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Nitrogen Fixation and Vegetative Regrowth of Alfalfa and Birdsfoot Trefoil after Successive Harvests or Floral Debudding[1]

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ABSTRACT

Nitrogenase-dependent acetylene reduction, leaf, herbage, and root growth, and total nonstructural carbohydrate accumulation of alfalfa (*Medicago sativa* L.) and birdsfoot trefoil (*Lotus corniculatus* L.) were compared to learn how nitrogen fixation capacity and vegetative growth respond to partial (75–85%) or total shoot and leaf removal, and floral debudding. Treatments were imposed on greenhouse-grown plants during two successive harvest cycles.

Both species displayed an initial decline in total nitrogenase activity within 2 days of harvest and a subsequent recovery of activity after 10 to 21 days. Rate of recovery varied with the amount of leaf area removed. Periodic flower removal did not significantly alter total nitrogenase activity of either species compared with the unharvested controls. In the first harvest cycle, partial leaf area removal did not affect nitrogenase activity of alfalfa, but activity of trefoil was reduced 56%. In the second harvest cycle, partial leaf area removal reduced total nitrogenase activity of alfalfa 46% and that of trefoil 69%. Complete leaf and shoot removal reduced total nitrogenase activity of alfalfa 78% after the first harvest and 86% after the second harvest.

Recovery of nitrogenase activity after harvest paralleled leaf area expansion in both species. After the initial decline following the first partial harvest, total nitrogenase activity and leaf area of alfalfa increased 170 and 500%, respectively. After the initial decline following the second partial harvest, nitrogenase activity and leaf area of alfalfa increased 280 and 800%, respectively. Partly harvested trefoil and completely harvested alfalfa showed similar response patterns. Release of bud dormancy and leaf area expansion after flowering of nonharvested alfalfa apparently caused an increase in nitrogenase activity, but patterns of acetylene reduction and leaf area were not otherwise closely correlated in controls of either species. Decline and accumulation of total nonstructural carbohydrates in both species varied with defoliation treatment. Patterns of nonstructural carbohydrates in root tissue were not closely related to the changes in total nitrogenase activity caused by shoot removal.

Nitrogen fixation in legumes requires a large expenditure of energy from photosynthates for nodule growth and function. TNA[3] estimated by the $N_2(C_2H_2)$ reduction assay (5) has been associated with photosynthate supply and partitioning in a number of species. Supplemental light (10), grafting of a second shoot (21), and CO_2 enrichment (6) enhanced TNA of soybeans (*Glycine max* L. Merr.). Higher PAR and LA accompanied increased TNA of several forage legumes (3, 4). Floral debudding and the associated promotion of vegetative growth stimulated nodule growth (16) and TNA (11) of pea (*Pisum sativum* L.). The TNA of soybeans declined after CO_2 deprivation (9), shading (10), defoliation (10), stem girdling (9), shoot excision (12), and prolonged darkness (12). Similarly, TNA decreased after exposure of subterranean clover to low light intensity (4), removal of pea shoots (13), and continuous darkening of pea and alder (23). The depression of TNA by such competing sinks as developing fruits (1, 7, 10) further indicates the importance of photosynthate to N_2-fixation.

The removal of photosynthetic tissue from forage legumes at harvest and the competition between vegetative regrowth and nodules for reserve carbohydrates and current photosynthate may adversely affect the capacity of nodules to sustain N_2-fixation. Previous investigators reported a decline in nodule numbers following herbage removal for several forage species (2, 24, 25). White clover (14, 17) and alfalfa (22) exhibited a pattern of initial decline and eventual recovery in TNA after harvesting.

The objectives of this research were to determine the response of N_2-fixation, leaf, herbage, and root growth, and TNC concentration to: (*a*) the partial loss and subsequent regrowth of photosynthetic tissue; and (*b*) floral debudding in two successive harvest cycles for alfalfa and birdsfoot trefoil (*Lotus corniculatus* L.). Complete defoliation was also imposed on alfalfa to determine its influence upon N_2-fixation. Alfalfa and trefoil were selected because of the difference in the level of carbohydrates stored in their roots (18). These experiments provided a test of the capacity of alfalfa with its extensive carbohydrate reserves and trefoil with its relatively small carbohydrate reserves to sustain N_2-fixation after the removal of a substantial portion of photosynthetic tissue.

MATERIALS AND METHODS

Alfalfa (*Medicago sativa* L., cv. 'Saranac') and birdsfoot trefoil (*Lotus corniculatus* L., cv. 'Leo') seedlings inoculated with *Rhizobium inoculum* (supplied by Nitragin Co., Milwaukee, WI)[4] were sown and cultured in a greenhouse sand bench. When the trefoil

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[3] Abbreviations: TNA, total nitrogenase activity; $N_2(C_2H_2)$ reduction, nitrogenase-dependent acetylene reduction; LA, leaf area; TNC, total nonstructural carbohydrates.

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and alfalfa were about 14 weeks old, the tops were removed and the roots were trimmed to 12 cm. Seedlings were then transplanted by pairs into 15 cm pots filled with sand. The center-to-center spacing of pots of alfalfa was 20 cm within and between rows. The center-to-center spacing of pots of trefoil was 28 cm within rows and 32 cm between rows. Although trefoil plants were more widely spaced, their decumbent growth habit resulted in mutual and self shading similar to that of alfalfa. Finely ground limestone was applied at the rate of 0.4 g/pot. Potassium and P were supplied as 0.4 g KH_2PO_4 and 0.2 g K_2SO_4/pot, but no N was added. Supplemental fluorescent light at a quantum flux density of 190 $\mu E\ m^{-2}\ s^{-1}$ was provided during a 16/8 h light/dark cycle at 25/20 C for alfalfa and 28/20 C for trefoil.

