TIBA and nitrogen incorporation, senescense and yield in a symbiotically grown vining dry bean (*Phaseolus vulgaris* L.) genotype[1]

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ABSTRACT

The growth regulator 2, 3, 5 triiodobenzoic acid (TIBA) was applied foliarly at the beginning of flowering (R1 stage) to a vining dry bean (*Phaseolus vulgaris* L.) genotype, grown symbiotically in a low nitrogen containing soil. Profiles of root nodule nitrogenase and leaf nitrate reductase activities, as well as of leaf chlorophyll content throughout the post-flowering period, indicate that TIBA reduced the duration of nitrogen incorporation and caused early senescence. An increase in the number of pods per plant as well as in dry matter harvest index, supports the theory that TIBA causes bean plants to shift from vegetative to reproductive growth. No seed yield increase occurred as a result of TIBA application. Symbiotic nitrogen fixation alone may not provide sufficient nitrogen to allow optimal development of an increased number of pods. Further trials under higher soil nitrogen levels are suggested.

RESUMEN

Efecto del ácido triiodobenzoico (ATIB) sobre la incorporación de nitrógeno, senescencia y rendimiento en un genotipo de habichuela trepadora (*Phaseolus vulgaris* L.), cultivado en condiciones simbióticas.

Un genotipo de habichuela trepadora (*Phaseolus vulgaris* L.) se cultivó en condiciones simbióticas en un campo con bajo nivel de nitrógeno. Se aplicó ATIB (50 g./ha.) foliarmente al comenzar la floración (etapa R1). Por medio de ensayos periódicos de nitrogenasa radicular y reductasa de nitrato foliar durante el período de posfloración, se observó que el ATIB redujo la duración de la incorporación de nitrógeno. El muestreo de clorofila foliar durante este mismo período demostró que estas mismas plantas también decayeron antes que el control. Un incremento en el número de vainas y el índice de cosecha coincidieron con la teoría de que el ATIB provoca en la planta un cambio de crecimiento vegetativo a uno reproductivo. No se observaron aumentos en el rendimiento de semillas como respuesta al ATIB. Se propone que la fijación del nitrógeno de por sí podría ser insuficiente para abastecer con nitrógeno el desarrollo de un mayor número de vainas. Se sugiere ensayos adicionales con más altos niveles de nitrógeno.

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INTRODUCTION

The substance 2,3,5 triiodobenzoic acid (TIBA) was responsible for up to 15% seed yield increase in soybean (*Glycine max* L.) under optimum growing conditions when applied foliarly at 50 g/ha at the beginning of flowering (3, 12). It is believed that it acts as an "antiauxin" in shifting the growth-reproduction balance toward reproduction (2). The increased seed yields have been attributed to a larger number of floral buds in the lower nodes (12). Optimized light interception and photosynthesis due to the subsequent formation of a triangular, as opposed to a flat canopy, may also contribute greatly (3). More pronounced TIBA effects on yield were noted in late maturing, large vegetative, as opposed to early maturing, less vegetative soybean cultivars (12).

The seeds of dry bean (*Phaseolus vulgaris* L.) play an important role as the major protein source for human consumption in many developing areas of the world. Nevertheless, by seed legume standards dry bean is considered an unimproved, low yielding crop (1). A major limitation to increased seed yields in this crop is its lack of responsiveness to fertilizer nitrogen; supplemental nitrogen creates predominantly vegetative structures at the expense of reproductive ones, especially in the vining bean genotypes (10). Another limitation is its low symbiotic nitrogen (N_2) fixation potential, which is considered about one half that of soybean (14). Two separate plant population experiments with diverse dry bean morphological types revealed that pod number per plant was the most sensitive yield component and the one most highly correlated to seed yield per plant (4, 13). This finding also holds true for *Vicia faba* L., *Vigna radiata* L., *Glycine max* L. and *Lentilla lens* L., seed legumes of economic importance (13). Pods per plant is also the economic yield component of dry bean most responsive to fertilizer nitrogen (7).

