the rate of recovery was fastest. At the termination of the experiment, the $N_2(C_2H_2)$ reduction activity on a per plant basis of the harvested plants exceeded that of the controls.

Nodule specific activity for $N_2(C_2H_2)$ reduction decreased sharply after harvesting and then recovered slowly (Fig. 2B). Between days 1 and 13, activity was lower in harvested plants than in the controls. By days 18 and 22, nodule specific activity in harvested plants was equal to that in the controls. In contrast to the pattern shown by harvested plants, nodule specific activity of the control plants decreased throughout the experiment as nodule mass was increasing (Fig. 1C).

Nodule Protease Activity. Protease activity in nodules from harvested plants increased rapidly from day 1 to a maximum at day 7, then declined rapidly until day 13 and stabilized at a value slightly greater than that of the control nodules (Fig. 3). In comparison, protease activity in nodules of control plants remained unchanged for the duration of the experiment.

Nodule Leghemoglobin and Soluble Protein. Nodule leghemoglobin concentration of harvested plants declined about 53% from day 0 to day 10 as compared to controls, then recovered rapidly from day 10 to 18 and stabilized at a concentration about 80% that of nodules from control plants (Fig. 4).

Soluble protein concentration (Fig. 5) in nodules from harvested plants inversely reflected protease activity (Fig. 3) and showed a pattern similar to that of leghemoglobin concentration (Fig. 4). Soluble protein in nodules from harvested plants declined rapidly between days 1 and 7, more slowly between days 7 and 10, and then increased rapidly from day 10 to 22. Soluble protein in nodules from control plants varied little during the experiment.

Nodule Nitrate Reductase. Nitrate reductase activity in nodules from harvested plants increased rapidly (40% within 24 hr), reaching a maximum between days 7 and 10 (Fig. 6). After day 10, activity declined rapidly until day 18. In contrast, nitrate reductase activity in nodules from control plants remained unchanged through day 18. Between days 18 and 26 nodule reductase activity from both harvested and control plants increased in parallel. There was no difference in activity between treatments by day 26.

Histology of Nodules. Changes in nodule anatomy were seen

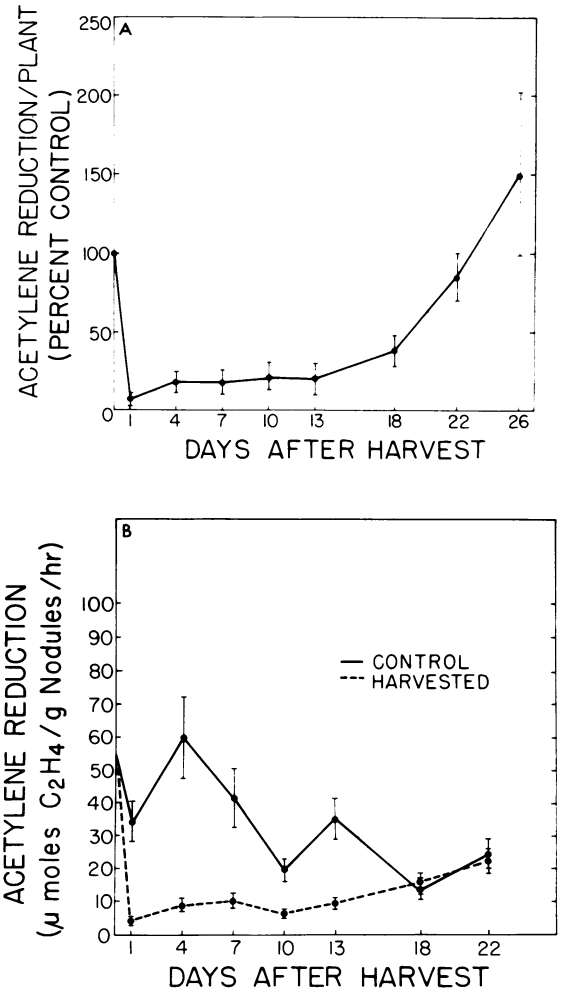

FIG. 2. Time course of total (A) and specific (B) $N_2(C_2H_2)$ reduction of harvested and control plants. Each point is the mean of three replicates ± SE.