After transplanting, the alfalfa and trefoil were allowed to regrow to 10% flowering before harvest. After this harvest, alfalfa was allowed to regrow for 21 days and trefoil for 37 days. Thereafter, each species was subjected to three treatments: control, floral debudding, and partial shoot removal (hereafter, partial harvest). Controls continued growth after the start of experiments on day 0 without further harvests. Debudding was removal of floral buds and flowers every third or fourth day starting at the onset of sampling on day 0. Partial harvests were performed twice during the experimental period. The first alfalfa harvest at 75% bloom on day 1 reduced leaf area by 75%. A second harvest (day 36) at 100% bloom removed 85% of the leaf area. The first trefoil harvest at 90% bloom on day 2 reduced leaf area by 85%. At 100% bloom (day 35), the second harvest removed 75% of the leaf area.

Each treatment was replicated four times in randomized complete blocks. Four pots (eight plants) per treatment were randomly selected for each species at each sampling, which was often divided equally between two successive days to facilitate handling of material. Alfalfa was sampled on days 0, 2, 9, 18, 34, 37, 46, 55, and 71. Trefoil was sampled on days 0, 4, 12, 20, 35, 39, 45, 57, and 71.

In a companion experiment to the three already described, a group of alfalfa plants from the original population was allowed to regrow for 30 days following a fourth harvest. Total shoot removal occurred at 85% bloom on day 1 of sampling and at 50% bloom 24 days later. Every leaf was removed and only 2 to 3 cm of stem remained as stubble. Four pots (eight plants) per sampling were randomly selected. These plants were assayed on days 0, 2, 5, 12, 25, 26, 27, 29, 36, and 52. The time of sampling for all experiments was standardized at about 4 h before the onset of darkness.

The TNA per plant, estimated by the $N_2(C_2H_2)$ reduction assay (5), was the measure of the N_2-fixation capacity of individual plants. Roots of plants from each pot were separated quickly, washed free of sand in 20 C water, and gently blotted to remove excess moisture. Detopped roots were placed in 1-liter jars equipped with serum stoppers. Sixty cc of air were withdrawn and replaced with an equal volume of C_2H_2. Root systems were incubated at 20 C. At intervals of 32 or 48 min., 0.5 ml of the gaseous atmosphere from the incubation chamber was withdrawn by a syringe and injected into a Varian 3700 gas chromatograph equipped with a 0.4 × 180 cm Poropak N column at 80 C oven temperature. The injector temperature was 80 C and the carrier gas was N_2 at a flow rate of 30 cc/min. Rate of C_2H_4 production from C_2H_2 was computed by a Varian CDS 111 microprocessor from a standard curve that was established daily.

The LA of individual plants was measured with a Hayashi Denko automatic area meter type AAM-5. Herbage and root mass were measured after the plant material was oven-dried at 70 C for at least 24 h. The TNC concentration of roots was analyzed by Smith's procedure (19).

RESULTS AND DISCUSSION

INTERSPECIFIC COMPARISONS

Control or Nonharvested Plants. The patterns of TNA for alfalfa and trefoil generally differed throughout the experiment, although rates for individual plants of the two species were similar. The TNA of alfalfa was about 6 μmol C_2H_4/h·plant for the first 38 days and rose to a maximum of 16 μmol C_2H_4/h·plant by day 72 (Fig. 1A). The TNA of trefoil rose from 4 to 12 μmol C_2H_4/h·plant within the initial 13 days, linearly declined to 6 μmol C_2H_4/h·plant over the next 33 days, and reached 9 μmol C_2H_4/h·plant during the final 26 days (Fig. 2A).

Despite similar proportional increases in herbage growth for alfalfa and trefoil over the sampling period, the patterns of LA expansion differed between the species (Fig. 1, B and C; Fig. 2, B and C). Leaf area of alfalfa expanded from about 300 to 950 cm^2/plant within the first 35 days. Thereafter, senescence of leaves followed by new growth from crown and axillary buds maintained LA at about 800 cm^2/plant. Leaf area of trefoil expanded more slowly and steadily from 375 to 900 cm^2/plant.

Root growth and TNC concentration also differed between the two species. Alfalfa increased root mass from 1.5 to 14 g/plant and TNC levels from 9 to 21% over the sampling period (Fig. 1, C and D). Root mass and TNC levels of trefoil remained constant at about 12 g/plant and 6%, respectively (Fig. 2, C and D).

Debudded Plants. The TNA of debudded alfalfa was about 5 μmol C_2H_4/h·plant for the initial 37 days and reached 12 μmol C_2H_4/h·plant on day 72 (Fig. 3A). The TNA of trefoil increased from 4 to 12 μmol C_2H_4/h·plant for the first 21 days, but subsequently declined to 5 μmol C_2H_4/h·plant on day 72 (Fig. 4A).

