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NITROGEN FIXATION IN ALFALFA: RESPONSES TO BIDIRECTIONAL SELECTION FOR...

VIANDS, D R ; BARNES, D K ; HEICHEL, G H 1 OF 1

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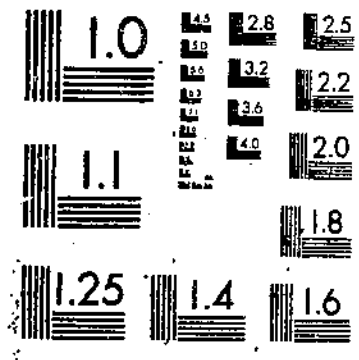

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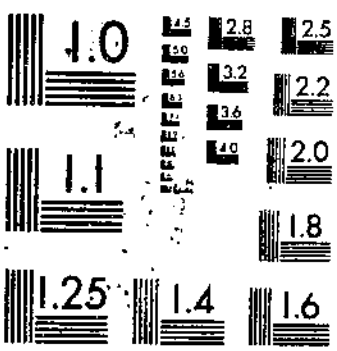

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Nitrogen Fixation in Alfalfa

Responses to Bidirectional Selection for Associated Characteristics

Abstract

Viands, D. R., Barnes, D. K., and Heichel, G. H. 1981. Nitrogen Fixation in Alfalfa—Responses to Bidirectional Selection for Associated Characteristics, U.S. Department of Agriculture Technical Bulletin 1643, 24 p.

Our objectives in this research were to determine responses to plant selection for characteristics associated with N_2-fixation in alfalfa and to develop a practical breeding program for N_2-fixation improvement. Each of two broad-based gene pools, MnNC and MnPL, was divided into subpopulations developed by conducting two cycles of bidirectional, recurrent, phenotypic selection for nitrogenase activity (NA) per plant, top dry weight, nodule mass, and fibrous root mass. We made additional subpopulations by conducting one cycle of bidirectional selection for a base index (A) that did not include NA and by conducting one cycle of selection at two selection intensities for high levels of a base index (B) that included NA. Plants for screening and for determining responses to selection were grown in low-nitrate greenhouse sandbenches containing a mixture of *Rhizobium* strains. We evaluated plants for the four selection characteristics plus nodule number, secondary root number, N concentration in plant tops, and total N in plant tops.

All characteristics except N concentration were changed significantly by two cycles of selection for each characteristic in either one or both gene pools. Two cycles of selection increased NA 66 percent in the MnNC gene pool and 61 percent in the MnPL gene pool. Nodule mass accounted for about 37 percent of the variation in NA. The other characteristics accounted for about 6 percent after we accounted for nodule mass. The remaining 57 percent of the variation in NA was unexplained.

Based on the results obtained, we recommend improving N_2-fixation in alfalfa by first selecting large plants with a large nodule mass and then screening those plants for NA. Evidence for nonadditive genetic effects for some characteristics suggests that the production of a hybrid type of cultivar would be most useful. Significant variation among 'Saranac', 'Agate', and the experimental subpopulations suggested that present cultivars differ in N_2-fixation potential.

We conducted field experiments and used the mass 15 isotope of N as a tracer of N metabolism to determine the seeding-year performance of selected populations from the greenhouse investigations. Additional objectives were to investigate the effects of stage of plant development, and herbage versus whole plant sampling, on assessments of N_2-fixation in the field.

During the seeding year, two experimental populations averaged about 43 percent of their N needs from symbiosis, compared with 36 percent for the standard cultivar 'Saranac'. The experimental populations fixed an average of about 148 kg/ha of N during the growing season, compared with 109 kg/ha for 'Saranac'. N_2-fixation was least (4 to 20 kg/ha) in the first and fourth harvest intervals and greatest (38 to 87 kg/ha) during the second or third harvest intervals.

In contrast to greenhouse evaluations of NA, we observed significant ($p<0.05$) differences in rates of N_2-fixation per plant in the field between the two experimental populations, and between the experimental populations and 'Saranac', over the growing season. Differences of 14 to 31 percent in average plant populations, however, resulted in smaller differences in N_2-fixation on a land area basis than would be expected from individual plant performance. The rates of N_2-fixation of field-grown plants mirrored growth rates, which varied with the changing field environment and especially with onset of dormancy.

Keywords: *Medicago sativa* L., *Rhizobium meliloti* Dang., recurrent phenotypic selection, acetylene reduction assay, root morphology, nonadditive genetic effects, base indexes, nitrogen-15, isotope dilution, sampling methods, nitrogenase activity.

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Nitrogen Fixation in Alfalfa

Responses to Bidirectional Selection for Associated Characteristics[1]

Donald R. Viands, Donald K. Barnes, and Gary H. Heichel[2]

Introduction and Literature Review

N_2-fixation in legumes is a process whereby *Rhizobium* spp. in association with the legume host plant reduce atmospheric N_2 to a form usable by the plant. Legumes have been used in crop rotations to improve the soil N content and tilth for subsequent grain crops. As N fertilizers became more abundant and less expensive, crop rotations containing legumes began to diminish in many parts of the United States. The energy crisis, beginning in 1973, prompted concern for the future supply and cost of N fertilizers made from fossil fuels. It created increased interest in the potential of N_2-fixation by legumes to substitute for N fertilizers.

Before 1976, most efforts to improve the N_2-fixation capacity of legumes involved attempts to discover improved strains of *Rhizobium* spp. When *Rhizobium* strains are applied to soil inhabited by indigenous strains, however, plant performance is generally not improved compared to noninoculated plants. Examples include soybean (*Glycine max* (L.) Merrill), where neither yield nor seed protein concentration was affected (*13*)[3] and alfalfa (*Medicago sativa* L.), where neither yield nor forage protein concentration was affected (*2*).

Host plants and the *Rhizobium* spp. have genetic control of the N_2-fixation process. Control of host plant has been demonstrated in several legumes by observing significant variation among cultivars and of host plant X *Rhizobium* strain interactions for yield and for N_2-fixation (*3, 7, 8, 10, 11, 16*). Inheritance studies have shown that the host plant helps to condition many steps in the N_2-fixation process. In red clover, *Trifolium pratense* L., genes influenced resistance to infection by *Rhizobium*, infection time, and numbers of nodules (*25, 26, 27*). In soybeans, nonnodulation was controlled by a single recessive gene (*37*), and in alfalfa, nonnodulation was controlled by two recessive genes (*32*). Effectiveness, or ineffectiveness, of nodules was reported to be heritable in red clover (*29, 30*); in alfalfa (*1, 32, 36*); in *Lotus* spp. (*9*); in subterranean clover (*T. subterraneum* L.) (*11*); in soybeans (*35*); and in peas (*Pisum* spp.) (*19*).

Based on observations that N_2-fixation was determined by the host plant as well as by the *Rhizobium* strain, suggestions were made concerning the possibility of breeding red clover for increased levels of effectiveness (*28*). Further reports showed improved N_2-fixation and yield in red clover (*31*). An increased nodule volume was shown in white clover (*T. repens* L.) during two cycles of selection but not during a third cycle (*24*). Other suggestions were made for breeding alfalfa for improved N_2-fixation and for selecting well-nodulated alfalfa plants (*10, 20*).

Heritable differences among alfalfa genotypes were first demonstrated for N_2-fixation as indicated by NA measured by the acetylene reduction assay (*34*). Recommendations were made that breeding for improved N_2-fixation be conducted by screening plants first for increased nodule mass and fibrous roots and then for increased NA (*34*). NA per plant was increased by 82 percent after one cycle of selection for NA in a nonfall-dormant, alfalfa population (*5*). Two of the recent studies (*5, 34*) reported that high top weight, high root weight, and increased nodule mass were associated with increased NA per plant. However, the causes of the associations were unclear.

Although the available data suggest that increasing the N_2-fixation capacity of alfalfa is possible, we must understand various interrelationships among morphological, physiological, and yield parameters before large-scale breeding programs are initiated. The research reported in this publication was designed to provide plant breeders and physiologists with background information on breeding for improved N_2-fixation capacity.

Our objectives were (1) to determine responses to bidirectional selection for the individual traits of NA per plant, nodule mass, top dry weight, and fibrous root mass, and also for base indexes consisting of NA and four morphological characteristics in two alfalfa gene pools; (2) to examine associations among NA and morphological characteristics; (3) to determine the best morphological characteristic, or combination of characteristics, for selection to improve the genetic potential for enhanced N_2-fixation in alfalfa; (4) to determine if breeding for characteristics associated with N_2-fixation affected N concentration and total N in plant tops; and (5) to compare N_2-fixation under field conditions of two experimental populations from the first cycle of the greenhouse recurrent selection program with that of a standard alfalfa cultivar. The scope of this research and the large amount of critical data prompted us to publish the results in a single document rather than in a series of papers.

Materials and Methods

Greenhouse Investigations

Plant Material—We conducted selection experiments with two genetically broad-based alfalfa gene pools. MnNC-4 was developed at St. Paul, Minn., by intercrossing plants resistant to bacterial wilt (caused by *Corynebacterium insidiosum* (McCull.) H.L. Jens.). Sixty plants each were selected from 'Agate', 'Atlantic', 'Dawson', 'Iroquois', 'Kanza', and MnP-B followed by three generations of intercrossing without selection and by one cycle of selection for seed yield in Idaho.

MnPL-6 is similar to 'Vernal' in

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[3] Italic numbers in parentheses refer to Literature Cited, page 17.

parentage. it was developed at St. Paul from plants selected on the basis of vigor and general appearance from a 3-year-old yield trial (*18*). The selected plants were transplanted to a spaced-plant nursery at Rosemount, Minn., where the less vigorous and disease-susceptible plants were eliminated over a 2-year period. Open-pollinated seed harvested from approximately 300 plants was the basic seed for the MnPL population. The population then was selected one cycle for resistance to bacterial wilt, one cycle for resistance to common leafspot caused by *Pseudopeziza medicaginis* (Lib.) Sacc., two cycles for resistance to Phytophthora root rot caused by *Phytophthora megasperma* Drechs., and one cycle for resistance to Fusarium wilt caused by *Fusarium oxysporum* Schlecht f. sp. *medicaginis* (Weimer) Snyd. & Hans. According to fall dormancy evaluations in Minnesota, MnNC-4 is less winter -hardy (similar to 'Ranger') than MnPL-6, which is very winter-hardy (similar to 'Vernal').

Plant Culture—All screening and evaluation studies for morphological traits and NA were conducted in greenhouse sand benches with low nitrate levels (12 to 14 p/m). Before planting, each greenhouse bench (3.75 × 1.91 × 0.15 m) was steamed, and then 360 g of 0-20-20 fertilizer, 450 g of lime, and a trace amount of micronutrients were incorporated into the sand. A mixture of five *R. meliloti* Dang. strains contained in a dry peat base from the Nitragin Company, Milwaukee, Wis., was incorporated into the sand before planting and also was applied as a slurry onto the sand after planting.

A mixture of *Rhizobium* strains was used rather than a single strain because we believed that it more closely represented a field situation (*34*). Seeds were planted in 5- × 5- × 13-cm plastic sleeves (two plants per sleeve and thinned to one plant 10 days after emergence) to prevent roots of each plant from entangling with those of neighboring plants. Plants were assayed at about midbloom stage of the first regrowth (about 12 to 14 weeks after planting).

Experimental Design—We divided each gene pool into subpopulations, which were developed by applying two cycles of recurrent phenotypic selection for high and low NA, high and low top dry weight, high and low nodule mass, and high and low fibrous root mass (table 1). Two additional subpopulations in each pool were developed by applying one cycle of selection for high and low Base Index A and using the equations:

$$I(\text{MnNC}) = 0.21\,T + 0.24\,N + 0.18\,F + 0.08\,S$$

and

$$I(\text{MnPL}) = 0.69\,T + 0.35\,N + 0.48\,F + 0.12\,S$$

where I = base index value, T = top dry weight, N = nodule mass score, F = fibrous root mass score, and S = secondary root number score.

Because improving NA was the primary goal, we derived weights for each independent variable by computing mean coefficients of regression over blocks, after fitting regression equations of NA on all independent variables listed in these base index equations.