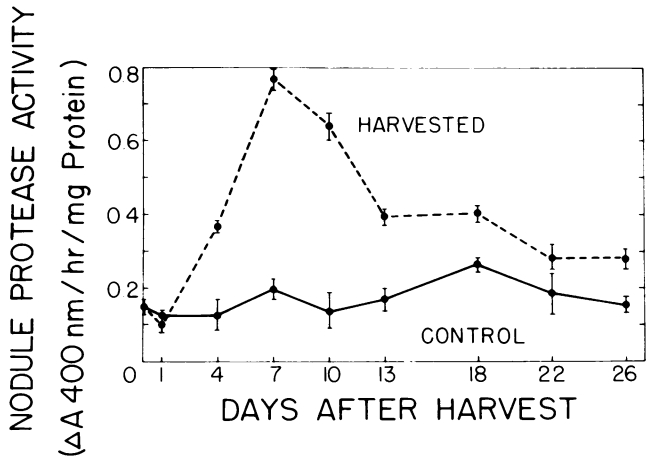

FIG. 3. Effect of harvest on alfalfa nodule protease activity. Each point is the mean of three replicates ± SE.

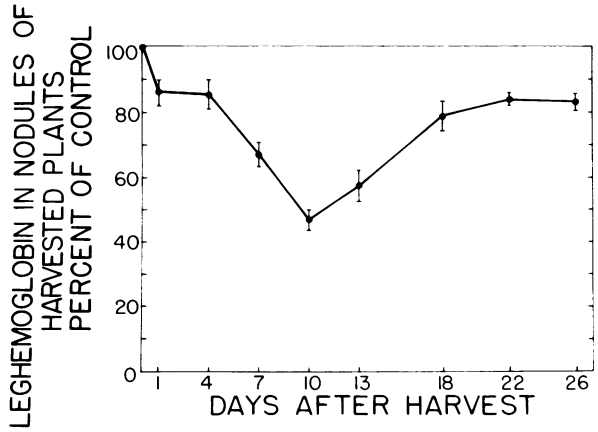

FIG. 4. Effect of harvest on leghemoglobin concentration in alfalfa nodules. Each point is the mean of three replicates ± SE.

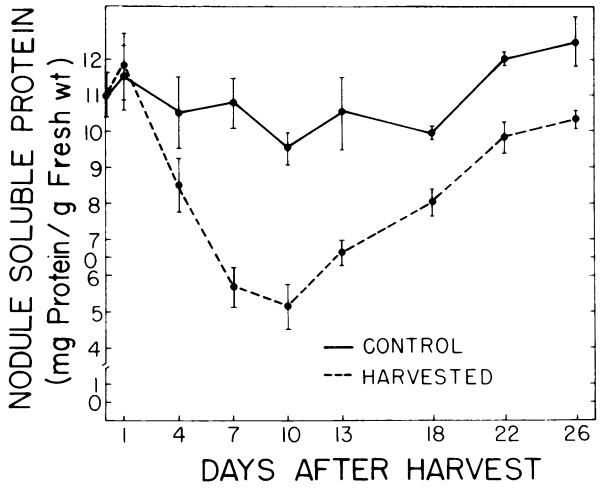

FIG. 5. Patterns of alteration of alfalfa nodule soluble protein as influenced by harvest. Each point is the mean of three replicates ± SE.

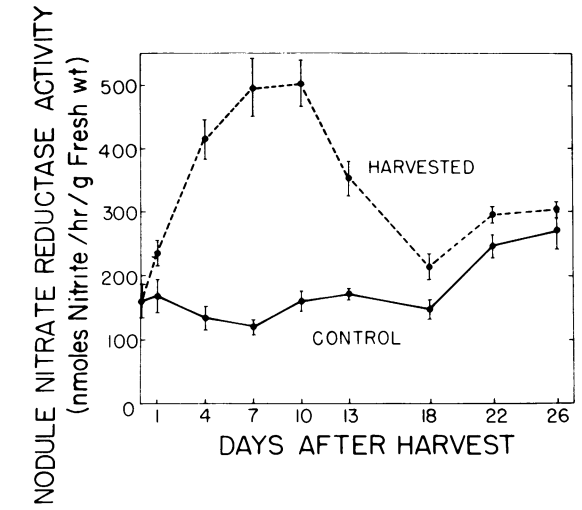

FIG. 6. Effect of harvest on alfalfa nodule nitrate reductase activity. Each point is the mean of three replicates ± SE.

