10. Foytik, J. 1981. Very low-cost nutritious diet plans designed by linear programming. J. Nutr. Educ. 13(2):63–66.
11. Free, W.J. and V.A. Vitelli. 1979. Using budgets for decision making. Economics of producing & marketing woody landscape plants. TVA Bul. Y-149:40–44. Muscle Shoals, Ala.
12. Gortzig, C.F. 1976. Profit maximization in commercial greenhouse floriculture under northern United States production and marketing conditions: A linear programming approach. PhD Diss., Michigan State Univ., East Lansing. (Diss. Abstr. 77-11,651).
13. Grace, S.B., J.M. Gerber, and E.R. Swanson. 1982. Economic analysis of alternative weed controls on seven vegetables. Ser. E. Ill. Agr. Econ. Staff Paper no. 82E-208, Urbana.
14. Harsh, S.B. 1975. A progress report on Teleplan activities. Dept. of Agr. Econ., Michigan State Univ., East Lansing.
15. Harter, W.G. 1967. The computer and linear programming as important instruments for decision making assistance for farmers. PhD Diss., The Ohio State Univ., Columbus. (Diss. Abstr. 68-8832)
16. Hillier, F.S. and G.J. Lieberman. 1980. Introduction to operations research. 3rd ed. Holden-Day, San Francisco.
17. Hironka, R. and B.H. Sonntag. 1978. A computer model for selecting feeding programs and formulating diets for maximum profit from feedlot cattle. Agr. Bul.—Alberta Univ., College of Agr., Edmonton 1(4):33–35.
18. McConnell, D.B. 1979. Kurume Azalea production system alternatives. Economics of producing & marketing woody landscape plants. TVA Bul. Y-149:20–25. Muscle Shoals, Ala.
19. McNiel, R. 1979. Pin Oak production system alternatives. Economics of producing & marketing woody landscape plants. TVA Bul. Y-149:32–33. Muscle Shoals, Ala.
20. Perry, F., Jr. (ed.). 1982. Nursery management and production costs: Burford Holly. Southern Coop. Ser. Bul. No. 274:1–35. Auburn Univ., Ala.
21. Phillips, T. and V. Ward. 1980.Programming for optimum crop combinations for a small nursery. Proceedings of SNA Res. Conf.—1980. Southern Nurserymen's Assn. p. 165–167.
22. Reyes, A.A., R.W. Blake, C.R. Shumway, and J.T. Long. 1981. Multistage optimization model for dairy production. J. Dairy Sci. 64:2003–2016.
23. Sabota, C.M. 1983. Linear programming of large and small container nurseries. PhD Diss., Univ. of Illinois, Urbana. (Diss. Abstr. 8410039)
24. Smith, C.N. 1979. Kurume Azalea cost of production budget. Economics of producing & marketing woody landscape plants. TVA Bul. Y-149:26–32. Muscle Shoals, Ala.
25. Smith, C.N. and D.B. McConnell. 1978. Effects of changes in prices of selected production inputs on azalea costs per container. Proc. Fla. State Hort. Soc. 91:217–220.
26. Sowell, R.S., D.H. Willits, P.V. Nelson, and A.J. Coutu. 1982. Optimizing greenhouse production management decisions using linear programming. Proc. Amer. Soc. Agr. Eng. St. Joseph, Mich. Paper 82-5021.
27. Vaut, G.A., R.L. Christensen, T.C. Slane, and J.F. Smiarowski. 1973. Greenhouse linear programming. Dep. Agr. & Food Economics, Univ. of Massachusetts, Amherst. Publication no. 93:1–29.
28. Walker, J.L. 1971. An economic model for optimizing the rate of timber harvesting. PhD Diss., Univ. of Washington.
29. Weitkamp, W.H., W.J. Clawson, D.M. Center, and W.A. Williams. 1980. A linear programming model for cattle ranch management. Univ. of Calif. Bul. 1900.
30. Westbrook, R.F. 1970. Applications of operations research in the forest products industry: II. Operations research in the forest products industry. Fourth Texas Ind. Wood Seminar. Texas Forest Serv. Texas A & M Univ. Sept. 17–18, 1970. p. 56–62.
31. White, E.A. (n.d.) Computer on the farm. Coop. Ext. Serv., The Pennsylvania State Univ., University Park.
32. Wright, R.D. 1979. Pin Oak cost of production budget. Economics of producing & marketing woody landscape plants. TVA Bul. Y-149:34–37. Muscle Shoals, Ala.