More than 800 plants of each gene pool were screened, and approximately 110 plants (14 pct) were chosen for each subpopulation in the first cycle of selection. Because of sampling time, environmental effects in the sand benches, and possible diurnal fluctuations in NA, plants for screening were blocked and 5 plants were selected from each 36-plant block. We used some plants as parents for more than one subpopulation in each pool. When intercrossing selections to produce seed for the next generation, racemes therefore were tagged to designate the subpopulation to which that seed belonged. Each subpopulation was independent in the second cycle of selection. Because of the size of the experiment in the second cycle of selection, only about 250 plants (seven 36-plant blocks) were screened in each subpopulation. We selected 7 plants (20 pct) per block for a total of about 50 plants (table 1).

In addition to these breeding subpopulations, we divided each gene pool into two subpopulations designated HB-B (20 pct) and HB-B (1 pct) after the first cycle of selection (table 1). The HB-B (20 pct) subpopulation was developed by selecting 20 percent (160) of the plants, and the HB-B (1 pct) subpopulation was developed by selecting 1 percent (10 plants) for high levels of Base Index B, comprised of the same characteristics as Base Index A plus NA values. For Base Index B, top dry weight and the root characteristics were weighted 1.0, and NA was weighted 1.5 times that of the other characteristics. Seed of the Syn. 1 generation of each subpopulation was produced in the greenhouse, and seed of the Syn. 2 generation was increased in Prosser, Wash., as part of the NC-83 Regional Seed

TABLE 1.—2-cycle scheme of bidirectional recurrent phenotypic selection for 6 plant characteristics in the MnNC and MnPL alfalfa gene pools

Gene pool generation MnNC	Gene pool generation MnPL	Selection cycle	Selection intensity (pct)	Selection scheme[1]
6	9	2	20	HT HN HF HA → HB-B (1 pct)
5	8	1	14	HT HN HF HA HB-A → HB-B (20 pct)
4	6	0		ORIGINAL GENE POOL
5	8	1	14	LT LN LF LA LB-A
6	9	2	20	LT LN LF LA

[1] H = selection for high levels.
L = selection for low levels.
T = top dry weight.
N = nodule mass score.
F = fibrous root mass score.
A = nitrogenase activity per plant.
B-A = Base Index A comprised of top dry weight, nodule mass score, fibrous root mass score, and secondary root number score, each weighted as described in the text.
B-B (1 pct) = Base Index B at the 1-percent intensity.
B-B (20 pct) = Base Index B at the 20-percent selection intensity.
(Base Index B was comprised of nitrogenase activity and the characteristics in Base Index A, each weighted as described in the text.)

Production Project. Responses to selection were evaluated in three experiments.

The MnNC experiment was planted in February 1978 and included the original MnNC gene pool and plants from both cycles of selection in the high and low directions for each characteristic from the MnNC pool. The experiment was planted in a randomized, complete block design with 70 blocks and 28 entries. Ten of the 28 entries represented the original pool so that each response curve would have an independent value for the original pool. For ease of discussion, values for the original pool, presented in "Results and Discussion," are the means of these 10 entries. Each of the 28 entries was represented by 1 plant per block.

At midbloom stage of the first regrowth (early May 1978), we assayed each plant for top dry weight and for NA per plant by using the acetylene-ethylene reduction assay (*15, 34*). The root systems of each plant were scored visually for nodule mass, nodule number, fibrous root mass, and secondary root number. All scores ranged from 1 (least) to 5 (most). For example, nodule numbers were classified into approximately the following numbers of nodules: 1 = 0 to 5 nodules, 2 = 6 to 30 nodules, 3 = 31 to 45 nodules, 4 = 46 to 70 nodules, and 5 = more than 70 nodules. Secondary root scores were classified as 1 = no secondary roots, 2 = 1 to 3 roots, 3 = 4 to 6 roots, 4 = 7 to 8 roots, and 5 = more than 8 roots.

The 70 blocks were combined pairwise in the analysis of variance for each characteristic, resulting in 35 replications. Computations for the analyses of variance were done on mean performance of the two plants within each entry per replication. Realized heritabilities were computed for each selection criterion, within each of the two cycles of selection, and for both the high and low direction of selection. Heritabilities were computed as the ratio of selection gain to selection differential (*14*).

In addition to these characteristics, we analyzed plant tops of each entry for N concentration by a micro-Kjeldahl procedure. Crown and root systems were not analyzed. Because of the large number of plants, we combined plant tops from each entry over 7 blocks at a time, which resulted in a total of 10 replications. We also combined all entries representing the original gene pool; therefore, analyses of variance for N concentration and total N in plant tops were computed on 19 entries.

The MnPL experiment was planted in June 1978 and was evaluated in early September. The numbers and types of entries and the types of measurements were the same as those in the MnNC experiment, except that N analyses were not made on the plant tops.

The MnNC-MnPL experiment was planted in March 1978 and was evaluated in late June to compare the two gene pools. We evaluated the two original gene pools, eight subpopulations after two cycles of selection for high levels of each plant characteristic, seven Base Index B subpopulations, and two cultivars ('Agate' and 'Saranac'). The same characteristics were evaluated as in the individual gene pool evaluations. The experiment was a randomized, complete block design with 19 entries and 34 blocks. Analyses of variance (blocks paired to total 17 replications) were computed for NA and the morphological characteristics. We planted an additional block in this experiment so that plant tops of each entry could be combined into seven replications of five blocks each for N analysis.

Field Investigations

We conducted our experiments at the Rosemount Experiment Station, University of Minnesota, St. Paul, during 1977 to assess under field conditions the progress of selection for morphological and physiological traits associated with N_2-fixation capacity. The isotope dilution (ID) technique that used the mass 15 isotope of N as a tracer of plant N metabolism (*17, 22*) was used to measure N_2-fixation instead of the acetylene reduction assay of NA. The ID technique is preferred to the acetylene reduction assay for field experiments because it integrates N_2-fixation over time, variations in the physical environment, and heterogeneity of the rhizosphere.

Soil Characteristics—Experimental plots were located on Port Byron silt loam, a fine-silty, mixed, mesic Typic Hapludoll soil with a record of alfalfa culture and inoculum application during 1971-75. The site was fallow throughout 1976 and was fertilized in October with 50 kg/ha P and 560 kg/ha K. Soil pH averaged 6.5 in the upper 15 cm of the profile. On May 3, 1977, immediately before onset of experiments, the soil averaged 3.7 percent organic matter, 12 p/m N as nitrate, and 5 p/m N as ammonium in the upper 15 cm of the profile. Ammonium-N was uniform with depth in the profile to 60 cm, while nitrate-N increased from 30 p/m at 15- to 30-cm depth to 51 p/m at 30- to 60-cm depth.

Plant Material—We conducted two experiments to assess the progress of selection. In one, N_2-fixation of the two populations (MnNC-5, MnPL-8; table 1) that had been developed by one cycle of recurrent selection at a 14-percent selection intensity for improvement (in the high direction) for traits associated with N_2-fixation was compared with that of 'Saranac', a standard cultivar. In the second, N_2-fixation of the HB-B (1 pct) and HB-B (20 pct) subpopulations (Syn. 1 generation) of MnNC-5 and MnPL-8 (table 1) was assessed to determine whether intensity of selection for traits associated with N_2-fixation affected performance in the field. In the absence of an alfalfa lacking nodules or having nodules ineffective for N_2-fixation, 'Rise' reed canarygrass (*Phalaris arundinacea* L.) was chosen as a perennial, non-nitrogen-fixing control species with seasonal growth characteristics similar to those of alfalfa.

Experimental Design—Each experimental plot was an undisturbed soil column 100 × 100 × 50 cm bounded on four sides by a redwood frame to prevent lateral dispersion of isotope and to allow unrestricted water percolation and root development. Before establishing alfalfa in the plots, they were sprayed uniformly with 350 ml water containing 0.4 g/m² ^{15}N as $(^{15}NH_4)_2SO_4$ with 42.4 atom percent ^{15}N in 1.0 g total N. Control plots for establishment with the non-nitrogen-fixing reed canarygrass were sprayed similarly with 6 g/m² total N from $(^{15}NH_4)_2SO_4$ with 7.1 atom percent ^{15}N. The N rate applied was insufficient to affect alfalfa nodulation (*16*). The isotope was mixed into the upper 13 cm of the profile immediately after application and allowed to equilibrate for 7 days with periodic stirring before planting.

We established experiments in five replicates of a randomized complete block design on May 10, 1977. All alfalfa entries were sown at equal seeding rates. Because of differences in establishment, population MnNC-5 averaged 168 plants/m²; population MnPL-8, 191 plants/m²; and the cultivar 'Saranac', 121 plants/m² at the first harvest. The HB-B (20 pct) subpopulation of MnNC-5 averaged 168 plants/m², and the HB-B (1 pct) subpopulation averaged 148 plants/m² at the first harvest. For MnPL-8, the HB-B (20 pct) subpopulation averaged 191 plants/m², and the HB-B (1 pct) subpopulation averaged 182 plants/m². Reed canarygrass plots established by hand seeding had an average stand density of 122/m² at first harvest. Alfalfa established as seed was inoculated before sowing with commerical *R. melioti* inoculum from Nitragin.

Plant Samples—Alfalfa and reed canarygrass were sampled when alfalfa was at 1 to 5 percent flower on July 1, August 2, September 15, and again after becoming dormant on October 20. On each date, 10 plants were chosen randomly and were dug from the seeded alfalfa plots, and 5 were chosen from the seeded reed canarygrass plots. Plants initially were washed free of soil in the field and subsequently were transported to the laboratory for nodule removal, rewashing, and rinsing. We clipped herbage remaining after whole-plant harvest on the first three dates to 4-cm stubble height and retained a subsample for a separate isotope analysis of the herbage. Herbage growth was insufficient for sampling at the fourth harvest. All whole plant (a composite of root, crown, and herbage) and herbage samples were dried to constant mass and ground to pass a 1-mm screen in a Udy cyclone mill. Nodules were excluded from all samples.

We determined total N concentration of ovendry plant samples by Kjeldahl analysis with distilled ammonium trapped in 0.1 N H_2SO_4, followed by titration to an acid endpoint with 0.1 NaOH. Kjeldahl distillates were reduced to 5 to 8 ml per sample and were stored in sealed shell vials until analysis for N isotope composition by mass spectrometry.

Calculating N_2-Fixation—Symbiotic N_2-fixation (SNF) was calculated as N fixed on a land area or plant basis (N_f) or proportion of total sample N derived from symbiosis (N_{sy}). Using the ID technique (*17, 22*), we calculated N_{sy} as 100-100 (atom percent excess ^{15}N in legume sample/atom percent excess ^{15}N in nonlegume sample). N fixed on a land area basis was $N_f = (N_{tv})$ (total N yield/m²). This method of calculation assumes that the grass and the legume took up combined forms of N in proportion to the amounts available, and the