through light microscopy by day 4 after harvesting (Fig. 7A). Compared to nodules from control plants, cells at the proximal end containing bacteroids had started to show disorganization. By 7 days after harvest, cells containing bacteroids were greatly disorganized and bacteroid numbers were reduced in nodules from harvested plants (Fig. 7B) compared to nodules from control plants (Fig. 8A). Ten days after harvesting, cells containing bacteroids were greatly reduced in nodules from harvested plants and only a small area immediately behind the apical meristem remained (Fig. 7C).

On day 4, numerous starch granules were present in cells containing bacteroids; by day 10 starch granules had become much less evident and those present were reduced in size (Fig. 9, C and D).

The apical meristem and vascular bundles remained intact and functional even with the massive disorganization that occurred in nodules of harvested plants (Fig. 9, A and B). Cells containing bacteroids started to increase by day 13 in nodules from harvested plants and at day 26 a large number of cells containing bacteroids was evident (Figs. 7D and 9E). Numerous starch granules were also observed in the new bacteroid region of nodules from harvested plants similar to nodules from control plants (Fig. 9, E and F), but nodule cells that became disorganized and lost bacteroids did not regenerate new bacteroids (Fig. 7D).

DISCUSSION

Harvesting (removal of 80% of the aerial portion of the plant) significantly altered $N_2(C_2H_2)$ reduction capacity, nodule enzymology, and nodule development in seedling alfalfa. New shoot growth in harvested plants commenced by day 13. Root dry matter accumulation in harvested plants was negligible over the course of the experiment. Similar observations for shoot and root growth after harvest have been presented by Smith and Silva (21).

In both control and harvested plants, changes in nodule fresh weight per plant corresponded closely to the changes observed in root dry weight per plant. There was no net change in nodule fresh weight per plant after harvest. This would indicate that a significant shedding of nodules did not occur after harvest and regrowth through one cycle. Even though nodule fresh weight per plant remained constant after harvest, the proportion of nodule cells containing bacteroids decreased up to day 10 and then increased between days 13 and 26 (Fig. 7). The formation of new bacteroid-containing cells occurred simultaneously with renewed shoot growth while root dry matter accumulation was unchanged during this interval. These observations suggest that energy provided by the aerial portion of the plant may be competitively utilized for renewed shoot growth and resumption of nodule growth. Hodgkinson (10) showed that export of ^{14}C-labeled photosynthate from leaves of new shoots of alfalfa to the roots occurred by 6 days after harvest. Competitive utilization of photosynthate by various plant parts has also been demonstrated in corn (16) and soybeans (13).

The 88% decline in $N_2(C_2H_2)$ reduction activity per plant 24 hr after shoot removal (Fig. 2A) was caused entirely by a drop in specific activity and is similar to that observed in white clover (15) and similar to the effect of stem girdling in soybeans (8).

Starch depletion, in contrast to the rapid decrease in $N_2(C_2H_2)$ reduction activity, did not occur until after day 4 (Fig. 9, C and D). Nonstructural carbohydrates have also been reported to become depleted in alfalfa roots after harvest (21) but not at a rate comparable to the observed decrease in $N_2(C_2H_2)$ reduction activity. These observations suggest that starch granules in nodules and root nonstructural carbohydrates are not readily available to sustain nodule activity in alfalfa. $N_2(C_2H_2)$ reduction activity remained low until shoot growth and nodule regrowth began (Figs. 1, 2, and 7). Although significant leaf area (20–30% of the initial area) remained on the lower portion of the harvested shoots before new shoot growth commenced, this tissue was either inadequate in supplying carbohydrate for $N_2(C_2H_2)$ reduction or the persistent decline in nodule activity was the result of factors other than photosynthate supply. In comparison, control plants showed a continuing decline in nodule specific activity from day 0 to day 22 (Fig. 2B). This decline occurred even though root growth, nodule fresh weight, and starch accumulation were increasing during this period. These results suggest that photosynthate may not be the only limiting factor to $N_2(C_2H_2)$ reduction in these plants.