Rates of herbage growth, LA expansion, root mass accumulation, and TNC accumulation were relatively greater in debudded alfalfa than in trefoil. During the experiment, herbage mass increased 400% in alfalfa and 130% in trefoil (Figs. 3C, 4C). Trefoil displayed no consistent LA expansion (Fig. 4B), but LA of alfalfa expanded nearly 3-fold within the initial 37 days (Fig. 3B). Thereafter, senescence of alfalfa leaves followed by new growth from crown and axillary buds maintained a constant LA. Root mass over the sampling period increased 940% for alfalfa and 130% for trefoil (Figs. 3C, 4C). The TNC concentration increased from 9 to 20% for alfalfa and from 6 to 10% for trefoil within the first 36 days (Figs. 3D, 4D). Neither species increased TNC levels thereafter.

Partial Harvest. Trefoil exhibited a relatively greater decline in TNA than did alfalfa after partial harvest. The first harvest of alfalfa caused no reduction in TNA (Fig. 5A), but decreased TNA of trefoil 56% within 2 days (Fig. 6A). The second harvest reduced TNA of alfalfa 46% and that of trefoil 69% within 2 days.

Recovery of TNA after both harvests was more rapid in alfalfa than in trefoil (Figs. 5A, 6A). Alfalfa exhibited essentially no decline of TNA in the first regrowth cycle after harvest, but TNA of trefoil required 11 days of regrowth to return to the pretreatment value. After the second harvest, alfalfa returned to the pretreatment TNA value within 10 days, but trefoil required 21 days. The differences in rapidity of recovery of TNA after each harvest closely mirrored the interspecific differences in LA expansion and herbage growth (Figs. 5 and 6). Altbough the root mass of trefoil remained constant at about 9 g/plant, the root mass of alfalfa increased from 1.3 to 5 g/plant during the experiment (Figs. 5C, 6C). Both species exhibited a slow decline and recovery in TNC concentration following partial harvest (Figs. 5D, 6D).

The greater decline in TNA for trefoil than alfalfa following partial harvest (Figs. 5A, 6A) may be related to the lower TNC level of trefoil (Figs. 5D, 6D), a characteristic that has been reported previously (19). Perhaps the higher TNC concentration of alfalfa roots than that of trefoil roots might allow the mobilization of more reserve carbohydrates to the nodules to offset the decrease in photosynthate supply by leaf removal. The contrasting patterns of TNA between the control plants of the two species further suggest that differences in photosynthate supply and partitioning between alfalfa and trefoil could mediate N_2-fixation capacity.

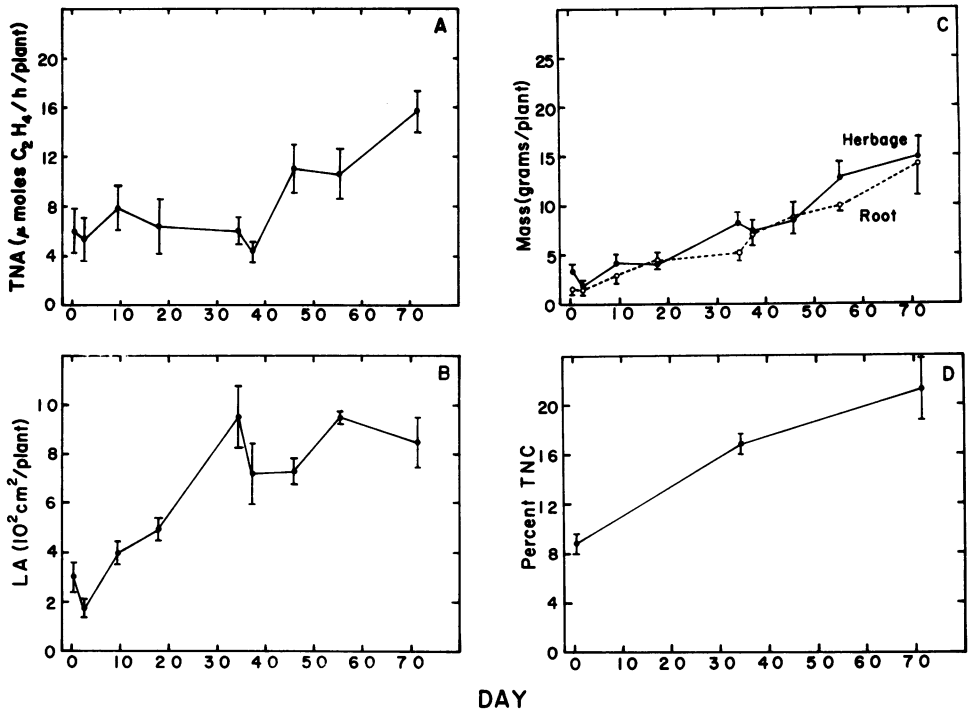

FIG. 1. Total nitrogenase activity, leaf area, herbage and root growth, and total nonstructural carbohydrate accumulation for control (nonharvested) alfalfa. Each point is a mean of four replicates ± SE.