J. Amer. Soc. Hort. Sci. 112(3):510-513. 1987.

Relationship of Plant Development to Nodulation in Determinate and Indeterminate Beans

Steven L. Morrison and Lisa M. Baird
Department of Botany, Connecticut College, New London, CT 06320

Additional index words. nitrogen fixation, acetylene reduction, ureides, amino acids, xylem exudate, *Phaseolus vulgaris*, *Rhizobium phaseoli*, symbiosis

Abstract. **The relationship of plant growth habit to establishment of a successful symbiosis between *Phaseolus vulgaris* L. and *Rhizobium phaseoli* was examined. A determinate and an indeterminate cultivar of bean were inoculated with a pure culture of *R. phaseoli* 127K44, and plant development was measured using the plastochron index. Time required to nodulate as determined by nodule count did not vary between plant types. The timing, duration, and overall success of the symbiosis as measured by increased acetylene reduction, dry weight, and transport of ureides and amino acids were related to the period of exponential leaf expansion in both the determinate and indeterminate cultivar.**

Establishment of a successful symbiosis for nitrogen fixation involves a complex sequence of genetic, physiological, and developmental interactions between the host legume and a strain of *Rhizobium* (4, 5). Many studies have focused on the role of the bacteria in symbiosis (4, 5). Recently, attention has been directed toward the role of the host in formation and maintenance of symbiotic associations and delineation of the extent to which this role can be manipulated (10).

Nodulation and nitrogen fixation in *P. vulgaris*, the common bean, is variable (8). An abundance of ineffective strains of *R. phaseoli* combined with the poor response of several *P. vulgaris* genotypes to effective strains of *Rhizobium* (9) have made routine study or application of symbiotic associations in this plant problematical. Recent reports have suggested that plant development in beans may play a significant role in limiting the establishment and duration of the symbiosis. A lack of coordi-

Received for publication 27 May 1986. This research was supported by National Science Foundation grant PCM-8312261 and a grant from the American Philosophical Society. We thank F. Bliss and R. Virginia for critical reading of the manuscript. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

nation between plant and rhizobial development has been shown to result in ineffective symbiosis in *P. vulgaris* 'Red Kidney' (1, 2). In these studies, manipulation of plant development through prevention of flowering increased vegetative growth, acetylene reduction activity, ureide transport, and nodule longevity (2). These data suggested that patterns or processes in plant development altered the effectiveness of a symbiosis with a given rhizobial strain.

Although the capacity for nitrogen fixation differs between determinate and indeterminate cultivars of *Phaseolus* (7–9), information on the role of host development and physiology in formation and maintenance of an effective symbiosis is lacking for both plant types. Our objective was to determine the relationship between plant development and the formation and maintenance of effective symbioses between *P. vulgaris* and *R. phaseoli*.

Materials and Methods

Seeds of *P. vulgaris* cv. Sanilac (determinate, type 1b) and Puebla 152 (indeterminate, type 3) (3) were surface-sterilized in 0.5% sodium hypochlorite (pH 7.0) for 2 min, rinsed, scarified, and planted in 25.4-cm (10-inch) pots in 2 sterilized sand : 1 perlite (v/v). Six seeds were planted per pot, then thinned for uniform shoot growth to three plants per pot ≈10 days after germination. Each seed was inoculated at planting with ≈5 × 10^8 cells of a pure culture of *R. phaseoli* 127K44 (gift of S. Smith, Nitragin Corp., Milwaukee, Wis.).

Plants were grown in environmental chambers using a 16-hr photoperiod. Day and night temperatures were 25° ± 2°C and 19° ± 2°, respectively. Relative humidity was 65% ± 3%. Light in the chambers was maintained at 550 $\mu mol \cdot s^{-1} \cdot m^{-2}$ with a mixture of high-intensity discharge lamps and mercury arc lamps. Inoculated plants were watered on alternate days for the first 14 days with a modified Hoagland solution containing 14 μM N; thereafter, minus N solution was used. On alternate days control plants were fed nutrient solution containing 140 μM N. Control plants did not nodulate during the experimental period.