Harvesting caused a rapid increase in nodule protease activity (Fig. 3). This increased activity was reflected by a decrease in nodule soluble protein concentration, leghemoglobin, and by a massive disorganization and loss of bacteroids in nodule cortical cells. By 13 days after harvest, $N_2(C_2H_2)$ reduction activity, nodule regrowth, nodule soluble protein concentration and leghemoglobin all began to increase with a concomitant decrease in nodule protease activity. These data indicate that in alfalfa, nodule senescence judged by histological disorganization and bacteroid loss after harvesting is localized and that renewed nodule growth and function appear to be mediated by shoot growth and decreased nodule protease activity. Defoliation and other types of stress have been shown to cause nodule senescence in a number of legumes (3, 7, 15, 24). It was not clear in those studies what mechanisms were associated with the observed nodule senescence or if the nodules recovered function and growth.

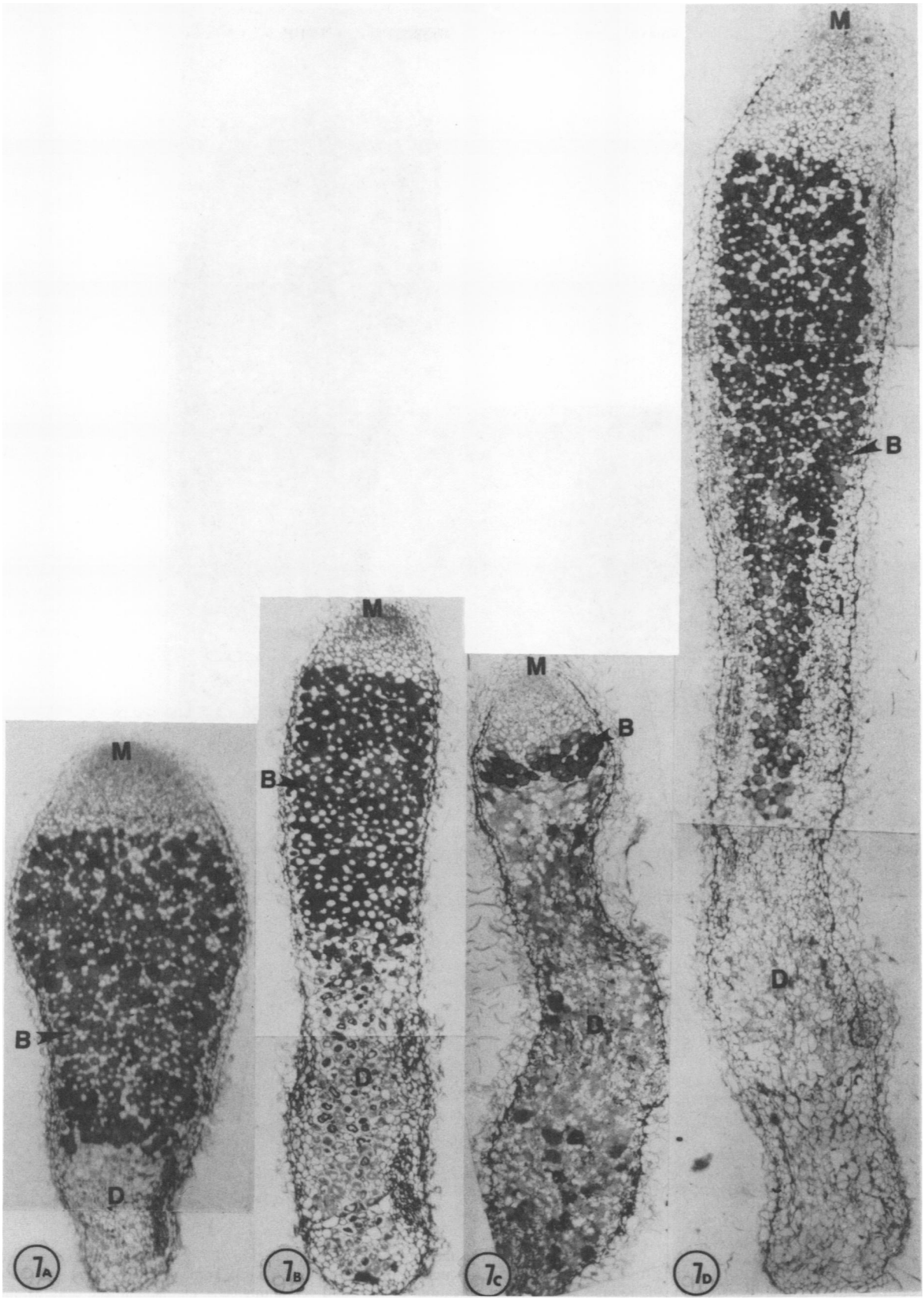

FIG. 7. Nodule deterioration and regrowth after harvest. (A): nodule 4 days after harvest. Note the loss of bacteroids from the disorganized cells at the base of the nodules. (B): nodule 7 days after harvest. The loss of bacteroids has progressed halfway up the nodule. (C): nodule 10 days after harvest. Only a few bacteroid-containing cells remain adjacent to the meristematic region. (D): nodule 26 days after harvest. The disorganized cells have not regenerated, however, the number of bacteroid-containing cells has increased with the renewed growth of the nodule. All nodules are × 50 magnification. B: bacteroid-containing cells; D: disorganized cells; M: meristem.