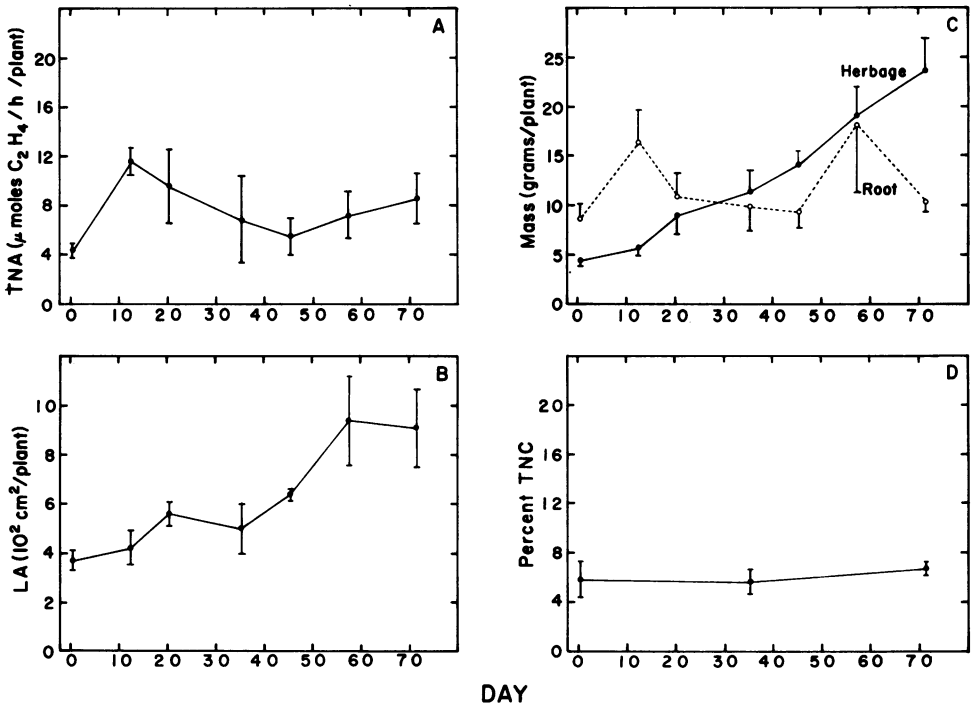

FIG. 2. Total nitrogenase activity, leaf area, herbage and root growth, and total nonstructural carbohydrate accumulation for control (nonharvested) trefoil. Each point is a mean of four replicates ± SE.

Nodule Numbers and Mass. Numbers and mass of trefoil nodules were always greater than those of alfalfa before and after the second harvest interval (Tables I and II). Nodule numbers, but not mass, of alfalfa decreased significantly 10 days after harvest (Table I), observations which agree with the results of Vance *et al.* (22) on alfalfa seedlings. The decline in nodule numbers after harvest is similar to reports with other species (2, 24, 25), although the maintenance of nodule mass suggests that alfalfa preferentially partitions photosynthate to nodule growth so that the capacity for nitrogen fixation is only temporarily impaired. Twenty days after harvest, numbers and mass of nodules were similar to the pretreatment values. Nodule mass of the harvested alfalfa plants doubled between the onset and termination of the second harvest interval, and nearly tripled on controls and debudded plants (Table I).

In contrast with alfalfa, nodule number of harvested trefoil declined significantly between the onset and termination of the

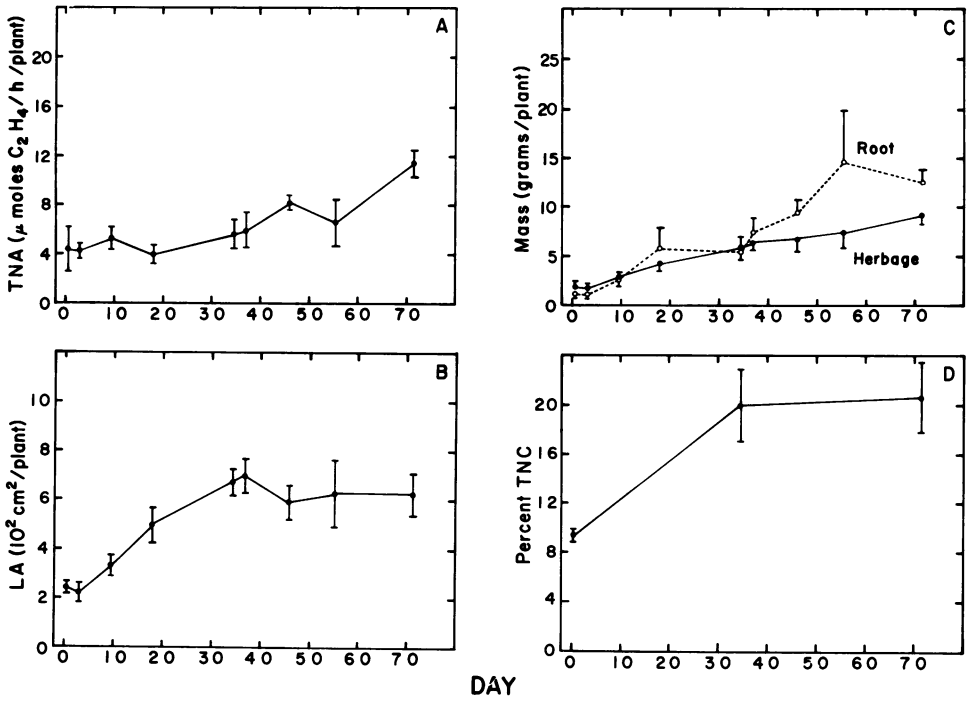

FIG. 3. Total nitrogenase activity, leaf area, herbage and root growth, and total nonstructural carbohydrate accumulation for debudded alfalfa. Each point is a mean of four replicates ± SE.