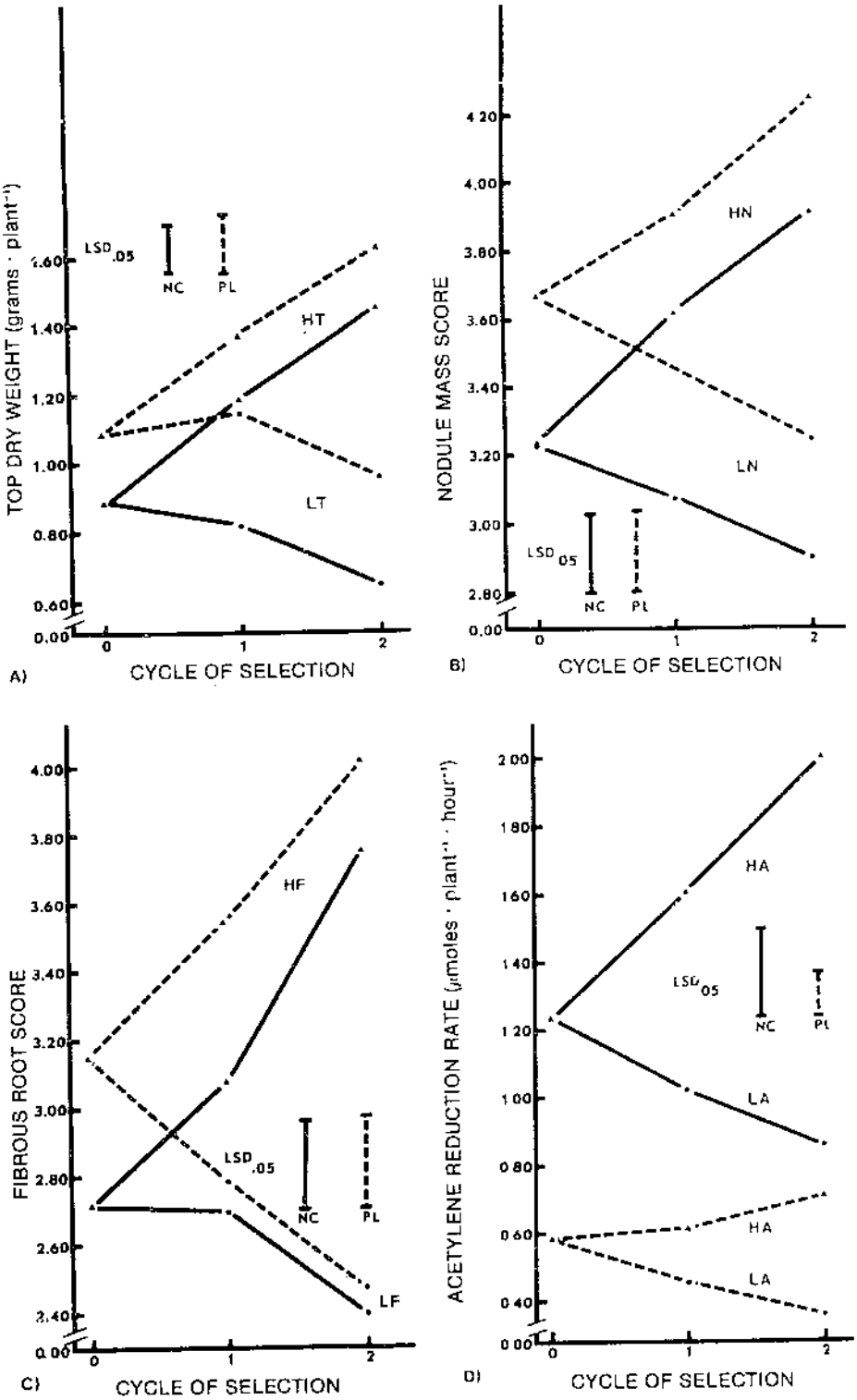

FIGURE 1.—Responses to two cycles of recurrent phenotypic selection for high and low levels of (A) top dry weight, (B) nodule mass score, (C) fibrous root mass score, and (D) nitrogenase activity per plant in the MnNC and MnPL alfalfa gene pools.

discrimination between ^{14}N (the more prevalent isotope) and ^{15}N (the isotope used as a tracer) during root and nodule uptake was inconsequential.

Results and Discussion

Responses to Selection: Greenhouse Investigations

Subpopulation Performance—Both the MnNC and MnPL gene pools showed significant responses to selection for each of the four plant characteristics when they were evaluated in the MnNC experiment and the MnPL experiment (fig. 1A-D). Highly significant ($P < .01$) differences were observed between the high and low subpopulations developed for each characteristic within each of the gene pools. Comparisons between the gene pools in figure 1 should not be made because the experiments were conducted at different times.

The MnNC pool responded linearly to two cycles of recurrent phenotypic selection for high and for low top dry weights (fig. 1A). After two cycles of selection, top dry weights in the pool were altered 65 percent in the high direction and 27 percent in the low direction compared to the original pool. The MnPL pool responded linearly to selection for high top dry weights with an increase of 50 percent (fig. 1A). Response in the low direction was not significant.

Responses to selection for nodule mass score were linear in MnNC with a 0.7 scoring unit increase after two cycles of selection for high mass and a 0.3 decrease after selection for low mass (fig. 1B). Responses to selection in MnPL were a 0.6 unit increase for high nodule mass and a 0.4 unit decrease for low nodule mass (fig. 1B).

Responses to selection for fibrous root score were linear and very large, especially in the high direction, in both pools (fig. 1C). After two cycles of selection in MnNC, alterations of fibrous root score were an increase of 1.0 scoring unit in the high direction and a 0.3 decrease in the low direction. Responses to selection in MnPL were a 0.8 unit increase for high root score and a 0.7 unit decrease for low root score.

Two cycles of selection in the MnNC pool for NA measured as acetylene reduction rate resulted in a 66-percent increase in the high direction and 31 percent decrease in the low direction (fig. 1D). MnPL responded significantly to selection only for low NA, resulting in a 38-percent decrease (fig. 1D). Although nonsignificant, the response to selection for high NA was 21 percent above the original pool. The low NA values of the HA and LA subpopulations of MnPL appeared to be due to environmental stress. In the MnPL experiment, some air pollution injury was noted during July and early August. Plants recovered after harvest, but we had to evaluate the regrowth when temperatures inside the greenhouse were 35° to 40°C. Reports have shown that very warm conditions will produce low NA values (23). More typical values were observed in the MnNC-MnPL experiment (June) when temperatures were 23° to 28° (fig. 2A). In that experiment, the MnPL subpopulation selected for high AR significantly was higher for AR than the original population.

The MnNC pool responded significantly to one cycle of selection for high Base Index A with changes of 11 percent and for low Base Index A with changes of 7 percent compared to the original pool (table 2). MnPL responded significantly only in the high direction with an 11-percent increase.

Response to selection for low levels of each characteristic was less than selection for high levels of each characteristic in both gene pools. Many of the selected plants at the low end of the distribution in each subpopulation grew slowly, flowered late, and had few flowers. Therefore, progenies of the poorest performers were often in lower frequencies than would have been expected in the next cycle of selection had all selected plants contributed equally to the interpollinations.

Differences in realized heritabilities were observed among the selected characteristics in MnNC (table 3). The heritabilities were similar in the first cycle of selection for high levels of all five characteristics. Heritabilities were low except in the second cycle of selection for high-fibrous root mass score. In the second cycle of selection, the four characteristics (excluding Base Index A) ranked in heritability from largest to smallest: fibrous root mass score, nodule mass score, top dry weight, and NA. Differences in heritabilities between characteristics were small, except for high-fibrous root mass score. The heritability of each characteristic was greater in subpopulations selected for increase in the characteristic than in subpopulations selected for a decrease in the characteristic.

All heritabilities in MnPL were

TABLE 2.—Responses to 1 cycle of bidirectional selection for Base Index A[1] in 2 alfalfa gene pools[2]

Subpopulation per gene pool[3]	Selection cycle	Base Index A	Characteristics comprising Base Index A: Acetylene reduction rate (μmoles C_2H_2/plant/hr)	Top dry weight (g/plant)	Nodule mass (score[4])	Nodule number (score[4])	Fibrous root (score[4])	Secondary root (score[4])
MnNC-5-HB-A	1	1.87	1.50	1.17	3.46	2.71	2.96	3.63
MnNC-4	0	1.68	1.24	.88	3.22	2.52	2.72	3.16
MnNC-5-LB-A	1	1.56	.83	.78	3.04	2.54	2.49	3.00
$LSD_{.05}$		.12	.27	.15	.24	.20	.27	.33
$LSD_{.01}$		.16	.36	.19	.31	.27	.36	.44
MnPL-8-HB-A	1	4.37	.60	1.44	3.87	2.91	3.31	3.55
MnPL-6	0	3.94	.58	1.09	3.67	2.87	3.15	3.22
MnPL-8-LB-A	1	3.74	.53	1.06	3.47	2.63	2.91	3.29
$LSD_{.05}$		.29	.14	.18	.25	.25	.28	.34
$LSD_{.01}$		.38	.18	.23	.33	.33	.38	.45

[1] Base Index A for each gene pool consisted of top dry weight, nodule mass score, fibrous root score, and secondary root score, each weighted as described in the text.

[2] The MnNC experiment was evaluated in May 1978, and the MnPL experiment was evaluated in September 1978.

[3] HB-A = subpopulation selected for high levels of Base Index A.
LB-A = subpopulation selected for low levels of Base Index A.

[4] Root morphological characteristics were scored 1 (least amount) to 5 (most amount).

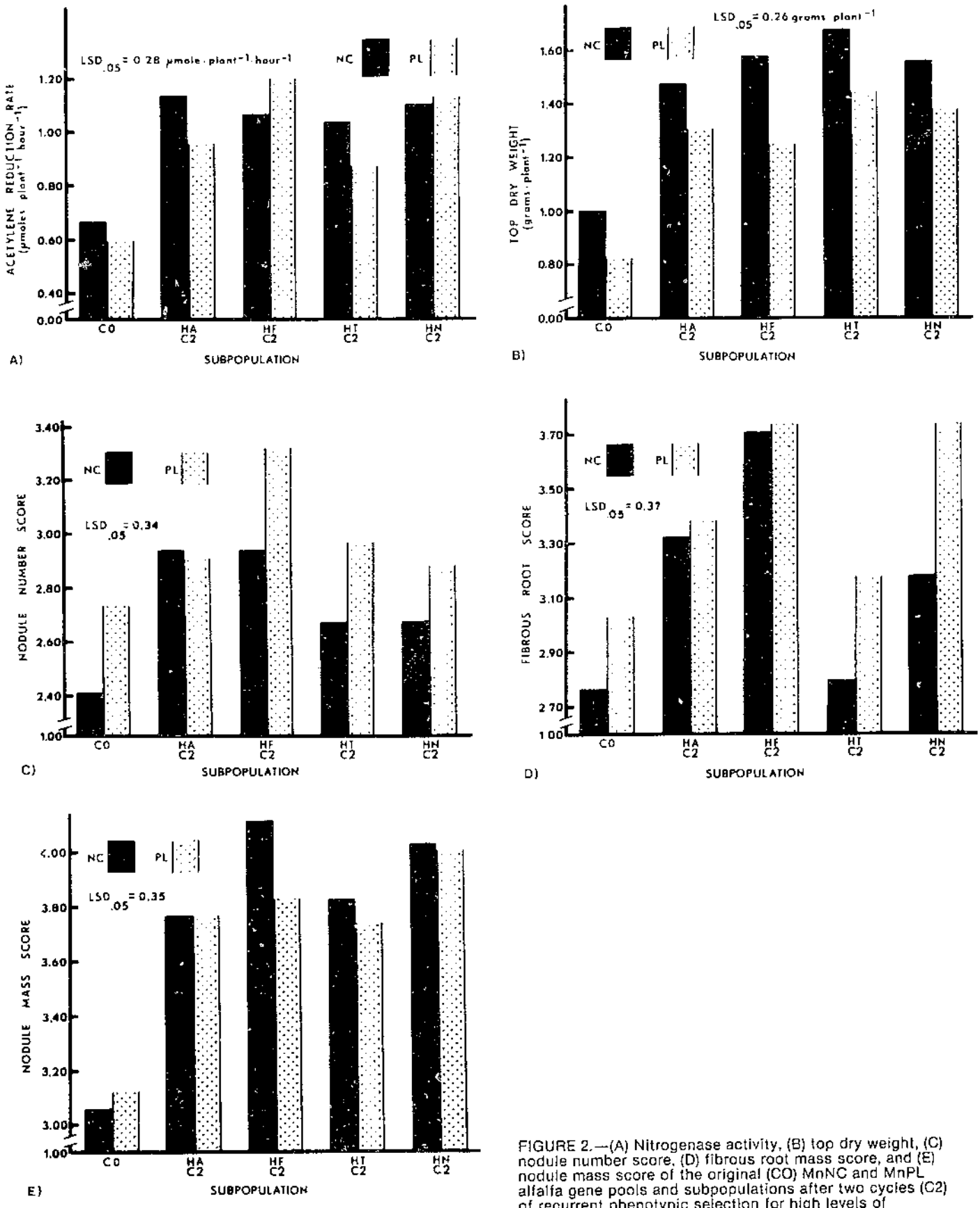

FIGURE 2.—(A) Nitrogenase activity, (B) top dry weight, (C) nodule number score, (D) fibrous root mass score, and (E) nodule mass score of the original (CO) MnNC and MnPL alfalfa gene pools and subpopulations after two cycles (C2) of recurrent phenotypic selection for high levels of nitrogenase activity per plant (HA), fibrous root mass score (HF), top dry weight (HT), and nodule mass score (HN) in each of the two gene pools (MnNC-MnPL experiment).