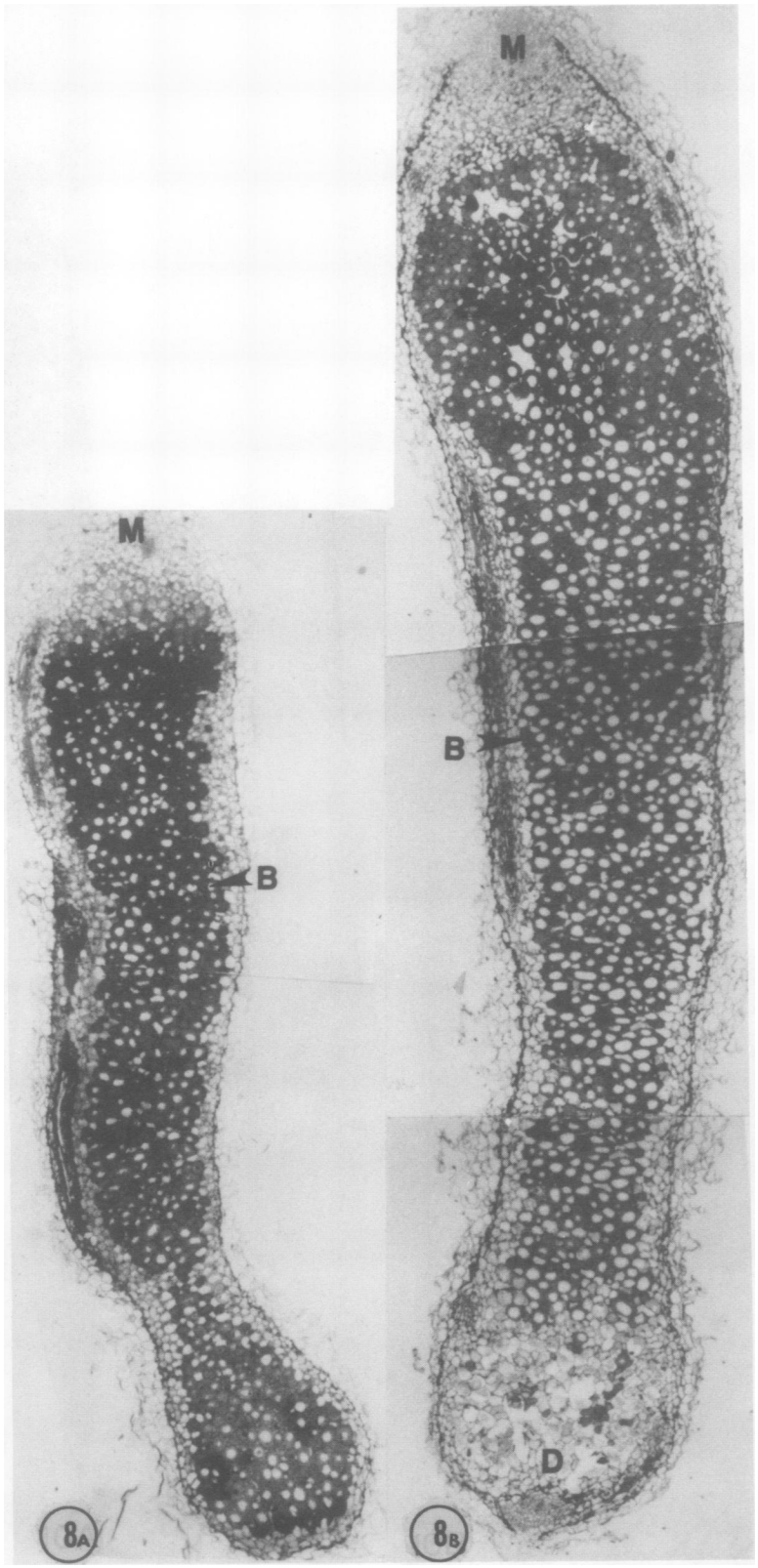

FIG. 8. Nodules collected from control plants (A): nodule from control alfalfa on the same day as Figure 9B (7 days). Bacteroid-containing cells are found throughout the nodule cortex. (B): nodule from control alfalfa on the same day as Figure 9D (26 days). Some cells at the base of the nodule have started to become senescent and disorganized. All nodules are × 50 magnification. B: bacteroid-containing cells; D: disorganized cells; M: meristem.

Although nodules reportedly become physically detached from perennial legume roots after harvest (3, 6, 7, 24, 25), our results showed no net loss in nodule mass of seedlings after shoot removal (Fig. 1C). Undoubtedly some loss of nodules may occur, but our observations suggest that there is a physiological and morphological adaptation of many existing nodules to the disorganization and bacteroid loss induced by harvesting.