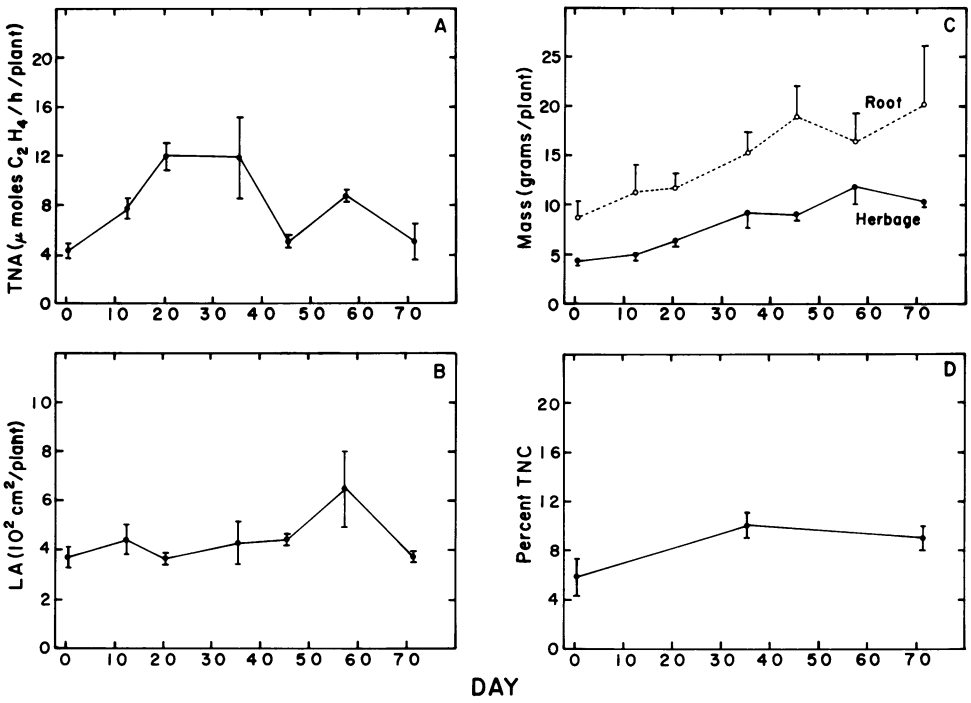

FIG. 4. Total nitrogenase activity, leaf area, herbage and root growth, and total nonstructural carbohydrate accumulation for debudded trefoil. Each point is a mean of four replicates ± SE.

second harvest interval, but nodule mass remained unchanged. Number and mass of trefoil nodules for the control and debudded plants remained unchanged after second harvest (Table II), in contrast with the significant increases in nodule mass that occurred on similarly treated alfalfa (Table I).

Although there was little difference in TNA between species within each treatment (Figs. 1 to 6), the large differences in nodule mass (Tables I and II) illustrate that alfalfa had a greater nitrogenase activity per unit mass of nodule than trefoil. Perhaps senescing trefoil nodules remained attached to roots longer than alfalfa nodules, or differences in nodule morphology resulted in alfalfa nodules being more efficient than trefoil nodules.

INTRASPECIFIC COMPARISONS

Control and Debudded Plants. Rates of TNA and patterns of LA, herbage growth, root growth, and TNC concentration were similar for control and debudded alfalfa (Figs. 1 and 3). During the initial 38 days TNA averaged 6 to 7 μmol C_2H_4/h·plant for controls and 4 to 5 μmol for debudded alfalfa (Figs. 1A, 3A).

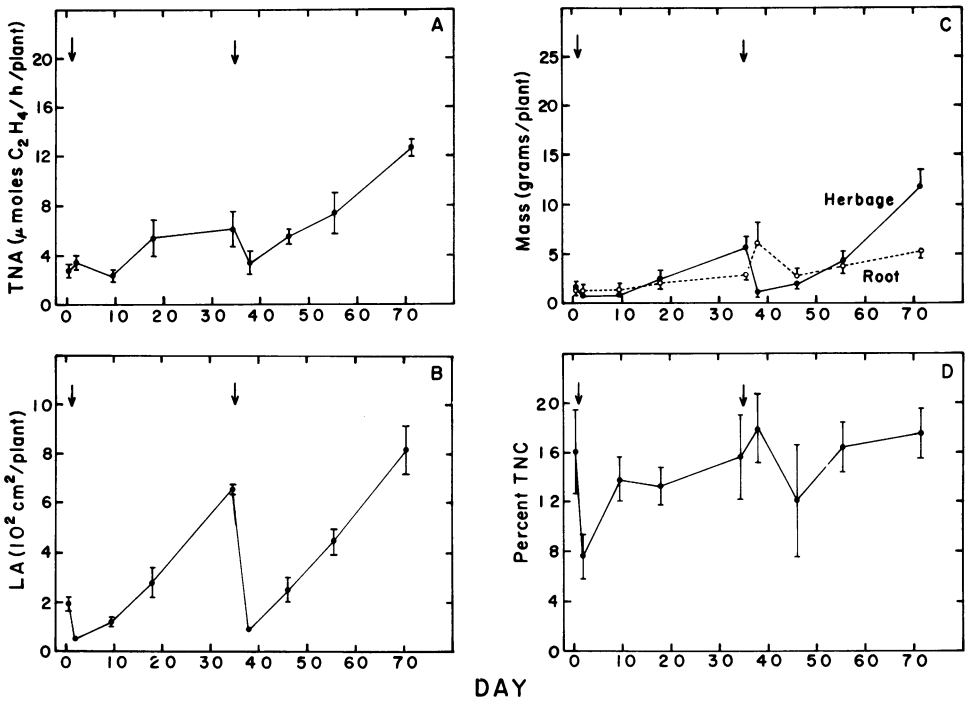

FIG. 5. Total nitrogenase activity, leaf area, herbage and root growth, and total nonstructural carbohydrate accumulation for alfalfa experiencing partial shoot removal in two successive cycles of harvest and regrowth. Arrows indicate time of harvest. Each point is a mean of four replicates ± SE.