TABLE 3.—Effect of 2 cycles of bidirectional selection for 5 characteristics in the MnNC and MnPL alfalfa gene pools

	Realized heritability			
	MnNC Selection cycle[1]		MnPL Selection cycle[1]	
Characteristic	1	2	1	2
High nitrogenase activity	0.25	0.25	0.01	0.06
Low nitrogenase activity	.29	.16	.08	.10
High top dry weight	.21	.32	.14	.24
Low top dry weight	.06	.22	.00	.28
High nodule mass score	.29	.35	.17	.45
Low nodule mass score	.05	.27	—	—
High fibrous root score	.29	.75	.27	.62
Low fibrous root score	.02	.33	.26	.30
High Base Index A	.28	—	.22	—
Low Base Index A	.17	—	.12	—

[1] The MnNC experiment was evaluated in May 1978, and the MnPL experiment was evaluated in September 1978.

low except for the second cycle of selection for high-nodule mass score and for high-fibrous root mass score (table 3). In the second cycle of selection, heritabilities were ranked from largest to smallest: fibrous root mass score, nodule mass score, top dry weight, and NA. This was the same ranking as in the MnNC gene pool. The pattern of higher heritabilites for the high selections, however, as compared to the low selections observed in MnNC was not as apparent in the MnPL gene pool. Heritabilities were larger for high levels than for low levels only in the first cycle of selection for top dry weight, for Base Index A, and in the second cycle of selection for fibrous root mass score.

The heritabilities in MnNC were larger than those in MnPL in 13 of 16 comparisons (table 3). Differences in heritabilities between the pools, however, were small, except for those involving NA and the first cycle of selection for low-fibrous root mass score. Those differences could be due to either genetic differences (genetic mechanisms and gene frequencies) between the gene pools or to environmental differences in which selection and evaluation were made. In both gene pools, realized heritabilities were larger in the second cycle of selection for high-fibrous root mass score than for any other characteristic (table 3). These high heritabilities reflected the large responses to selection for high-fibrous root mass score and suggested relatively simple genetic control for this characteristic. The higher heritabilities in the second cycle compared to the first cycle of selection for both nodule mass score and fibrous root mass score could have been due to scoring by different persons. The person scoring the original pools from which parents were selected generally scored higher for these two characteristics than the person scoring all other cycles of selection and the final evaluation experiments. The low heritabilities for NA in the MnPL pool were reflective of the small responses observed.

TABLE 4.—Effect of 2 cycles of bidirectional selection in the MnNC alfalfa gene pools on 5 characteristics[1]

		Characteristics associated with N_2-fixation					
Subpopulation per gene pool	Selection trait and cycle	Top dry weight (g/plant)	Acetylene reduction rate (μmoles C_2H_4/plant/hr)	Nodule mass (score[2])	Nodule number (score[2])	Fibrous root (score[2])	Secondary root (score[2])
	Top dry weight[3]						
MnNC-6-HT	2	1.45	1.94	3.84	2.91	3.07	3.83
MnNC-5-HT	1	1.19	1.55	3.56	2.76	2.84	3.51
MnNC-4	0	.88	1.24	3.22	2.52	2.72	3.16
MnNC-5-LT	1	.82	1.18	3.29	2.56	2.83	3.21
MnNC-6-LT	2	.64	.75	2.83	2.26	2.51	2.67
$LSD_{.05}$		.15	.27	.24	.20	.27	.33
$LSD_{.01}$		.19	.36	.31	.27	.36	.44
	Nodule mass score[4]						
MnNC-6-HN	2	1.25	1.86	3.91	2.73	3.29	3.97
MnNC-5-HN	1	1.14	1.49	3.61	2.76	2.84	3.54
MnNC-4	0	.88	1.24	3.22	2.52	2.72	3.16
MnNC-5-LN	1	.98	1.13	3.16	2.46	2.61	3.10
MnNC-6-LN	2	.89	.96	2.90	2.40	2.34	3.04
$LSD_{.05}$		.15	.27	.24	.20	.27	.33
$LSD_{.01}$		.19	.36	.31	.27	.36	.44
	Fibrous root mass score[5]						
MnNC-6-HF	2	1.11	1.97	3.81	3.06	3.76	3.93
MnNC-5-HF	1	1.18	1.62	3.59	2.81	3.09	3.70
MnNC-4	0	.88	1.24	3.22	2.52	2.72	3.16
MnNC-5LF	1	.97	1.17	3.29	2.60	2.70	3.47
MnNC-6-LF	2	.81	.94	3.01	2.40	2.40	2.90
$LSD_{.05}$		.15	.27	.24	.20	.27	.33
$LSD_{.01}$		.19	.36	.31	.27	.36	.44
	Nitrogenase activity per plant[6]						
MnNC-6-HA	2	1.33	2.06	3.94	3.07	3.33	4.11
MnNC-5-HA	1	1.14	1.60	3.64	2.77	3.01	3.67
MnNC-4	0	.88	1.24	3.22	2.52	2.72	3.16
MnNC-5-LA	1	.94	1.02	3.17	2.56	2.51	3.33
MnNC-6-LA	2	.80	.85	3.13	2.49	2.64	2.73
$LSD_{.05}$		.15	.27	.24	.20	.27	.33
$LSD_{.01}$		.19	.36	.31	.27	.36	.44

[1] The MnNC experiment was evaluated in May 1978.

[2] Root morphological characteristics were scored 1 (least amount) to 5 (greatest amount).

[3] HT = subpopulation selected for high levels of top dry weight.
LT = subpopulation selected for low levels of top dry weight.

[4] HN = subpopulation selected for high nodule mass.
LN = subpopulation selected for low nodule mass.

[5] HF = subpopulation selected for high fibrous root mass.
LF = subpopulation selected for low fibrous root mass.

[6] HA = subpopulation selected for high nitrogenase activity.
LA = subpopulation selected for low nitrogenase activity.

In one study, a heritability of 0.78 was reported for NA in a non-winter-hardy alfalfa population (5). The heritabilities for NA in our experiments were much lower, but we computed our values with a different procedure. Their computation for heritability was (progeny mean ÷ original population mean) ÷ (selected parental mean ÷ original population mean). Our heritabilities were computed as (progeny mean − original population mean) ÷ (selected parental mean − original population mean). In both studies the original population was tested simultaneously with the progenies. Had they computed their heritability by the method we used, they would have obtained a value of 0.25. This is the same heritability we obtained for the high NA subpopulation in the MnNC gene pool (table 3). Another difference between their research and ours was that they evaluated seedling growth, whereas we evaluated the first regrowth after the seedling growth.

Correlated Characteristics—Developing subpopulations by individual selection programs provided us a unique opportunity to study the interrelationships among morphological and physiological characteristics associated with N_2-fixation. Two cycles of selection for top dry weight in the MnNC gene pool significantly altered NA, nodule mass score, nodule number score, and secondary root number score (table 4). The associated traits responded in the same direction as the changes in top dry weights. Fibrous root mass score was altered significantly by selection for high top dry weight but not by selection for low top dry weight. In the MnPL gene pool (table 5), selection for either high or low top dry weight did not significantly change NA, nodule number score, and fibrous root mass score. Nodule mass score and secondary root number score were affected significantly after two cycles of selection for high and low top dry weights (table 5).

Two cycles of selection for high nodule mass score significantly increased all characteristics in both pools except nodule number score in MnPL (table 5). Selection for low nodule mass score significantly decreased NA and fibrous root mass score in both pools. Nodule number score and secondary root number score were lower numerically in both pools after two cycles of selection for low nodule mass score. Top dry weights were not affected by selection for low nodule mass score in either gene pool.

Two cycles of selection for high-fibrous root mass score significantly increased all six characteristics in both gene pools (tables 4 and 5). Selection for low-fibrous root mass score significantly lowered NA in both pools and nodule mass score and nodule number score in MnPL. Again, top dry weight responded least of all characteristics to selection for fibrous root mass score in both pools. After the first cycle of selection for low-fibrous root mass score, top dry weight was higher numerically, while levels of all other characteristics were lower than the original pools.

Two cycles of selection for high NA significantly increased all the other characteristics in the MnNC pool (table 4). Selection for low NA decreased the other

TABLE 5.—Effect of 2 cycles of bidirectional selection in the MnPL alfalfa gene pools on 5 characteristics[1]

Subpopulation per gene pool	Selection trait and cycle	Characteristics associated with N_2-fixation: Top dry weight (g/plant)	Acetylene reduction rate (μmoles C_2H_2/plant/hr)	Nodule mass (score[2])	Nodule number (score[2])	Fibrous root (score[2])	Secondary root (score[2])
	Top dry weight[3]						
MnPL-9-HT	2	1.63	0.64	3.93	2.81	3.16	3.74
MnPL-8-HT	1	1.38	.55	3.76	2.69	3.00	3.41
MnPL-6	0	1.09	.58	3.67	2.87	3.15	3.22
MnPL-8-LT	1	1.14	.51	3.71	2.76	3.04	3.43
MnPL-9-LT	2	.95	.50	3.43	2.74	2.84	2.81
$LSD_{.05}$		.18	.14	.25	.25	.28	.34
$LSD_{.01}$		.23	.18	.33	.33	.38	.45
	Nodule mass score[4]						
MnPL-9-HN	2	1.66	.85	4.26	2.97	3.64	3.96
MnPL-8-HN	1	1.28	.78	3.91	2.86	3.16	3.51
MnPL-6	0	1.09	.58	3.67	2.87	3.15	3.22
MnPL-8-LN	1	1.23	.62	3.89	3.00	3.29	3.33
MnPL-9-LN	2	1.08	.37	3.26	2.74	2.74	3.11
$LSD_{.05}$		.18	.14	.25	.25	.28	.34
$LSD_{.01}$		.23	.18	.33	.33	.38	.45
	Fibrous root mass score[5]						
MnPL-9-HF	2	1.39	.84	4.31	3.39	4.01	3.79
MnPL-8-HF	1	1.16	.69	4.03	2.97	3.54	3.56
MnPL-6	0	1.09	.58	3.67	2.87	3.15	3.22
MnPL-8-LF	1	1.34	.51	3.46	2.70	2.79	3.24
MnPL-9-LF	2	1.13	.41	3.24	2.53	2.47	3.14
$LSD_{.05}$		.18	.14	.25	.25	.28	.34
$LSD_{.01}$		.23	.18	.33	.33	.38	.45
	Nitrogenase activity per plant[6]						
MnPL-9-HA	2	1.45	.70	4.07	2.94	3.21	3.76
MnPL-8-HA	1	1.40	.61	3.79	2.86	3.07	3.46
MnPL-6	0	1.09	.58	3.67	2.87	3.15	3.22
MnPL-8-LA	1	1.13	.45	3.54	2.60	2.86	3.26
MnPL-9-LA	2	1.17	.36	3.21	2.66	2.56	2.85
$LSD_{.05}$		.18	.14	.25	.25	.28	.34
$LSD_{.01}$		.23	.18	.33	.33	.38	.45

[1] The MnPL experiment was evaluated in September 1978.
[2] Root morphological characteristics were scored 1 (least amount) to 5 greatest amount).
[3] HT = subpopulation selected for high levels of top dry weight.
LT = subpopulation selected for low levels of top dry weight.
[4] HN = subpopulation selected for high nodule mass.
LN = subpopulation selected for low nodule mass.
[5] HF = subpopulation selected for high fibrous root mass.
LF = subpopulation selected for low fibrous root mass.
[6] HA = subpopulation selected for high nitrogenase activity.
LA = subpopulation selected for low nitrogenase activity.

characteristics, but only secondary root score decreased significantly. In MnPL, two cycles of selection for high NA significantly increased top dry weight, nodule mass score, and secondary root number score (table 5). Selection for low NA in MnPL significantly decreased nodule mass score, fibrous root mass score, and secondary root number score. Top dry weight did not decrease after selection for low NA.

Responses to selection shown in tables 4 and 5 suggested that all six characteristics were associated positively with each other. These associations were confirmed by the positive correlation coefficients of the characteristics in all combinations when computed on individual plant data in the MnNC experiment and in the MnPL experiment (table 6). Researchers reported positive correlations among these same characteristics but in different alfalfa populations (*5, 34*). Partial correlation efficients were computed to determine more direct relationships among the characteristics in MnNC and MnPL. The significant responses of associated characteristics in tables 4 and 5 were usually a reflection of higher partial correlations compared to characteristics with uncorrelated responses (table 6).