The conspicuous features of this adaptation were evident after

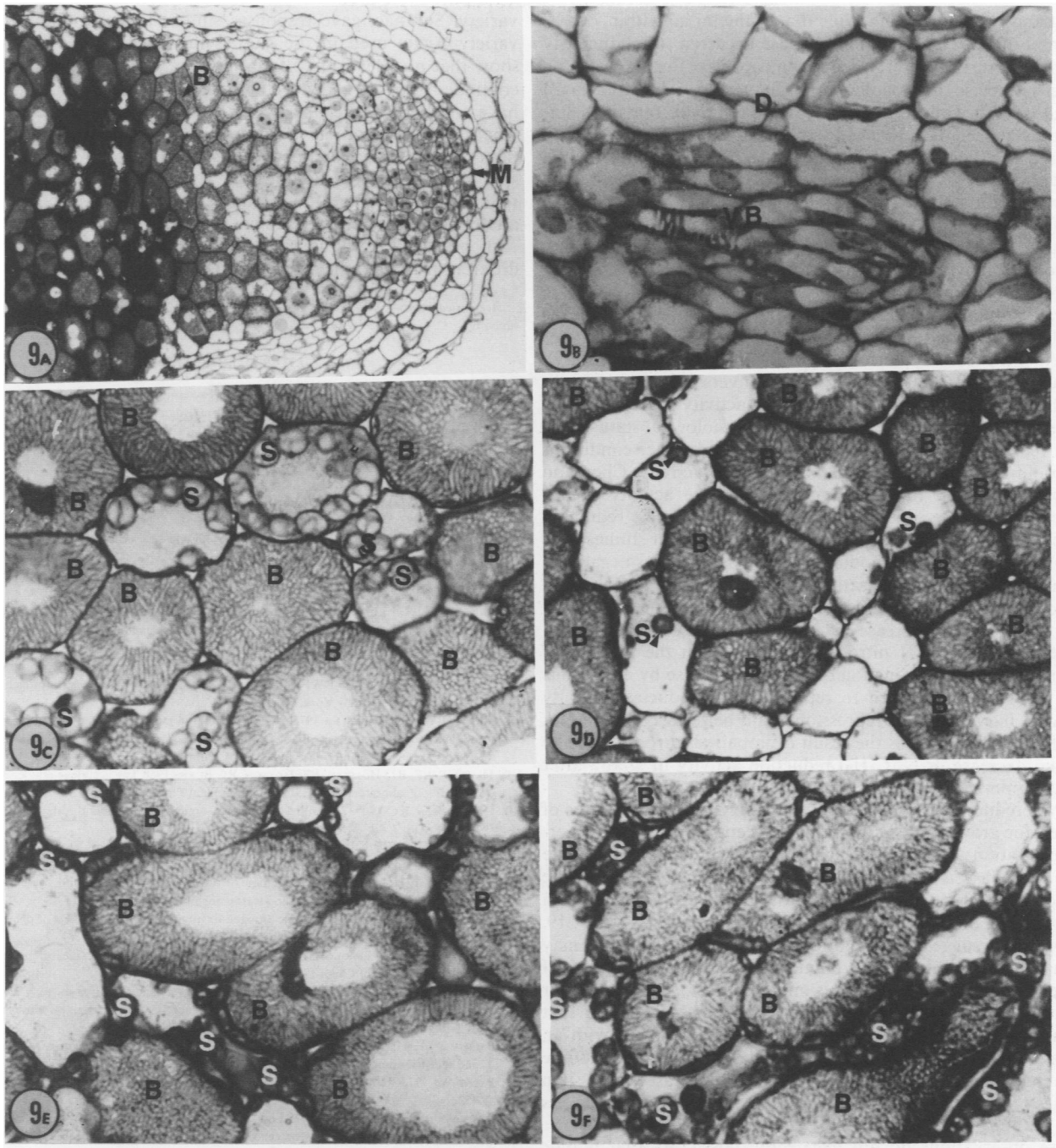

FIG. 9. (A): apical meristem from a nodule 13 days after harvest. (B): vascular bundle adjacent to disorganized and collapsed cortical cells from a nodule 13 days after harvest. Note both the apical meristem and the vascular bundle are still intact and apparently not senescent. (C): bacteroid-containing cells from nodules 4 days after harvest. Note the presence of many starch granules. (D): bacteroid-containing cells from nodules 10 days after harvest. Note reduction in number and size of starch granules. (E): bacteroid-containing cells from nodules 26 days after harvest. Note the presence of many starch granules. (F): bacteroid-containing cells from nodules of control plants sampled the same day as Figure 9E. Note a similar amount of starch present as in Figure 9E. All photos are × 600 magnification. B: bacteroid-containing cells; D: disorganized cells; M: meristem; S: starch granules; VB: vascular bundle.

histological examination of the nodules. Bacteroid-containing cells deteriorated following harvest (Fig. 7), while the apical meristem (Fig. 9A), the vascular bundles (Fig. 9B), and the infection threads (Johnson and Vance, unpublished) of the nodules remained intact. By about day 13 after harvest, existing nodules began to regenerate bacteroid-containing cells with increased starch, leghemoglobin, and soluble protein. Shoot regrowth began again by day 13 and increased $N_2(C_2H_2)$ reduction activity was evident by day 18. In other forage legumes defoliation or grazing has been shown to cause shedding of nodules with apparent reinfection and formation of new nodules (3, 24, 25). These authors made no observation on the capacity of nodules to recover from senescence. This adaptive ability of alfalfa nodules would appear to be an efficient mechanism by which nitrogen fixation capacity could be retained without reinfection and establishment of an entirely new nodule population. The observed ability of nodules to regrow and to continue to fix nitrogen after harvest may also be important in explaining why alfalfa has a rapid regrowth potential as compared to many other forage legumes.