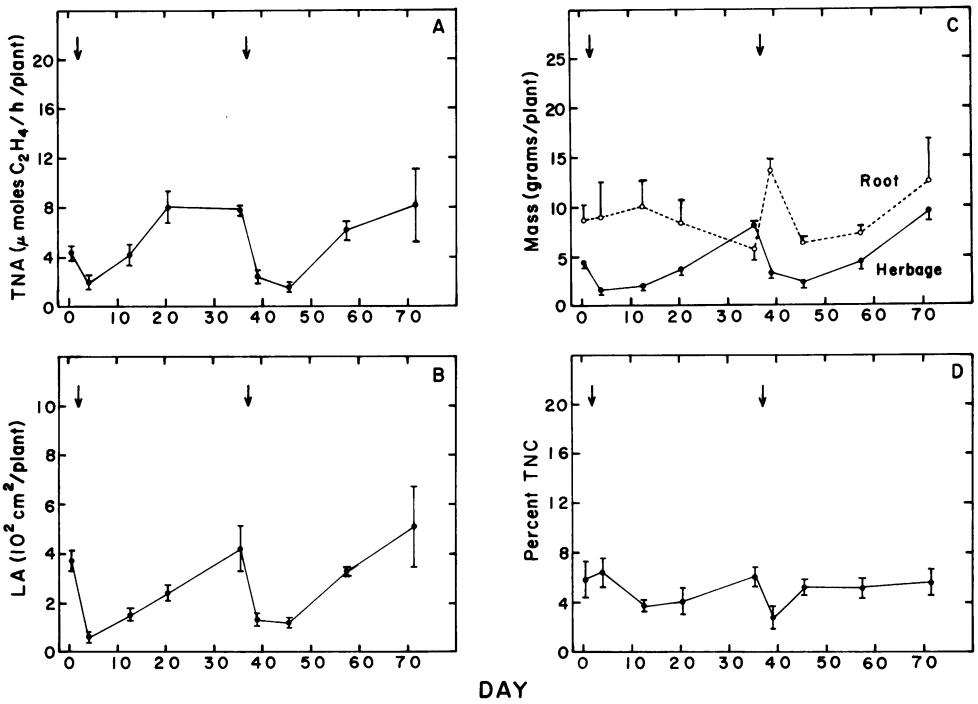

FIG. 6. Total nitrogenase activity, leaf area, herbage and root growth, and total nonstructural carbohydrate accumulation for trefoil experiencing partial shoot removal in two successive cycles of harvest and regrowth. Arrows indicate time of harvest. Each point is a mean of four replicates ± SE.

Thereafter, TNA increased to about 16 μmol C_2H_4/h·plant for controls and 12 μmol C_2H_4/h·plant for debudded plants by day 72. The increase in TNA of controls was coincidental with pod development, which was absent on the debudded plants. Leaf area expanded to about 950 cm^2/plant for controls on day 34 and 700 cm^2/plant for debudded alfalfa on day 37 (Figs. 1B, 3B). Leaf senescence followed by new growth from crown and axillary buds maintained LA at about 800 cm^2/plant for controls and 600 cm^2/plant for debudded plants during the rest of the experiment. Herbage mass increased 350% for controls over the sampling period and 400% for debudded alfalfa (Figs. 1C and 3C). Root mass increased 840% during the experiment for controls and 940% for debudded alfalfa. Both treatments attained maximum TNC levels of 20% (Figs. 1D and 3D).

Although control and debudded trefoil had similar rates of TNA per plant, they exhibited contrasting patterns of TNA, LA, herbage growth, root growth, and TNC concentration (Figs. 2 and 4). The TNA of controls reached about 12 μmol C_2H_4/h·plant on

Table I. *Number and Mass of Nodules on Control, Debudded, and Partly Harvested Alfalfa Plants Sampled Before the Second Harvest, and During or After Herbage Regrowth*

Sampling Day	Control		Debudded Control		Harvested Control	
	Number[a]	Mass[a]	Number[a]	Mass[a]	Number[a]	Mass[a]
		mg		*mg*		*mg*
34	298 ± 61	70 ± 18	185 ± 41	42 ± 7	323 ± 56	72 ± 6
46					198 ± 14	57 ± 11
55					283 ± 64	87 ± 23
71	465 ± 117	189 ± 21	340 ± 68	119 ± 9	364 ± 56	147 ± 16

[a] Mean of eight plants ± SE.