Improving N_2-Fixation By Selecting For Individual Morphological Characteristics—Because the acetylene reduction assay for NA requires more labor and equipment than determinations of the other characteristics, selecting for a correlated morphological characteristic instead of NA would be desirable when improving N_2-fixation. Data obtained from the MnNC-MnPL experiment (fig. 2) were useful in evaluating the possibility of substituting selection for a morphological characteristic for the acetylene reduction assay of NA. In this experiment, both gene pools were evaluated simultaneously under nonlimiting environmental conditions. Responses to selection for NA were large in both pools. Contrary to the low response to selection for high NA when evaluated in the MnPL experiment (fig. 1D), response in the same MnPL subpopulation was 61 percent when evaluated in the MnNC-MnPL experiment (fig. 2A).

The NA values for subpopulations after two cycles of selection for high levels of NA, fibrous root mass score, top dry weight, or nodule mass score in each gene pool are presented in figure 2A. Consistent with the MnNC experiment (table 4), the MnNC-MnPL experiment (table 5) showed that selection for each of the characteristics increased NA over the original population about equally in the MnNC gene pool (fig. 2A). In the MnPL pool, selection for each of the four characteristics increased NA over the original population. The selection for fibrous root mass score and nodule mass score, the characteristics most highly correlated with NA (table 6), increased NA the most.

Top dry weights of subpopulations within gene pools in the MnNC-MnPL experiment were similar after two cycles of selection for each of the four characteristics (fig. 2B). In both pools, the best numerical, though nonsignificant, improvement in top dry weight was from the selection for top weight, as such, but it also resulted in the lowest NA in MnPL (fig. 2A). The MnNC subpopulations all had higher top dry weights (not all significantly) than those of MnPL (fig. 2B), but no significant differences were shown in NA between the two gene pools after two cycles of selection (fig. 2A). These data and the results in tables 4 to 6 indicated a substantial degree of independence between top weights and NA and demonstrated that selection based on top weights does not always improve NA.

The nodule number scores of subpopulations within gene pools, after two cycles of selection for each of the four characteristics, are presented in figure 2C. In the MnNC gene pool, selection both for high fibrous root score and for NA increased nodule number score. In the MnPL gene pool, only selection for high fibrous root score significantly increased nodule number score. In both pools, selection for the one or two characteristics having the highest partial correlations with nodule number score increased nodule number significantly (table 6). All other subpopulations were similar to the original populations. The nodule mass scores of all selected subpopulations for both gene pools were greater significantly than the original populations (fig. 2E).

The fibrous root scores of subpopulations, selected for high levels of NA, fibrous root score, and nodule mass score, were greater significantly than those of the original populations in both gene pools (fig. 2D). Selection for increased top weight, which has a negative partial correlation with fibrous root score (table 6), did not change the fibrous root score in either the MnNC or MnPL populations. Apparently, an increase in fibrous root mass score provided

TABLE 6.—Correlation coefficient[1] matrices of 6 characteristics

Characteristic	Top dry weight		Nodule mass score		Nodule number score		Fibrous root score		Secondary root score	
	Simple	Partial	Simple	Partial	Simple	Partial	Simple	Partial	Simple	Partial
Computed on 1,949 individual plants in the MnNC experiment										
Nitrogenase activity per plant	0.51	0.23	0.67	0.35	0.44	0.19	0.44	0.15	0.40	0.00
Top dry weight			.57	.30	.22	−.06	.22	−.18	.55	.38
Nodule mass score					.44	.15	.49	.24	.49	.14
Nodule number score							.44	.25	.26	.02
Fibrous root score									.36	.19
Computed on 1,942 individual plants in the MnPL experiment										
Nitrogenase activity per plant	.28	.14	.54	.29	.34	.01	.50	.18	.24	−.12
Top dry weight			.41	.26	.11	−.02	.16	−.22	.47	.38
Nodule mass score					.49	.12	.68	.45	.49	.20
Nodule number score							.61	.42	.26	.01
Fibrous root score									.40	.15

[1] Simple and partial correlation coefficients at the 0.01 significance level = ±0.06.

greater numbers of potential nodule sites. Conversely, the selection for factors directly involved with N_2-fixation, such as NA and nodule mass score, increased fibrous root mass score.

Responses to selection did not indicate the best substitute for measuring NA. Coefficients of determination were computed by stepwise regression analysis to determine how much of the variation from NA was attributable to each of the morphological characteristics. We computed the coefficients of determination from the MnNC experiment and the MnPL experiment because of the large plant numbers and because of the large variability from both the high and low subpopulations compared to the MnNC-MnPL experiment. In both gene pools, nodule mass score accounted for much more of the variation from NA than any other characteristic (table 7). The remaining characteristics accounted for, at most, a total of 8 percent additional variation after nodule mass score was taken into account. The partial correlations support the regression analyses concerning the degrees of relationship between NA and the four associated characteristics (table 6).

These results suggested that nodule mass score would be the best morphological characteristic in a selection program to improve N_2-fixation; however, 50 percent or more of the variation from NA in the two pools was unexplained by the characteristics observed (table 7). The NA of the nodules also would be an important selection criterion for improving N_2-fixation.

TABLE 7.—Variation in NA associated with each of 5 morphological characteristics in the MnNC and MnPL alfalfa gene pools[1]

	Percentage of variation of NA per gene pool	
	MnNC	MnPL
Nodule mass score	42	31
Nodule mass squared	1	0
Nodule number score	3	0
Top dry weight	3	1
Fibrous root score	1	2
Secondary root score	0	1
Proportion unexplained	50	65

[1] Computed by stepwise regression in the MnNC experiment and the MnPL experiment, respectively.

Improving N_2-Fixation By Multiple Characteristic Selection— We conducted one cycle of selection for Base Index A (included all characteristics except NA) to determine if multiple trait selection would alter NA more than individual trait selection. When evaluated in the MnNC experiment and in the MnPL experiment, the subpopulations selected for high Base Index A (not evaluated in the MnNC-MnPL experiment) showed no advantage over one cycle of individual trait selection in the high direction in both pools (tables 2, 4 and 5). NA was lower significantly after selection for low Base Index A than after one cycle of selection for low levels of the other individual characteristics in MnNC (tables 2, 4, and 5). Selection for Base Index A also affected most of the other characteristics, but only top dry weights and secondary root number scores were altered significantly in the high direction in both pools. Nodule mass score was changed significantly in the high direction in MnNC (table 2).

After one cycle of selection for Base Index B, which included NA values, the HB-B (1 pct) and HB-B (20 pct) subpopulations in each gene pool significantly were higher for most characteristics than their respective original pools (fig. 3A-E). The HB-B (1 pct) subpopulations were expected to be higher than the HB-B (20 pct) subpopulations because of the higher selection intensities. We intended to evaluate both the Syn. 1 and Syn. 2 generations of both the HB-B (1 pct) and HB-B (20 pct) subpopulations in each gene pool. Unfortunately, we had insufficient seed of the Syn. 1 generation of the MnNC HB-B (20 pct) subpopulation to test.

In the Syn. 2 generation of the MnNC subpopulations, HB-B (1 pct) performed better than did HB-B (20 pct) for NA, fibrous root mass score, and nodule mass score (fig. 3A, D, and E). In the MnPL gene pool, HB-B (1 pct) performed similar to HB-B (20 pct) in the Syn. 1 generation for all characteristics. The Syn. 2 generation of HB-B (1 pct) had higher nodule mass score than Syn. 2 of HB-B (20 pct), however, because of a decrease in nodule mass from the Syn. 1 to the Syn. 2 generation of the latter (fig. 3E).

In 11 of 15 comparisons, the levels of characteristics decreased (not all significantly) in the Syn. 2 generation compared to the Syn. 1 generation (fig. 3A-E). We observed significant decreases for top dry weights in MnNC HB-B (20 pct) and in MnPL HB-B (1pct) (fig. 3B) and for nodule mass score in MnPL HB-B (20 pct) (fig. 3E). Because entries were randomized within each block, most of the differences between entries should have been genetic. Therefore, these significant decreases in parameter values and those that were statistically nonsignificant suggested that the characteristics were influenced by nonadditive genetic effects, especially for top dry weight and nodule mass score.

The check cultivar 'Saranac' performed better than the cultivar 'Agate' for all the characteristics (fig. 3). 'Agate' performed similarly to the original gene pools for the various characteristics while 'Saranac' performed similarly to the subpopulations after one cycle of selection for Base Index B.

Percent And Total N Of Plant Tops—Selection in the MnNC experiment did not appear to change the N concentration in plant tops (table 8). We observed a few statistically significant differences, but we do not believe that biologically these were significant because in no case after two cycles of selection were the differences between the high and low populations significant. We also did not observe any significant differences in N concentration in the MnNC-MnPL experiment (table 9)

Total N in plant tops of the MnNC subpopulations was changed in the same direction as was each of the characteristics for which selection was done (table 8). Because little variation for N concentration existed among entries, top dry weight was the primary component that altered total N. Changes in total N in the MnNC subpopulations were consistent in both the MnNC experiment and the MnNC-MnPL experiment (tables 8 and 9).

After two cycles of selection for high levels of the four characteristics in MnPL, we observed no alterations for N concentration in plant tops when evaluated in the MnNC-MnPL experiment (table 9). Like the MnNC pool, the MnPL subpopulations increased in total N in plant tops because of increases in top dry weights. Single-degree-of-freedom comparisons indicated that N concentration and total N in plant

tops were higher in the MnNC subpopulations collectively than in the MnPL subpopulations collectively (table 9).

Plant tops of the HB-B (20 pct) and HB-B (1 pct) subpopulations in both gene pools had about equal N concentration and higher total N than those of the original gene pools (table 10). Only the Syn. 2 generation of HB-B (20 pct) in MnNC was not different significantly from the original pool for total N in plant tops. The Syn. 2 generation of the subpopulations in both pools had significantly less total N in plant tops than the Syn. 1 generation because the Syn. 2 generation had lower yields. The higher yield of total N in plant tops of 'Saranac' than for 'Agate' was due to greater yield in 'Saranac' because N concentrations were similar in both cultivars (table 10).

Responses to Selection: Field Investigations

N_2-Fixation in The Seeding Year—N_2-fixation of seeding year stands of MnNC-5, MnPL-8, and

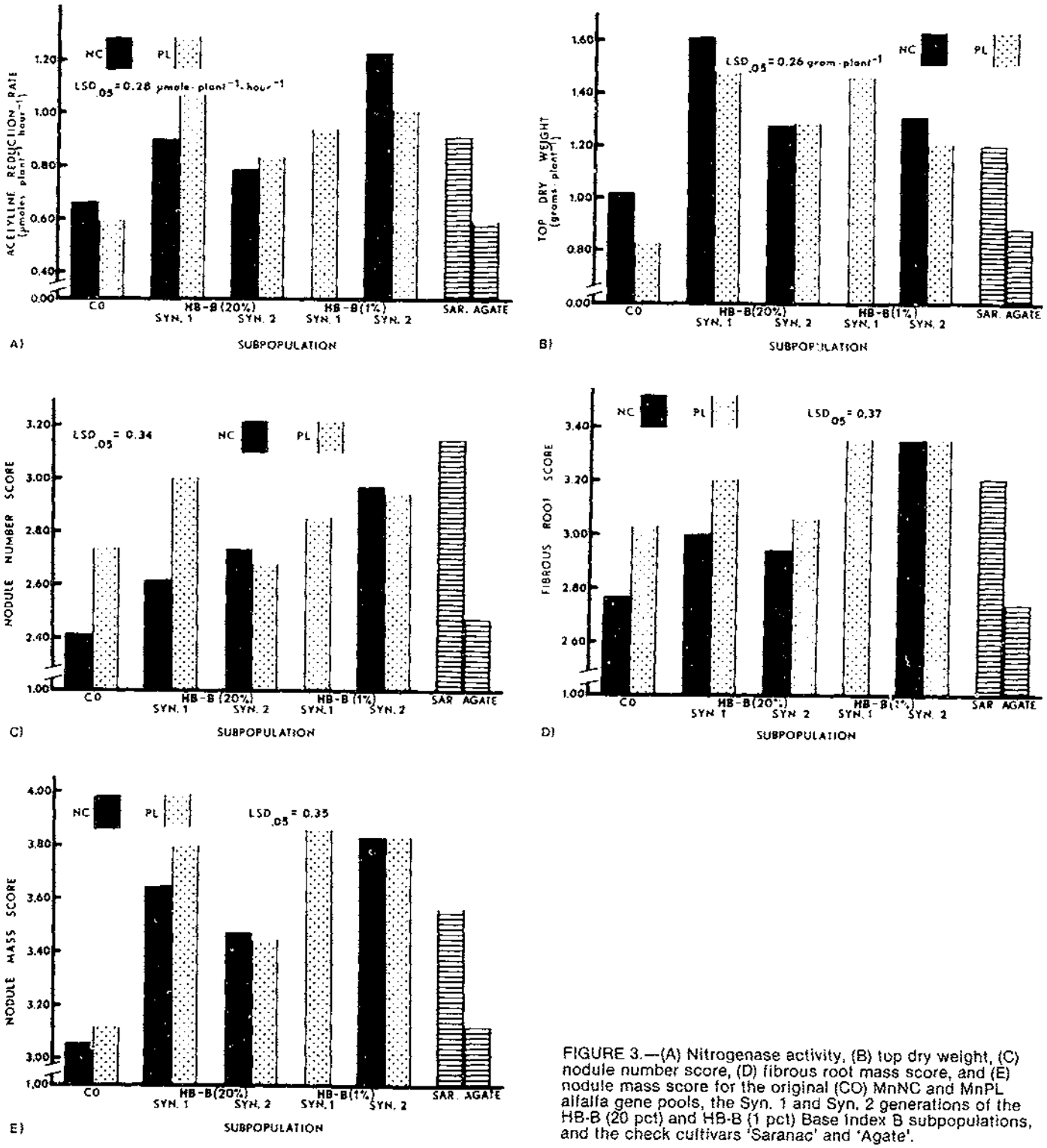

FIGURE 3.—(A) Nitrogenase activity, (B) top dry weight, (C) nodule number score, (D) fibrous root mass score, and (E) nodule mass score for the original (CO) MnNC and MnPL alfalfa gene pools, the Syn. 1 and Syn. 2 generations of the HB-B (20 pct) and HB-B (1 pct) Base Index B subpopulations, and the check cultivars 'Saranac' and 'Agate'.