Plant development was measured using the plastochron index (PI) (6) and plotted as a function of chronological age. PI calculations were based on the length of the middle lamina of trifoliolate leaves from 25 plants. Measurements were made on alternate days using a reference length of 15 mm to minimize manual manipulation of the leaves. The term "plastochron" refers to the number of days required for successive leaves to reach the reference length.

Six plants were decapitated 1 cm above soil level at weekly intervals and xylem sap collected for a period of 15 min as it exuded from the cut stump. The first 25 μl was counted as part of the sample volume but was not assayed to minimize contamination. Replicate samples were combined and net volume measured, and then frozen for subsequent analysis of ureides (18), total amino acids (13), and individual amino acids by HPLC following precolumn derivatization with *o*-phthal-dialdehyde (17). Entire root systems were harvested for the acetylene reduction (AR) assay (11) following collection of xylem exudate. Upon completion of the assay, nodules were removed from the root and counted. Root, shoot, and nodule material was dried for 72 hr at 70°C for determination of dry weight.

Net transport of ureides, total amino acids, and the amino acid composition of xylem exudate were compared at plastochron indices corresponding to early vegetative (V2), late vegetative (V5–V7), and early pod development (R3) stages of growth. Experiments were terminated at mid-pod development (R5–R6) (12).

Results

Leaf expansion in shoots of the indeterminate 'Puebla' was initially slower than in the determinate 'Sanilac'. Growth analysis of inoculated 'Puebla' and 'Sanilac' showed that the duration of the plastochron for 'Sanilac' became constant ≈21 days after planting (DAP) and remained constant until flowering occurred at the apex 43 DAP (Fig. 1). During this time, the plastochron for 'Sanilac' was 5.9 days. In 'Puebla', a constant plastochron of 3.9 days was reached 30 to 32 DAP, and the length of the plastochron remained constant until early pod fill.

Measurable AR was detected in both cultivars at a plant plastochron index of 2 to 3, when the second trifoliolate leaf was ≈30% expanded. AR in 'Puebla' and 'Sanilac' was not significantly different at this point (Fig. 2), then increased in each cultivar as the plastochron became constant. Subsequent decline in AR in 'Sanilac' occurred during flowering and early pod fill. In 'Puebla', AR increased through the first 49 days of growth before leveling off at 16 μmol/plant per hr as growth slowed.

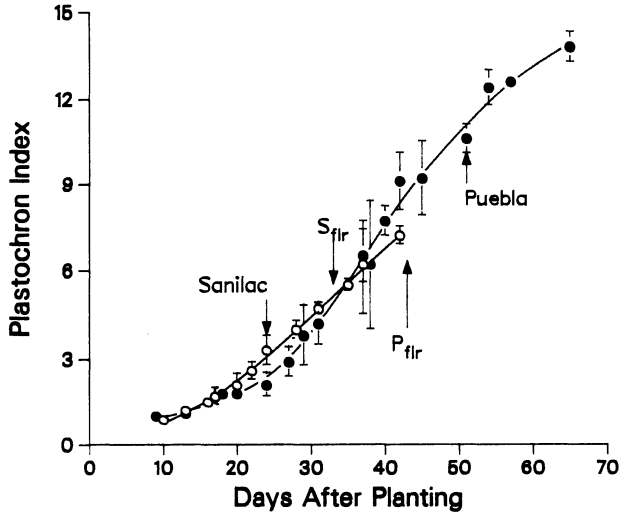

Fig. 1. Change in plastochron index with time for determinate 'Sanilac' and indeterminate 'Puebla' cultivars of beans inoculated with *R. phaseoli* 127K44. P_{flr} and S_{flr} are the times at which transition to flowering was first observed. Vertical bars represent SE.