Table II. *Number and Mass of Nodules on Control, Debudded, and Partly Harvested Trefoil Plants Sampled Before the Second Harvest and Again After Herbage Regrowth*

Sampling Day	Control		Debudded Control		Harvested Control	
	Number[a]	Mass[a]	Number[a]	Mass[a]	Number[a]	Mass[a]
		mg		*mg*		*mg*
35	753 ± 281	423 ± 222	1,009 ± 270	378 ± 52	1,613 ± 562	461 ± 167
71	1,007 ± 842	502 ± 315	726 ± 237	539 ± 256	782 ± 288	318 ± 87

[a] Mean of two plants ± SE.

day 13 and subsequently decreased, but debudded trefoil achieved a maximum TNA of 12 μmol C_2H_4/h·plant more slowly and maintained it from day 20 to 36 before declining (Figs. 2A and 4A). Leaf area of control trefoil expanded about 140% during the experiment, with debudded plants displaying no consistent changes of LA (Figs. 2B and 4B). Herbage mass increased 440% for controls and only 130% for debudded plants (Figs. 2C and 4C). Controls maintained root mass of about 12 g/plant and TNC levels at 6% (Fig. 2, C and D), but debudded trefoil doubled root mass and TNC concentration during the experiments (Fig. 4, C and D).

In contrast with results from floral debudding of peas (11), our periodic floral debudding of alfalfa and trefoil did not greatly alter TNA compared with controls. Our results suggest that regulation of N_2-fixation in these two forage legume species is less strongly associated with flowering than in the pea. Because pod development was concurrent with the period of increasing TNA in alfalfa controls, N_2-fixation was apparently less sensitive to competitive sinks for photosynthate than in soybeans (10), peas (1), and cowpeas (7).

In contrast with the insensitivity of trefoil TNA to floral debudding, debudding of trefoil clearly favored the partitioning of dry matter to the root compared with the shoot. These observations support the concept (15, 18) that trefoil TNC accumulation is limited by the preferential utilization of photosynthate for continued shoot growth and flowering. Termination of flowering by debudding (Fig. 4) or by onset of fall dormancy (18) favors the partitioning of photosynthate to trefoil roots.

Partial and Total Harvest of Alfalfa. Compared with controls, partly and totally harvested alfalfa exhibited marked reductions in TNA that were proportional to the severity of harvest (Figs. 5A and 7A). No reduction in TNA followed the initial 75% shoot and leaf removal, but the second harvest with 85% shoot and leaf removal caused a 46% decline of TNA within 2 days. Decrease of TNA following total shoot removal was 78% 2 days after the first harvest and 86% 1 day after the second harvest.

The TNA of alfalfa experiencing partial and total harvest increased with growth of new leaves (Figs. 1, 5, and 7). Between days 10 and 35 after the 75% shoot and leaf removal, TNA increased 170% as LA expanded 500% and herbage regrew 560% (Fig. 5, A, B, and C). Between days 38 and 72 after the 85% shoot and leaf removal, TNA rose 280% as LA expanded 800% and herbage regrew 980%.

Totally harvested alfalfa displayed a relatively greater increase in TNA following its initial postharvest decline than did control or partly harvested plants, but like partly harvested plants, the extent of recovery in TNA after total harvest was relatively less than LA expansion or herbage regrowth. Between day 5 and 25 following the first total harvest, TNA rose 500% as LA expanded 1,300% and herbage regrew 900% (Fig. 7, A, B, and C). After the second total harvest, TNA rose 600% as LA expanded 5,500% and herbage regrew 1,000% between days 29 and 52.

Root mass and TNC concentration exhibited the expected responses to shoot removal and were much less for partly and totally harvested alfalfa than for controls. During the experiment, root mass increased 840% for controls, 300% for partly harvested alfalfa, and 180% for totally harvested alfalfa (Figs. 1C, 5C, and 7C). Concurrently, TNC levels of controls rose from about 9 to 21%. Those of partly harvested alfalfa exhibited only a brief decline after harvest but otherwise remained similar to the preharvest value (Figs. 1D and 5D).

In contrast, TNC concentrations of totally harvested alfalfa declined to less than half of preharvest values after shoot removal and returned to their original values during herbage regrowth (Fig. 7D). The absence of competition for photosynthate between roots and shoot regrowth enabled the unharvested alfalfa to partition more carbohydrates to the root in comparison with harvested plants. The residual photosynthetic tissue of the partly harvested alfalfa apparently limited the utilization of reserve carbohydrates for regrowth so that there was less decline in TNC levels than in totally harvested alfalfa.

DISCUSSION

The decline of TNA of alfalfa and birdsfoot trefoil with herbage removal and its recovery with the onset and extent of vegetative regrowth (Figs. 5–7) were similar to that observed in white clover (14, 17) and illustrate the interdependence of N_2-fixation of perennial legumes and canopy photosynthetic capacity. There is now substantial evidence that herbage removal in alfalfa causes temporary senescence of nodule tissue with nodule renewal dependent