TABLE 8.—Nitrogen in plant tops of original MnNC alfalfa gene pool and of subpopulations resulting from 2 cycles of bidirectional selection for 5 characteristics (MnNC experiment)

Type of subpopulation	Selection cycle	Selected characteristic: Nitrogenase activity	Top dry weight	Nodule mass score	Fibrous root mass score	Base Index A
		Percent				
High	2	2.98	2.75	2.71	2.95	—
High	1	2.91	2.86	2.80	2.88	2.84
Original	0	2.95	2.95	2.95	2.95	2.95
Low	1	2.89	2.83	2.85	2.88	2.82
Low	2	2.74	2.84	2.88	2.79	—
[1]$LSD_{.05}$				0.17		
[1]$LSD_{.01}$				0.23		
		Total N (mg/plant)				
High	2	38.0	39.6	33.7	32.8	—
High	1	33.2	33.8	31.7	33.9	33.3
Original	0	26.0	26.0	26.0	26.0	26.0
Low	1	27.2	23.0	27.9	27.7	22.0
Low	2	21.7	18.8	25.2	22.4	—
[2]$LSD_{.05}$				4.9		
[2]$LSD_{.01}$				6.4		

[1] LSD's may be used to compare any 2 N concentration values in the table.
[2] LSD's may be used to compare any 2 total N values in the table.

TABLE 9.—Nitrogen concentration and total N in plant tops of the original MnNC and MnPL alfalfa gene pools and of subpopulations resulting from 2 selection cycles for high levels of 4 characteristics (MnNC-MnPL experiment)

Subpopulation per gene pool[1]	Selection cycle	N concentration (pct)	Total N (mg/plant)
MnNC-4	0	2.74	28.3
MnNC-6-HA	2	2.86	42.3
MnNC-6-HT	2	2.68	44.5
MnNC-6-HN	2	2.75	42.7
MnNC-6-HF	2	2.78	43.6
Mean		2.76	40.3
MnPL-6	0	2.58	21.4
MnPL-9-HA	2	2.68	34.7
MnPL-9-HT	2	2.69	38.4
MnPL-9-HN	2	2.65	36.2
MnPL-9-HF	2	2.61	31.9
Mean		2.64	32.5
$LSD_{.05}$		.22	7.8
$LSD_{.01}$		.29	10.3

[1] HA = subpopulation selected for high level of nitrogenase activity per plant.
HT = subpopulations selected for high levels of top dry weight.
HN = subpopulations selected for high levels of nodule mass.
HF = subpopulations selected for high levels of fibrous root mass.

'Saranac' measured either as N_f or N_{sy} increased from the first to the second or third harvest and then declined to the fourth harvest (table 11). On a seasonal basis, MnNC-5 and MnPL-8 significantly exceeded the N_f and N_{sy} of 'Saranac', but differences in N_2-fixation between MnNC-5 and MnPL-8 were not apparent. Harvest by entry interactions for N_f were significant at the third harvest. MnNC-5 and MnPL-8 averaged about 24 percent more N from N_{sy} and 35 percent more N fixed on an N_f than 'Saranac'. The three entries averaged about 41 percent of their total N needs from N_{sy} during the seeding year.

During the second or third harvest intervals, MnNC-5 derived nearly two-thirds of its total N from N_{sy}. In comparison, reports show that some soybeans obtain 39 to 66 percent of their seasonal N needs from N_{sy} (*4, 12, 21*). White clover (*T. repens* L.) and subterranean clover (*T. subterraneum* L.) derived more than 80 percent of their N budget from the atmosphere (*6, 33*). On an N_f, the three alfalfa entries in this study averaged about 13.5 g/m² (135 kg/ha) of fixed N over the growing season of the establishment year (table 11). This

TABLE 10.—Nitrogen concentration and total N in plant tops of original MnNC and MnPL alfalfa gene pools and of subpopulations resulting from 1 cycle of selection for Base Index B[1] (MnNC-MnPL experiment)

Subpopulation per gene pool	N concentration (pct)	Total N (mg/plant)
MnNC-4 (original pool)	2.74	28.3
MnNC-5 HB-B (20 pct) (Syn. 1)	2.59	41.5
MnNC-5 HB-B (20 pct) (Syn. 2)	2.65	33.8
MnNC-5 HB-B (1 pct) (Syn. 2)	2.75	36.5
MnPL-6 (original pool)	2.58	21.4
Mn-PL-8 HB-B (20 pct) (Syn. 1)	2.63	38.0
MnPL-8 HB-B (20 pct) (Syn. 2)	2.50	31.9
MnPL-8 HB-B (1 pct) (Syn. 1)	2.63	38.3
MnPL-8 HB-B (1 pct) (Syn. 2)	2.54	30.7
'Agate'	2.82	24.6
'Saranac'	2.74	33.2
$LSD_{.05}$	.22	7.8
$LSD_{.01}$	.29	10.3

[1] Base Index B consisted of nitrogenase activity per plant, top dry weight, nodule mass score, fibrous root score, and secondary root score, each weighted as described in the text.

TABLE 11.—Nitrogen-fixation of 3 alfalfa cultivars measured by the isotope dilution technique at Rosemount, Minn.

Harvest	'SARANAC' N_f[1]	'SARANAC' N_w[2]	MnNC-5-HB-A N_f	MnNC-5-HB-A N_w	MnPL-8-HB-A N_f	MnPL-8-HB-A N_w
	g/m²	*Percent*	*g/m²*	*Percent*	*g/m²*	*Percent*
1 (7-1-77)	0.4 ± 0.2	11.1 ± 5.1	2.0 ± 0.6	29.4 ± 8.2	1.3 ± 0.3	25.6 ± 6.1
2 (8-2-77)	3.8 ± 0.4	54.8 ± 5.0	4.8 ± 0.5	63.3 ± 7.1	5.7 ± 0.3	64.8 ± 3.2
3 (9-14-77)	5.4 ± 0.7	52.1 ± 6.8	8.7 ± 0.4	66.8 ± 3.1	4.7 ± 0.4	58.5 ± 5.2
4 (10-17-77)	1.3 ± 0.5	24.2 ± 10.0	1.5 ± 0.2	18.8 ± 11.7	.8 ± 0.4	20.1 ± 10.9
Total N_f (g/m²) or mean N_w (pct)	10.9	35.5 ± 10.7	17.0	44.6 ± 12.0	12.5	42.3 ± 11.3

[1] Symbiotic nitrogen fixation expressed on a land area basis.
[2] Proportion of nitrogen in the total plant derived from symbiosis.

rate of N_2-fixation is similar to that measured for subterranean (*33*) and white clovers (*6*) by isotope techniques but 30 to 50 percent more than most values measured on soybeans (*4, 12, 21*).

Comparisons Among Entries—N_2-fixation of field communities of MnNC-5 and MnPL-8 differed from that of 'Saranac' but not from one another (table 11). Because of the significant entry by harvest interactions and the 37-percent difference in plant population among the entries, we made further comparisons for individual plants.

N_2-fixation per plant varies with N_{dfa} and with the total N yield of the plant. Total N yield varies with the average N concentration of the plant and the total plant yield. Over the four harvests, the total dry matter yield of MnPL-8 was less significantly than that of MnNC-5, and, in turn, the yields of MnNC-5 and MnPL-8 were less than that of 'Saranac'. This was also the trend observed in the greenhouse studies. A significant entry by harvest interaction was evident for total yield of the three entries (fig. 4). Herbage yields of the three entries were similar at the first two harvests, and only at the third harvest was the herbage yield of 'Saranac' significantly greater than that of MnPL-8 or MnNC-5 (data not shown). The similar herbage yields of MnNC-5 and MnPL-8 are consistent with earlier greenhouse results (fig. 2) and suggest that the differences in dry matter yield shown in figure 4 were attributable to contrasting root growth of the two entries.

The N concentration in whole plants of the three entries declined significantly throughout the season (fig. 5), but no differences were found among entries in tissue N concentration. Nevertheless, the product of total dry matter yield and N concentration gave the expected significant difference in total N per plant among the three entries.

Combining total dry matter yield (fig. 4), N concentration (fig. 5), and N_{dfa} values (table 11) for the three entries allowed comparisons among rates of growth (GR, 10^3 mg dry weight/plant/day) and symbiotic N_2-fixation (SNF, mg N/plant/day) averaged over each of the four seeding year harvests (figs. 6, 7, and 8). For all entries, patterns of GR and SNF were similar throughout the season.

In the first harvest intervals, GR and SNF were low, but they increased several fold through the second or third harvest. Because the onset of dormancy occurred more quickly in MnPL-8 than in either MnNC-5 or 'Saranac', the latter two entries continued to grow and to fix N until later in the season. For this reason, GR and SNF were greater significantly for 'Saranac' and MnNC-5 than for MnPL-8 for all harvest intervals except for the first and last. Similarly, GR and SNF of MnNC-5 significantly exceeded that of MnPL-8, except during the fourth harvest interval.

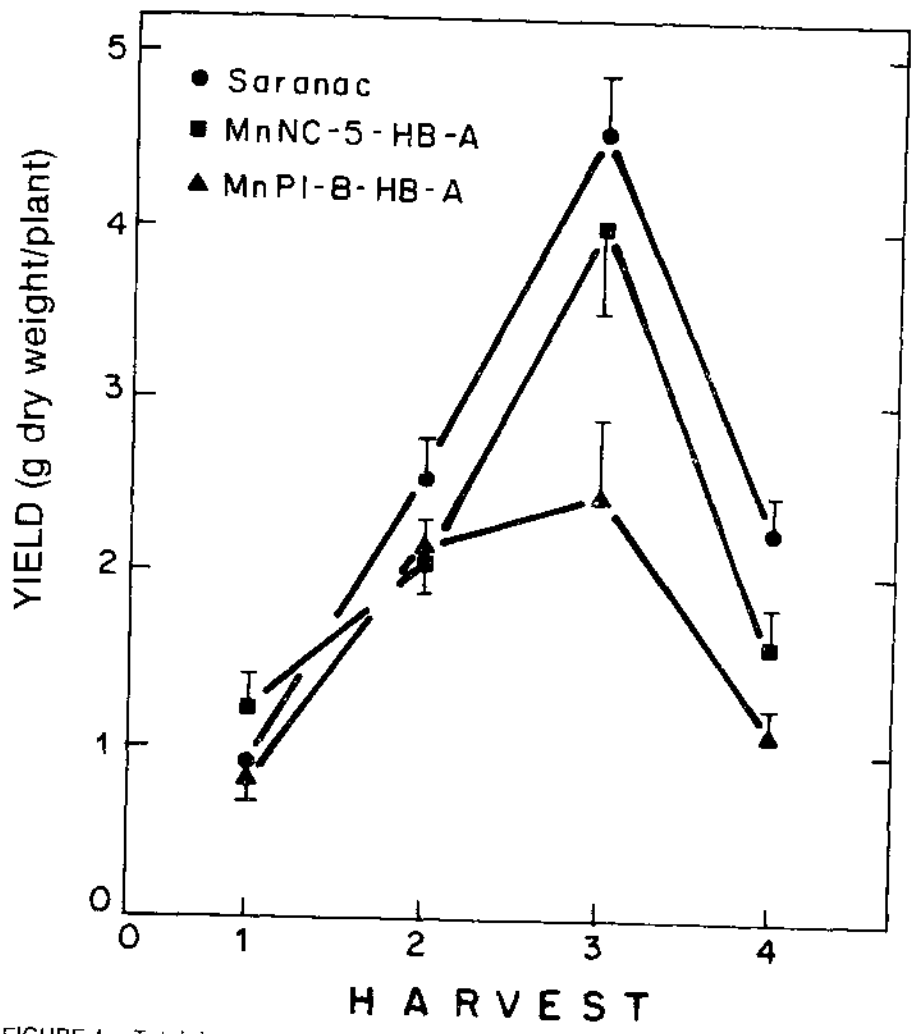

FIGURE 4.—Total dry matter yield per plant of three alfalfa populations established from seed and harvested at four intervals in the seeding year. Values are mean ± SE.