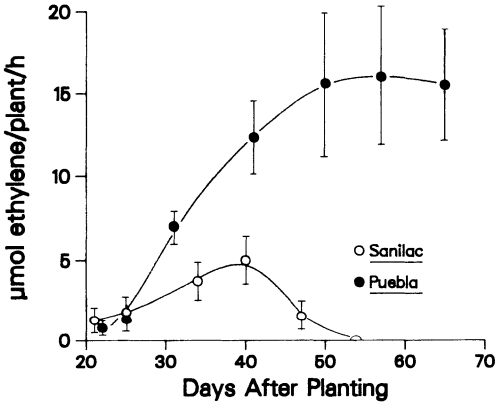

Fig. 2. Acetylene reduction as a function of plant age in the determinate 'Sanilac' and indeterminate 'Puebla' inoculated with *R. phaseoli* 127K44. Vertical bars represent SE.

Dry weight accumulation in roots and shoots of both cultivars did not differ significantly between the cultivars during the first 28 days (Fig. 3). Dry weight increase in 'Puebla' between 30 and 65 DAP was partitioned to the shoot. By the conclusion of the experiment, the shoot : root ratio for 'Puebla' was 7.4. In 'Sanilac', the shoot : root ratio at flowering was 1.3 and did not increase significantly thereafter.

Greater than 97% of ureides were transported as allantoin in both cultivars. At 21 DAP, ureide concentration in 'Sanilac' xylem exudate was twice that of 'Puebla'. The maximum concentration of ureide transported per milliliter of sap was equal in 'Sanilac' and 'Puebla' at days 35 and 47, respectively (Fig. 4). Amino acid concentration in xylem sap exudate was initially <0.25 $\mu mol \cdot ml^{-1}$ in both cultivars (Fig. 4), then increased and appeared to plateau at a time corresponding to maximum AR in each cultivar. A second increase (34% to 39%) in the concentration of amino acids in the xylem exudate occurred after AR reached a maximum and began to decline.

Amino acid analysis at plastochron indices equivalent to early, mid-vegetative, and early reproductive phases of growth (Fig. 5) showed that xylem exudate of 'Sanilac' contained between 10% and 20% glutamine and 15% to 25% asparagine, as well as significant quantities of aspartate and alanine. Asparagine concentration in the xylem exudate of 'Puebla' increased from 10% to 27% of the total during the growth period, while glutamine concentrations in 'Puebla' remained below 10%.

Calculation of total amino acid and ureide transport at these developmental stages (Table 1) showed that the larger volume of xylem sap transported by 'Puebla' throughout the experiment resulted in 1.5 to 3 times more ureide and 1.5 to 2 times more amino N being transported from 'Puebla' roots than 'Sanilac' roots.

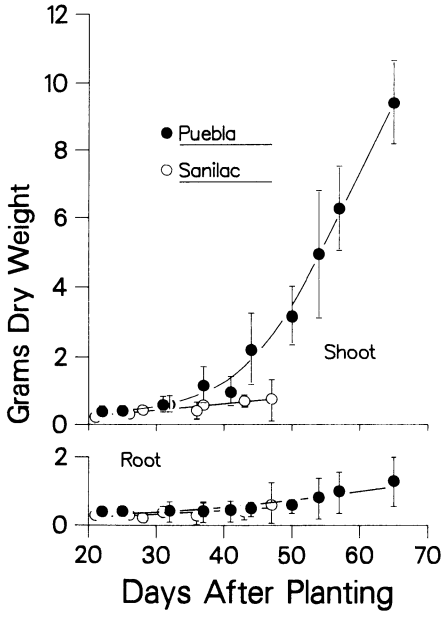

Fig. 3. Change in root and shoot dry weights for determinate 'Sanilac' and indeterminate 'Puebla' inoculated with *R. phaseoli* 127K44. Vertical bars represent SE.

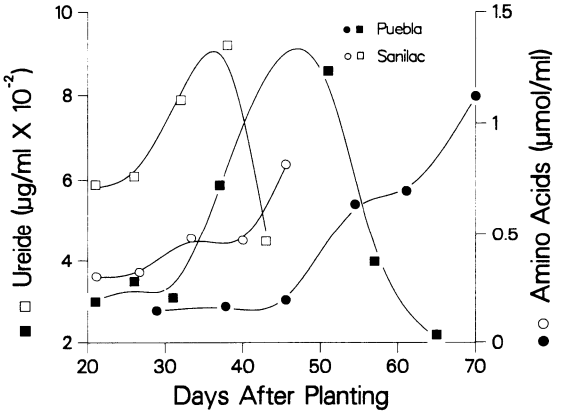

Fig. 4. Ureide and amino acid transport in xylem exudate of determinate 'Sanilac' and indeterminate 'Puebla' inoculated with *R. phaseoli* 127K44.