Nitrate reductase activity of nodules rapidly increased after

harvest and reached a value triple that of the initial within 7 days. The onset of increased nitrate reductase occurred simultaneously with the decline in $N_2(C_2H_2)$ reduction capacity (Fig. 2, A and B). Nitrate reductase in nodules from harvested plants increased through day 10 while $N_2(C_2H_2)$ reduction activity remained unchanged. Between days 13 and 18 nodule nitrate reductase activity decreased to a level similar to that observed in the control while concomitantly $N_2(C_2H_2)$ reduction activity began to increase. The time course for nodule nitrate reductase activity was similar to that of protease activity, leghemoglobin, and soluble protein concentration, but the patterns of nodule nitrate reductase activity and protease activity were the converse of those for leghemoglobin and protein concentration.

Nitrate reductase has been observed in the nodules of soybeans (1, 5, 19) and peas (4). Nodule nitrate reductase in soybeans was found to be positively correlated with nitrogen fixation (5, 19). We found nodule nitrate reductase to be inversely associated with $N_2(C_2H_2)$ reduction. This discrepancy in activity associated with nitrogen fixation may be attributable to physiological status since nitrate reductase in soybean was not measured under conditions that would cause reduced $N_2(C_2H_2)$ reduction activity. Chen and Phillips (4) showed increased nitrate reductase activity in pea nodules which were senescent and had low $N_2(C_2H_2)$ reduction activities as the result of applying $NaNO_3$. In recent studies with *Centrosema pubescens* (2), nodule nitrate reductase was negatively correlated with nitrogenase activity. Because the level of NO_3^- in our rooting medium was less than 1 μg/g throughout the experiment, the observed increase in nitrate reductase may reflect a change in regulation of nitrogen metabolism in the nodules of seedlings rather than induction of nitrate reductase by exogenous NO_3^-. Ammonia is known to repress nitrate reductase activity (17). After harvesting this repression could be released because of less NH_4 being formed as the result of impaired nitrogenase. The observed decrease in $N_2(C_2H_2)$ reduction activity in alfalfa after harvest supports this interpretation.

Nitrate reductase can have an assimilatory (provide a source of nitrogen for growth) or dissimilatory (accepts electrons in anaerobic respiration) function (17). It is not clear if legume nodule nitrate reductase is assimilatory or dissimilatory (1, 5, 19). The root nodule bacteria that infect alfalfa and soybeans have been shown to have both types of nitrate reductase functions (5, 17, 19, 20). Our data suggest that alfalfa nodule nitrate reductase may play a role in supplying the plant with an alternate source of nitrogen while nitrogen fixation is impaired during regrowth after harvest. Randall (personal communication) has suggested that soybean nodule nitrate reductase can provide the plant with an alternative source of nitrogen. In his studies with soybean, nodule nitrate reductase increased as nitrogenase decreased and stem exudates of plants fed ^{15}N enriched with $NaNO_3$ contained excess ^{15}N in the fully reduced nitrogen fraction.

These observations do not negate the possibility that nodule nitrate reductase may be dissimilatory and function to accept electrons that could be liberated during the period of senescence observed after harvest. Cell degradation could lead to fermentative microsites in the nodule. Respiration of bacteroids and nodule tissue could convert areas of the nodule to anerobic conditions and these anaerobic portions are then ready to express nitrate reductase activity.

A third alternative would be that both assimilatory and dissimilatory nitrate reductase activities are occurring. This would provide a source of capturing electrons. Our results suggest a contribution of nodule nitrate reductase to the total nitrogen economy of the plant.

This study provides evidence for harvest-induced changes in nodule function and development in alfalfa. A second alfalfa variety, Saranac, gave responses similar to those obtained with variety Agate used in the studies reported here. These studies show that nodules from alfalfa have an adaptive mechanism for continued growth and development after harvest and may also have an alternate mechanism for supplying nitrogen to the plant when nitrogen fixation is impaired after harvest. Although the patterns of metabolism of roots and nodules following harvest are still unclear, the regeneration of nodules, the increased nitrate reductase activity and the reestablishment of nitrogen fixation in alfalfa following shoot removal may depend upon processes of carbon and nitrogen metabolism within the nodule that are quite different from those necessary for shoot growth.

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