N_2-Fixation And Selection Intensity—The N_{dfa} calculated from herbage or from whole plant samples of the HB-B (20 pct) and HB-B (1 pct) subpopulations of MnNC-5 and MnPL-8 (table 12) showed the same seasonal progression as the alfalfa in other experiments (table 11 (*17*)). N_2-fixation was low in the first harvest interval, greatest in the second or third harvest interval, and least at the fourth harvest. Differences in N_{dfa} among harvests were significant, but absence of selection intensity by harvest interactions showed that the two subpopulations of each entry had similar N_2-fixation characteristics. This conclusion was reinforced by the observation that intensity of selection had no significant effect upon whole plant, dry-matter yields, herbage yields, or herbage N concentration (data not shown), in conformity with the results from the Syn. 1 generations of MnNC and MnPL from greenhouse experiments (fig. 3; table 10). Yields of herbage, whole plants, and the whole plant N concentrations, however, varied significantly with harvest.

Averaged over three harvests (herbage and whole plant samples) or four harvests (whole plant samples only), a consistent but

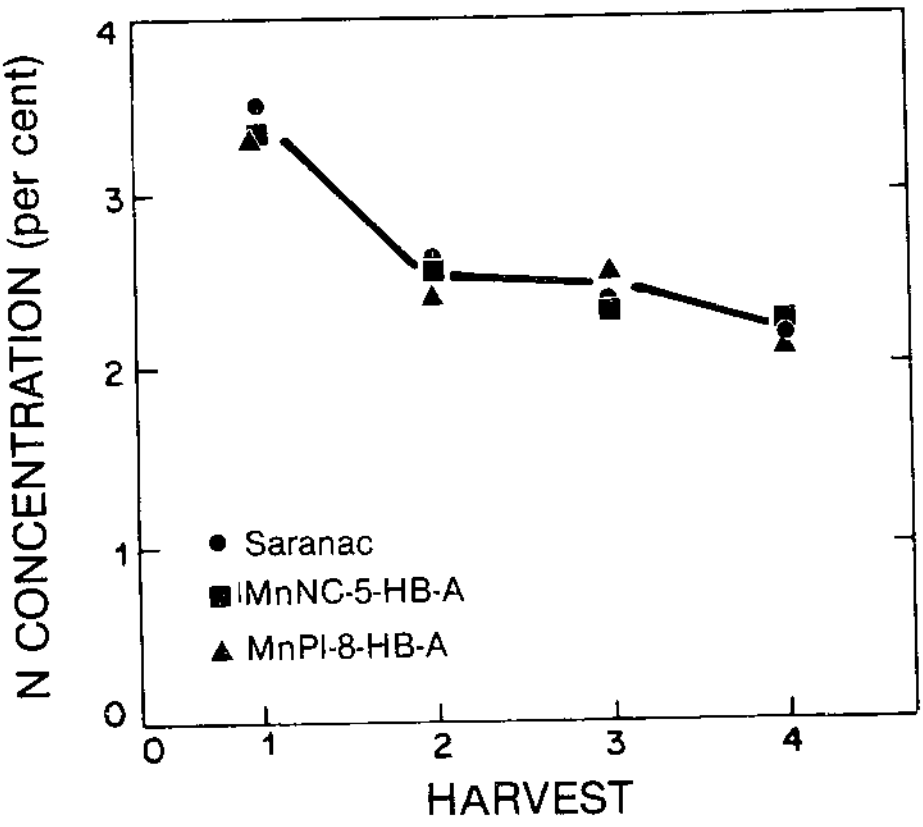

FIGURE 5.—Total plant N concentration for three alfalfa populations established from seed and harvested at four intervals in the seeding year. Standard errors are too small to indicate with mean values.

generally statistically insignificant tendency existed for HB-B (1 pct) subpopulations to have smaller N_{sy} values than the HB-B (20 pct) subpopulations for both entries. The only exception was when herbage samples of the HB-B (1 pct) subpopulation of MnNC-5 averaged significntly less N_{sy} than those of the HB-B (20 pct) subpopulation, which clearly was attributable to the differential performance at the first harvest (table 12). Imposing a higher intensity of selection was of no consequence, therefore, in improving either N_{sy} or the associated characters of yield and N concentration in field-grown plants after one cycle of recurrent selection.

Using Herbage To Measure N_{sy}.—Using herbage samples to assess N_{sy} would offer advantages to using whole plants because of ease of sampling and the nondestruction of whole plants of germplasm that may be in scarce supply. The N_{sy} of herbage and whole plant samples differed significantly among harvests for the HB-B (1 pct) and HB-B (20 pct) subpopulations of each entry (table 12), and the tissue source by harvest interaction was significant for the HB-B (1 pct) subpopulation of MnNC-5. This substantiates the view that in some plant tissues, differential partitioning of isotope within the plant may cause contrasting estimates of N_{sy} made from herbage or whole plant samples at a single growth stage (*17*). Comparisons of tissue sources over harvests showed that N_{sy} was greater significantly for herbage samples than for whole plant samples of the HB-B (1 pct) subpopulation of MnPL-8 (table 12) but not for any other selection intensity and entry combination. These results suggest that herbage samples will give results of N_{sy} often comparable to those of whole plants when herbage N_{sy} results are averaged over several harvests.

Greenhouse versus Field Performance—Although unselected MnNC-4 and MnPL-6 and the subsequent cycles of these entries selected for increased NA per plant were similar in performance at each stage of selection when tested in the greenhouse (figs. 2A and 3A), we found significant differences in N_2-fixation per plant between MnNC-5 and MnPL-8 in the field (fig. 6). The contrast between greenhouse and field performance may have occurred because the greenhouse selections were grown under a long photoperiod in a relatively narrow range of temperatures and were evaluated at one stage of growth. The field-grown plants responded to the continuously changing natural environment for 5 months and were sampled on several occasions.

The differences in dormancy between the entries clearly influenced the N_2-fixation of field-grown material (fig. 6), but not in the same material when grown in a noninductive environment in the

TABLE 12.—Nitrogen-fixation assessed from herbage and whole plant samples of subpopulations resulting from 2 levels of selection intensity in 2 alfalfa gene pools

Harvest	Selection intensity (pct)	Subpopulation: MnNC-5 tissue sample, Herbage	Subpopulation: MnNC-5 tissue sample, Plants	Subpopulation: MnPL-8 tissue sample, Herbage	Subpopulation: MnPL-8 tissue sample, Plants
		N_2 from symbiosis (pct)			
1	20	27.1 ± 7.1	20.4 ± 8.2	21.3 ± 4.7	20.5 ± 6.2
	1	11.7 ± 4.8	22.3 ± 8.8	30.6 ± 7.4	24.2 ± 2.6
2	20	70.4 ± 6.9	63.3 ± 7.1	76.9 ± 1.9	64.8 ± 3.2
	1	68.5 ± 5.9	54.9 ± 6.8	74.4 ± 5.0	62.5 ± 13.2
3	20	66.8 ± 8.3	66.8 ± 3.1	66.6 ± 6.3	57.4 ± 5.2
	1	64.4 ± 8.3	64.6 ± 5.5	55.3 ± 14.9	57.4 ± 6.4
4	20	—	18.8 ± 5.6	—	20.1 ± 6.0
	1	—	19.6 ± 5.8	—	18.1 ± 5.2
Mean (3 harvests)	20	54.8	53.2	54.9	48.0
	1	48.2	47.2	53.4	47.5
Mean (4 harvests)	20	—	44.6	—	40.7
	1	—	40.3	—	40.6

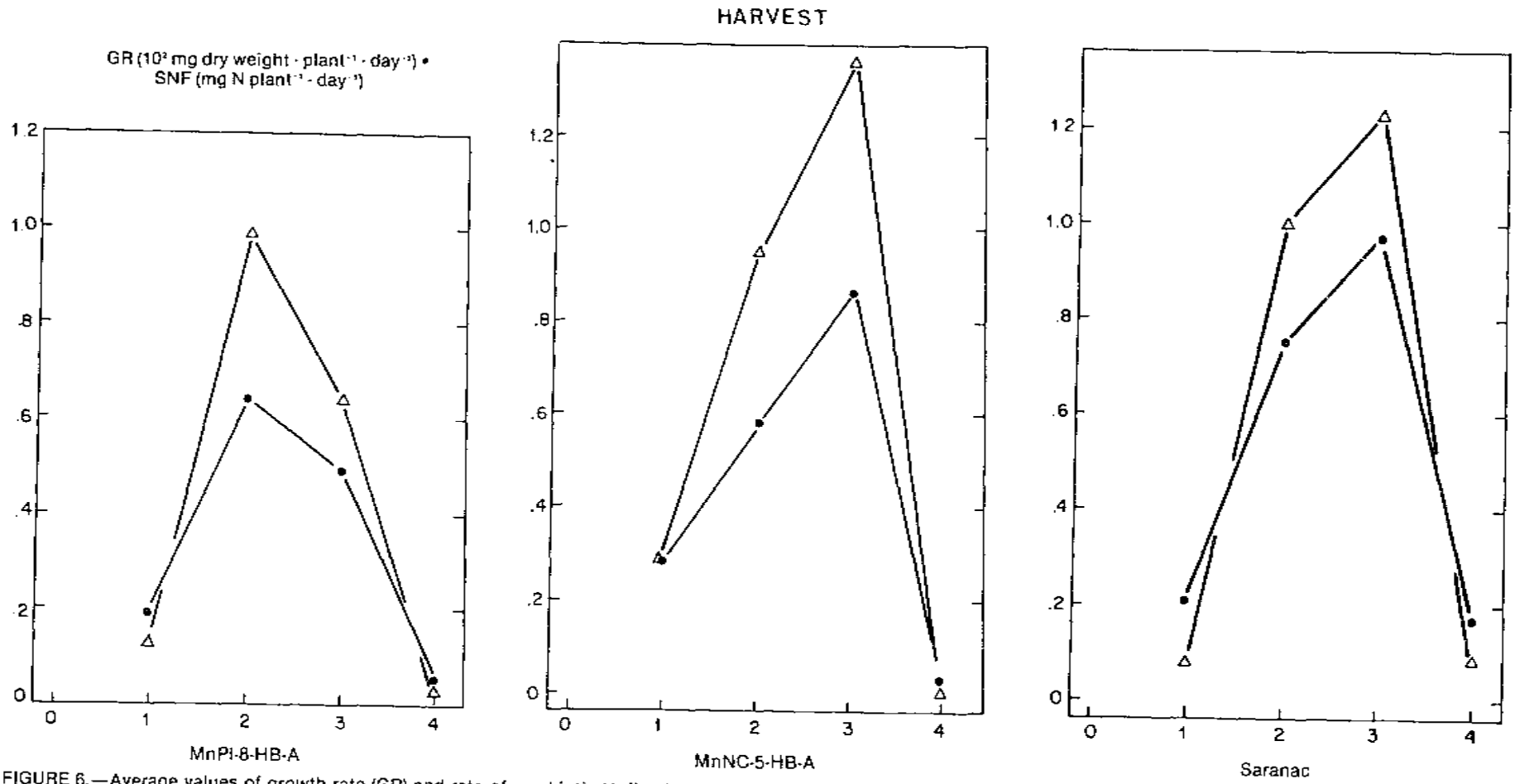

FIGURE 6.—Average values of growth rate (GR) and rate of symbiotic N_2-fixation (SNF) over each of four harvest intervals in the seeding year. Standard errors are too small to indicate with mean values.

greenhouse (figs. 2 and 3). This suggests caution in attempts to predict the field performance for N_2-fixation of alfalfas with varying dormancy responses from greenhouse tests under noninductive conditions. Although differences in dormancy response may be equally evident in the N_2-fixation of crop communities (table 11) and of individual plants (fig. 6), small differences in plant populations in crop communities may obscure differences in N_2-fixation that are apparent in individual plants. Thus, field evaluation of germplasm for N_2-fixation capability is an essential component of a selection program for N_2-fixation.