Discussion

Our experiments demonstrated that the plastochoron index, as a function of chronological age, was an effective means of comparing plant development with the status of N_2 fixation activity in these cultivars. The development of an effective symbiosis, as determined by change in AR, profiles of ureide and amino acid transport in xylem exudate, and an increase in dry weight, occurred as the plastochron approached a constant value. These results support those of Snyder and Bunce (16), who concluded that plastochron rate provided a sensitive means of comparing growth of variously treated cultivars of soybeans grown under similar conditions and was highly sensitive to the nitrogen status of the plant.

On the basis of duration of plastochron, an effective symbiotic association was established ≈12 days later in the indeterminate 'Puebla' than in the determinate 'Sanilac'. N_2 fixation in 'Puebla', however, continued well into pod fill.

The relationship between plastochron and physiological parameters indicative of successful nitrogen fixation is complex. Not only is the shoot important as a source of photosynthate for nodule development and maintenance (15), but senescence of lower leaves in bean has been shown to alter photosynthate

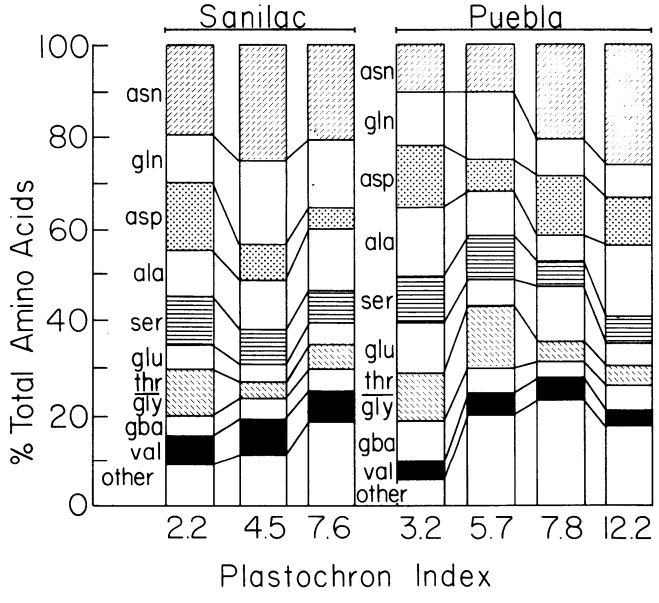

Fig. 5. Composition of amino acids in xylem exudate at chronological ages corresponding to early vegetative (V2), late vegetative (V5–V7), and early pod development (R3). The determinate bean 'Sanilac' and indeterminate bean 'Puebla' were both inoculated with *R. phaseoli* 127K44.

Table 1. Volume of sap exudate and net transport of ureides and amino acids by *P. vulgaris* cvs. Sanilac and Puebla.

Cultivar	Plastochron index	Growth stage[z]	Exudate (μl/plant per hr)	Ureides (μg/plant per hr)	Amino N (nmol/plant per hr)
Sanilac	2.5	V2	60 ± 30[y]	35	19
	5.5	V5/R1	113 ± 25	95	52
	NA[x]	R3	110 ± 63	52	91
Puebla	2.2	V2	163 ± 21	57	29
	7.5	V7/R1	218 ± 22	166	46
	12.3	R3	303 ± 38	179	206

[z]Growth stages; V2 (early vegetative); V5-7 (late vegetative); R1 (early flowering); R3 (early pod development).
[y]± SE.
[x]NA: Plastochron index was not measured, as flowering had occurred at the apex.

partitioning and to limit N_2 fixation (19). Of particular interest, Malik (14) showed that the presence of developing leaves in soybean was more important to duration of the symbiosis than total leaf area. Plant age and senescence in Malik's study appeared to contribute to symbiotic decline despite grafting to double leaf area. These reports are particularly interesting in the context of comparison of symbiotic development in determinate and indeterminate cultivars of *P. vulgaris*. The results suggest that, in *P. vulgaris*, the physiological patterns of effective symbiosis with a single rhizobial strain are similar. Duration of the symbiosis, however, appears related to the potential for extended shoot growth, as evidenced by an indeterminate growth habit.