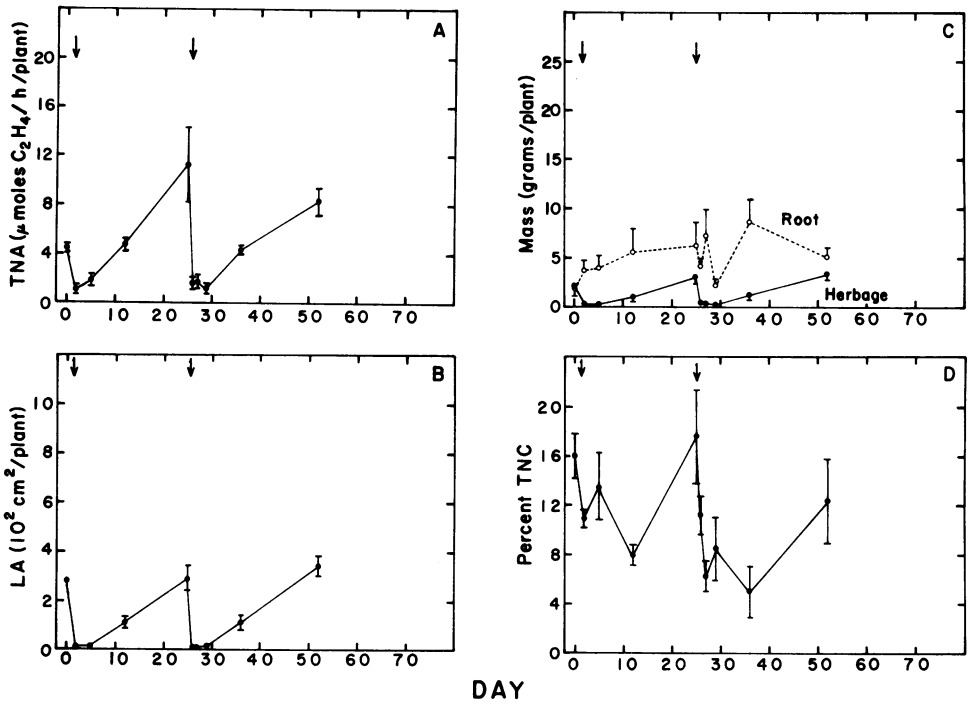

FIG. 7. Total nitrogenase activity, leaf area, herbage and root growth, and total nonstructural carbohydrate accumulation for alfalfa experiencing total shoot removal in two successive cycles of harvest and regrowth. Arrows indicate time of harvest. Each point is a mean of four replicates ± SE.

upon vegetative regrowth (22). The observations that carbohydrates remobilized from roots support vegetative regrowth (8, 20) raise the question of the possible participation of reserve carbohydrates in sustaining N_2-fixation after herbage removal and during regrowth. The evidence in Figures 5 to 7 clearly shows that the changes in TNC after harvest and during regrowth were markedly different in phase and amplitude than those of TNA and LA, suggesting a secondary role of root nonstructural carbohydrates in sustaining of N_2-fixation after harvest. This position is further supported by observations of maintenance of stored starch in nodules temporarily experiencing harvest-induced senescence (22). Furthermore, the repeated observations (Figs. 5–7) that leaf and herbage regrowth were always relatively greater than the recovery of TNA after harvest suggests that nodules are weaker sinks for photosynthate and remobilized TNC than aerial regrowth.

Factors other than LA are apparently involved in mediating TNA of perennial legumes. In control and debudded alfalfa (Figs. 1 and 3), TNA remained unchanged during the initial 35 days of the experiment, but LA increased several-fold in both treatments. This suggests that leaf aging with resultant loss in photosynthetic efficiency, mutual shading, or the diversion of photosynthate to root growth in lieu of nodules were significant constraints to nodule activity. In control and debudded trefoil (Figs. 2 and 4) increased TNA occurred without concurrent changes in LA, suggesting a possible interrelationship of nodule function with temporary storage of photosynthate or with the carbon metabolism of leaves of this species. After release of crown and axillary bud dormancy, a significant increase in TNA of control alfalfa between days 37 and 46 (Fig. 1) occurred simultaneously with leaf regrowth to replace that lost by senescence (Fig. 1B). These observations suggest that endogenous controls on photosynthate partioning and availability are important in nitrogen fixation of forage legumes.

Despite the implication that storage carbohydrates of roots may be of limited importance in sustaining nitrogen fixation of forage legumes, the evidence that TNA and nodulation of trefoil are more severely affected than that of alfalfa by successive harvests (Figs. 5 and 6, Tables I and II) suggests that the decline of nodule function after harvesting or grazing may be a factor in the well known (15, 18) poorer persistence of trefoil. Perhaps the short duration of an apical meristem in trefoil nodules causes nodule function of this species to be particularly sensitive to herbage loss. The evidence suggests the possible greater role of pools of readily metabolized carbohydrates in the roots or crowns of alfalfa than in trefoil, and the possible greater reliance of trefoil upon residual photosynthetic tissue to sustain N_2-fixation after harvest.

These experiments clearly show that the response of N_2-fixation of perennial forage legumes to the interruption of current photosynthate supply caused by herbage removal varies with species and with severity of harvest. The dependence of TNA upon partial herbage removal and subsequent herbage regrowth was greater for birdsfoot trefoil than for alfalfa. Total herbage removal reduced N_2-fixation of alfalfa more than did partial herbage removal. N_2-fixation of unharvested controls or of plants subjected to floral debudding was independent of LA except at release of vegetative bud dormancy late in flowering. N_2-fixation of both species varied with harvest treatment, but it differed in phase and in amplitude from the changes in TNC resulting from herbage removal and floral debudding. Mechanisms of partitioning of current and stored photosynthate are important to the adaptation of N_2-fixation of forage legumes to short-term interruption in photosynthate supply.

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