Concluding Discussion

Greenhouse experiments with the two diverse gene pools indicated that progress was made by breeding for NA and morphological characteristics associated with N_2-fixation. The linearity of responses over two cycles of selection suggested that progress could continue by further selection in these pools.

Because NA and the observed morphological characteristics were correlated in all combinations, selection for one characteristic generally affected all others. In the MnPL gene pool, top dry weight often did not respond like the other characteristics within the same subpopulation. Selection for large top dry weights in MnPL did not increase NA as much as selection for the root morphological characteristics. Therefore, we believe that selection for top weights alone will not result in the best improvement in N_2-fixation.

Selection for nodule mass score greatly increased NA in both gene pools. Because nodule mass score was related more directly to NA than were top dry weights or the other root morphological characteristics in both pools, we concluded that nodule mass score would be the most dependable morphological characteristic for selection to improve N_2-fixation. Because little variation from NA was explained by the remaining morphological characteristics after accounting for nodule mass, additional selection for the remaining characteristics would add very little to improve N_2-fixation. However, initial selection based on top growth would save digging many of the plants because top dry weights are associated with N_2-fixation and because a major breeding objective is to increase forage yield.

Based on these results, we recommend that large numbers of plants should be screened initially for large plants with large nodule mass when breeding for improved N_2-fixation in alfalfa. We prefer to use first regrowth (about 12-week-old plants) for analysis rather than seedling growth. The first regrowth stage provides more uniformity in plant size, and it is not a function of seedling vigor, which agrees with previous recommendations (*34*).

Significant progress can be made by selection for the morphological characteristics alone. Because those characteristics account for only about 45 percent of the variation caused by NA, we believe that plants selected for morphological characteristics should be screened further for NA to measure the effectiveness of nodules. Because temperature and light quality both are important to N_2-fixation (*23*), we have concluded that the best period for greenhouse NA evaluations in Minnesota is from March to June. Growth chamber facilities could allow year-round evaluations.

Although total N in plant tops was improved, no alterations in N concentration generally occurred in plant tops in these experiments. When the original MnNC pool and both the high- and low-MnNC subpopulations were evaluated (MnNC experiment), the correlation coefficient (using means of combined plants as described in "Materials and Methods") between N concentration and top dry weight was -0.18 ($P<.05$). When the original pools and only the high subpopulations after two cycles of selection were evaluated (MnNC-MnPL experiment), the same type of correlation was 0.10 ($P<.05$) in MnNC and 0.16 ($P<.05$) in MnPL. Others reported significant correlations of 0.28 between N concentration and yield in nonfall dormant alfalfa populations selected for high NA and 0.31 for low NA (*5*). The high degree of independence indicated by these low correlations suggested the possibility of selecting for yield and N_2-fixation followed by selecting for N concentration to improve forage quality. Yield and N concentration both were higher significantly in the MnNC subpopulations collectively than in the MnPL subpopulations in the MnNC-MnPL experiment.

Decreases in levels of some characteristics in the Syn. 2 generation in our selected subpopulations suggested that these characteristics had some nonadditive genetic effects. Heterosis for NA was observed in progenies of some alfalfa single crosses and inbreeding depression in S_1 progenies (*34*). If nonadditive genetic effects are important, some type of hybrid cultivars would be advantageous to maximize the level of N_2-fixation. Further experimentation is needed to determine the inheritance for each of the characteristics.

Differences observed among 'Agate', 'Saranac', and our selected subpopulations for NA, top dry weight, and the root morphological characteristics associated with N_2-fixation suggested that alfalfa cultivars now on the market may differ in N_2-fixation potential. When initiating a N_2-fixation breeding program, we feel that evaluating adapted varieties for NA would be desirable before deciding on parental germplasm sources.

Using the mass 15 isotope of N as a tracer of N metabolism provides new insights into N_2-fixation of alfalfa and permits a better understanding of the seasonal patterns of N_2-fixation in field-grown materials. Comparisons of field and greenhouse performance for N_2-fixation illustrate some of the strengths and some of the constraints of selecting for physiological and morphological characteristics associated with NA. The isotope technique is a valuable tool in a breeding program for N_2-fixation and for assessing how traits investigated in controlled environments mediate N_2-fixation in the field.

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Literature Cited

(1) Aughtry, J. D., Jr.
1948. Effects of genetic factors in *Medicago* on symbiosis with *Rhizobium*. New York Agricultural Experiment Station, Memorandum No. 280.

(2) Barnes, D. K.
1978. Effect of coated seed on nodulation, establishment, and yields of alfalfa. *In* Proceedings of the 8th Annual Alfalfa Symposium, Bloomington, Minn., March 7-8, p. 52-55.

(3) Burton, J. C., and P. W. Wilson.
1939. Host plant specificity among the *Medicago* in association with root-nodule bacteria. Soil Science 47:293-303.

(4) Deibert, E. J., M. Bijeriego, and R. A. Olson.
1979. Utilization of ^{15}N fertilizer by nodulating and nonnodulating soybean isolines. Agronomy Journal 71:717-723.

(5) Duhigg, P., B. A. Melton, and A. A. Baltensperger.
1978. Selection for acetylene reduction rates in 'Mesilla' alfalfa. Crop Science 18:813-816.

(6) Edmeades, D. C., and K. M. Goh.
1978. Symbiotic nitrogen fixation in a sequence of pastures of increasing age measured by a ^{15}N dilution technique. New Zealand Journal of Agricultural Research 21:623-628.

(7) Erdman, L. W., and J. C. Burton.
1938. Strain variation and host specificity of *Rhizobium leguminosarum* on new pea varieties. Proceedings of the Soil Science Society of America 3:169-175.

(8) _____ and U. M. Means.
1953. Strain variation of *Rhizobium meliloti* on three varieties of *Medicago sativa*. Agronomy Journal 45:625-629.

(9) Gershon, D.
1961. Genetic studies of effective nodulation in *Lotus* spp. Canadian Journal of Microbiology 7:961-963.

(10) Gibson, A. H.
1962. Genetic variation in the effectiveness of nodulation of lucerne varieties. Australian Journal of Agricultural Research 13:388-399.

(11) _____
1964. Genetic control of strain-specific ineffective nodulation in *Trifolium subterraneum* L. Australian Journal of Agricultural Research 15:37-49.

(12) Ham, G. E.
1978. Use of ^{15}N in evaluating symbiotic N_2 fixation of field-grown soybeans. *In* Isotopes in biological dinitrogen fixation, International Atomic Energy Agency, Vienna, Austria, p. 151-162.

(13) _____ V. B. Cardwell, and H. W. Johnson.
1971. Evaluation of *Rhizobium japonicum* inoculants in soils containing naturalized populations of *Rhizobia*. Agronomy Journal 63:301-303.

(14) Hanson, W. D.
1963. Heritability. *In* W. D. Hanson and H. F. Robinson, eds., Statistical genetics and plant breeding. National Academy of Sciences-National Research Council Publication 992:125-140, Washington, D.C.

(15) Hardy, R. W. F., R. D. Holsten, E. K. Jackson, and R. C. Burns.
1968. The acetylene-ethylene assay for N_2-fixation: laboratory and field evaluation. Plant Physiology 43:1185-1207.

(16) Heichel, G. H., and C. P. Vance.
1979. Nitrate-N and *Rhizobium* strain roles in alfalfa seedling nodulation and growth. Crop Science 19:512-518.

(17) _____ D. K. Barnes, and C. P. Vance.
1981. Nitrogen fixation of alfalfa: the seeding year. Crop Science 21:330-335.

(18) Hill, R. R., Jr., M. W. Pedersen, L. J. Elling, and others.
1971. Comparison of expected genetic advance with selection on the basis of clone and polycross progeny-test performance in alfalfa. Crop Science 11:88-91.

(19) Holl, F. B.
1975. Host plant control of the inheritance of dinitrogen fixation in the *Pisum-Rhizobium* symbiosis. Euphytica 24:767-770.

(20) Leach, G. L.
1968. The effectiveness of nodulation of a wide range of lucerne cultivars. Australian Journal of Experimental Agriculture and Animal Husbandry 8:323-326.

(21) Legg, J. O., and C. Sloger.
1975. A tracer method of determining symbiotic nitrogen fixation in field studies. *In* E. R. Klein and P. D. Klein, eds., Second International Conference on Stable Isotopes, Oak Brook, Ill., October 20-23, 1975, p. 661-666.

(22) McAuliffe, C., D. S. Chamblee, H. Uribe-Arango, and W. W. Woodhouse, Jr.
1958. Influence of inorganic nitrogen on nitrogen fixation of legumes as revealed by ^{15}N. Agronomy Journal 50:334-337.

(23) Munns, D. N., V. W. Fogle, and B. G. Hallock.
1977. Alfalfa root nodule distribution and inhibition of nitrogen fixation by heat. Agronomy Journal 69:377-380.

(24) Mytton, L. R., and D. G. Jones.
1971. The response to selection for increased nodule tissue in white clover (*Trifolium repens* L.) *In* T. A. Lie and E. G. Mulder, eds., Biological nitrogen fixation in natural and agricultural habitats. Plant and Soil, Special Volume, Martinus Nijhoff, The Hague, p. 17-25.

(25) Nutman, P. S.
1946. Genetical factors concerned in the symbiosis of clover and nodule bacteria. Nature 157:463-465.

(26) _____
1949. Nuclear and cytoplasmic inheritance of resistance to infection by nodule bacteria in red clover. Heredity 3:263-291.

(27) _____
1952. Host factors influencing infection and nodule development in leguminous plants. Proceedings of the Royal Society of London, Section B, 139:176-185.

(28) _____
1954a. Symbiotic effectiveness in nodulated red clover. I. Variation in host a[illegible] in bacteria. Heredity [illegible]:35-46.

(29) _____
1954b. Symbiotic effectiveness in nodulated red clover. II. A major gene for ineffectiveness in the host. Heredity 8:47-60.

(30) _____
1957. Symbiotic effectiveness in nodulated red clover. III. Further studies on inheritance of ineffectiveness in the host. Heredity 11:157-173.

(31) _____
1971. Selection for increased nitrogen fixation in red clover. *In* T. A. Lie and E. G. Mulder, eds., Biological nitrogen fixation in natural and agricultural habitats. Plant and Soil, Special Volume, Martinus Nijhoff, The Hague, p. 27-31.

(32) Peterson, M. A.
1980. Inheritance of five ineffective nitrogen fixation traits in alfalfa. M.S. thesis. Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul.

(33) Phillips, D. A., and J. P. Bennett.
1978. Measuring symbiotic nitrogen fixation in rangeland plots of *Trifolium subterraneum* L. and *Bromus mollis* L. Agronomy Journal 70:671-674.

(34) Seetin, M. W., and D. K. Barnes.
1977. Variation among alfalfa genotypes for rate of acetylene reduction. Crop Science 17:783-787.
(35) Vest, G., and B. E. Caldwell.
1972. *rj4*—A new gene conditioning ineffective nodulation in soybean. Crop Science 12:692-693.
(36) Viands, D. R., C. P. Vance, G. H. Heichel, and D. K. Barnes.
1979. A plant-mediated ineffective nitrogen fixation trait in alfalfa (*Medicago sativa* L.). Crop Science 19:905-908.
(37) Williams, L. F., and D. L. Lynch.
1954. Inheritance of a non-nodulating character in the soybean. Agronomy Journal 46:28-29.

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