The objective of this experiment was to find out how TIBA affects not only morphological parameters and seed yield but also the metabolic processes of nitrogen incorporation in symbiotic dry bean under limiting soil nitrogen conditions. In addition to agronomic measurements, such as those carried out in several soybean/TIBA trials (2, 3, 9, 12), the study reported herein used seasonal assays of nitrogenase and nitrate reductase activities, measure of symbiotic and soil derived nitrogen incorporation, respectively. Leaf chlorophyll was also measured seasonally. Through these metabolic parameters we attempted to define the effect of TIBA on nitrogen incorporation capacity, its relationship to senescence and its effect on the seed yield potential of dry bean.

MATERIALS AND METHODS

This experiment was conducted at the Rutgers University Vegetable Research Farm in New Brunswick, N. J., during the 1988 season. The germplasm utilized was the *Phaseolus vulgaris* L. genotype "Puebla 152-

B," a large black-seeded, vining, indeterminate landrace of Mexican origin. Seeds were inoculated with a peat-based commercial preparation of *Rhizobium phaseoli* strains 127 K 17, 127 K 44, and 127 K 45, obtained from the Nitragin Co. of Milwaukee, WI. Seeds were planted in 50-cm rows June 24, 1988. Upon emergence, plants were thinned to 10 cm, to obtain a final plant population of 200,000 plants/ha. Plots consisted of four rows; the two inner ones were used for harvest, whereas the outer ones served as guard rows. One row was used for seasonal measurements and the other for final yield data.

The soil, a "Sassafras sandy loam," previously under rye cover crop for 2 years, tested as follows: 206, 103, 1607, 316 kg/ha for P, K, Ca, Mg, respectively, all considered to be high. Nitrate and ammonia levels were 11 and 8 kg/ha, respectively, both considered very low. In order to enhance nitrogen fixing symbiosis no nitrogen fertilizer was applied. Sprinkler irrigation was applied as needed throughout the season.

TIBA was applied foliarly with a hand sprayer at the R1 (first flower) growth stage at a rate of 50 g/ha. It was dissolved in 200 liters of H_2O, using 0.01% Tween 20 (Fisher Scientific Co. Fairlawn NJ)[4] as a surfactant.

Plant sampling dates for above ground fresh weight, crown root nitrogenase activity, leaf NRA and chlorophyll were at 28, 32, 35, 40, 46, 49 days after R1 stage. Data was obtained from the mean SD of 4 randomly chosen groups consisting of three consecutive plants for each sampling date.

Nitrogenase activity was assayed with the C_2H_2 reduction method (15). Soil-root cores (15 cm × 10 cm id) were taken from the field, placed in 1000 ml mason jars and incubated in 10% acetylene at 30° C for 1 hour. The C_2H_4 produced was determined with a Perkin-Elmer (Sigma 4) gas chromatograph equipped with a H_2/air flame ionization detector and a stainless steel column packed with Poropack N (192 × 0.3 cm). The column was operated at 100° C with N_2 as the carrier gas.

Leaf nitrate reductase activity (NRA) was assayed by the *in vivo* method (8). The uppermost fully expanded trifoliate leaves were superimposed and 1 cm-diameter disks taken with a cork borer. Subsamples (0.2 g) were incubated in modified medium containing 100 mM KPO_4 buffer (pH 7.5), 50 mM KNO_3, 1% v/v propanol, and 0.01% Tween 20. Samples were incubated for 60 min at 30° C in the dark, and 0.2 ml aliquots removed for colorimetric NO_2 determination (8).

[4]Trade names in this publication are used only to provide specific information. Mention of a trade name does not constitute a warranty of equipment or materials by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other equipment or materials.

Chlorophyll concentration was determined by extraction with aqueous acetone (80% v/v) in the dark, from leaf disks used previously for NRA. The acetone extracts were centrifuged at 2000 g for 10 min and the supernatant used for the colorimetric determination of chlorophyll (11).

Dry matter and seed yield measurements were carried out at physiological maturity (R7 growth stage), 96 days after germination (52 days after flowering), by randomly harvesting four replications consisting of five consecutive plants. Plants were dried to 1% moisture, and yields were reported at 14% moisture. Using statistical analysis system (SAS), we performed analysis of variance with all replicates. Visual observations were made throughout the growing season.