Literature Cited

1. Baird, L.M. and B.D. Webster. 1982. Morphogenesis of effective and ineffective root nodules in *Phaseolus vulgaris* L. Bot. Gaz. 143:41–51.
2. Baird, L.M., G. Sarath, and B.D. Webster. 1983. Effects of prevention of flowering on the vegetative growth of bean plants inoculated with an "ineffective" strain of *Rhizobium phaseoli*. Bot. Gaz. 144:225–230.
3. CIAT. 1977. Descripción de los hábitos de crescimento de *Phaseolus vulgaris* L. utilizados por el Programa de Frijol del CIAT, Centro Internacional de Agricultura Tropical Informe Anual, Cali, Colombia.
4. Dart, P.J. 1974. The infection process, p. 381–429. In: A. Quispel (ed.). The biology of nitrogen fixation. North Holland, Amsterdam.
5. Dart, P.J. 1975. Legume root nodule initiation and development, p. 467–506. In: J.G. Torrey and D.T. Clarkson (eds.). The development and function of roots. Academic, London.
6. Erickson, R.O. and F.J. Michelini. 1957. The plastochron index. Amer. J. Bot. 44:297–305.
7. Graham, P.H. 1981. Some problems of nodulation and symbiotic nitrogen fixation in *Phaseolus vulgaris*: a review. Field Crops Res. 4:93–112.
8. Graham, P.H. and J. Halliday. 1977. Inoculation and nitrogen fixation in the genus *Phaseolus*. In: J.M. Vincent, A.S. Whitney, and J. Bose (eds.). Exploiting the legume–rhizobium symbiosis in tropical agriculture. Univ. Hawaii Coll. Trop. Agr. Misc. Pub. 145.
9. Graham, P.H. and J.C. Rosas. 1977. Growth and development of indeterminate bush and climbing cultivars of *Phaseolus vulgaris* L. inoculated with *Rhizobium*. J. Agr. Sci., Cambridge 88:503–508.
10. Hardarson, G. and R.A. Lie. 1984. Breeding legumes for enhanced symbiotic nitrogen fixation. Nijhoff/Junk, The Hague, Netherlands.
11. Hardy, R.W.F., R.P. Holsten, E.K. Jackson, and R.C. Burns. 1968. The acetylene-ethylene assay for nitrogen fixation; laboratory and field evaluation. Plant Physiol. 43:1185–1207.
12. Lebaron, M.J. 1974. Developmental stages of the common bean plant. Univ. of Idaho College of Agr. Current Info. Ser. 228.
13. Lee, Y.P. and T. Takahashi. 1966. An improved colorimetric determination of amino acids with the use of ninhydrin. Anal. Biochem. 14:71–77.
14. Malik, N.S.A. 1983. Grafting experiments on the nature of the decline in N_2 fixation during fruit development in soybean. Physiol. Plant. 57:561–564.
15. Phillips, D.A. 1980. Efficiency of symbiotic nitrogen fixation in legumes. Annu. Rev. Plant Physiol. 31:29–49.
16. Snyder, F.W. and J.A. Bunce. 1983. Use of the plastochron index to evaluate effects of light, temperature and nitrogen on growth of soya bean (*Glycine max* L. Merr.). Ann. Bot. 52:895–903.
17. Umgat, H., P. Kucera, and L.-F. Wen. 1982. Total amino acid analysis using pre-column fluorescence derivatization. J. Chromatogr. 239:463–474.
18. Vogels, G.D. and C. van der Drift. 1970. Differential analyses of glyoxylate derivatives. Anal. Biochem. 33:143–157.
19. Waters, L., P.J. Breen, H.J. Mack, and P.H. Graham. 1980. Translocation of ^{14}C-photosynthate, carbohydrate content, and nitrogen fixation in *Phaseolus vulgaris* L. during reproductive development. J. Amer. Soc. Hort. Sci. 105:424–427.