RESULTS

After application of 50 g/ha TIBA at flowering, some upper leaves showed puckering and distortion, although subsequent growth appeared normal. Aboveground fresh weight increased rapidly between 28 and 32 days after flowering, then more slowly until 50 days, without weight differences between TIBA and control plants (fig. 1).

Distinct morphological differences between TIBA and control plants were observed at physiological maturity, although no seed yield differences were found (table 1). Total aboveground dry weight was lower in the TIBA-treated plants, although their dry matter harvest index was

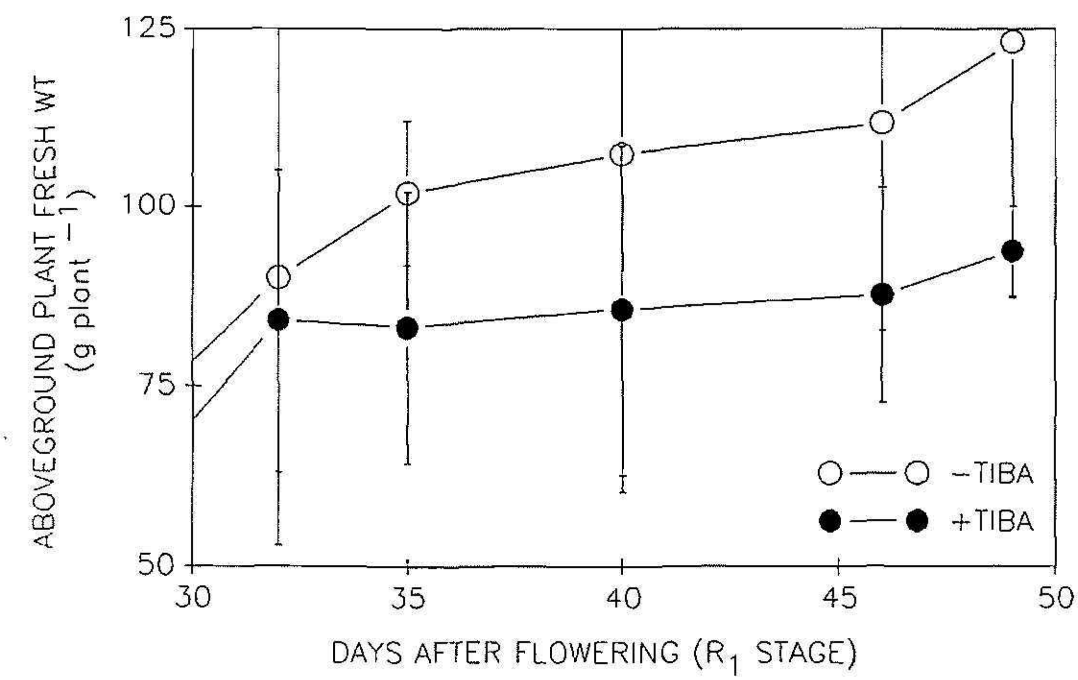

FIG. 1.—Effect of TIBA on postflowering above ground fresh weight gain of field grown *Phaseolus vulgaris* L. plants. Each point in the figure represents the mean of four replicate samples ± − SD.

TABLE 1.—*Effect of TIBA on seed yield, above ground dry matter, harvest index, pod number, height and days to maturity of field grown* Phaseolus vulgaris *L. plants*

Measurement	– TIBA	+ TIBA	
Seed yield, g/plant	10.0	9.0	
kg/ha	2000.0	1800.0	
Above ground dry matter, g/plant	18.6	15.1	*[1]
Dry matter harvest index	0.54	0.60	*
Pod number	26.2	32.0	*
Plant height, cm	143.0	96.4	*
Days to maturity	96	90	*

[1]Significantly different at $P < 0.05$.

higher (table 1). The number of pods in the TIBA plants was greater at 32, versus 26 in the control plants (table 1). The former also reached pronouncedly less height, 96.4 cm, than the control, 143 cm (table 1). No indication of a triangular canopy was evident in the TIBA treatment, as was reported in soybean (3). However, there was an increase in branching, which was manifested by a bushy appearance. TIBA-treated plants reached physiological maturity 6 days before the control plants (table 1).

Nitrogenase activity was higher in the control at 28 days and after 40 days (fig. 2). Nodulation appearance did not vary from the control.

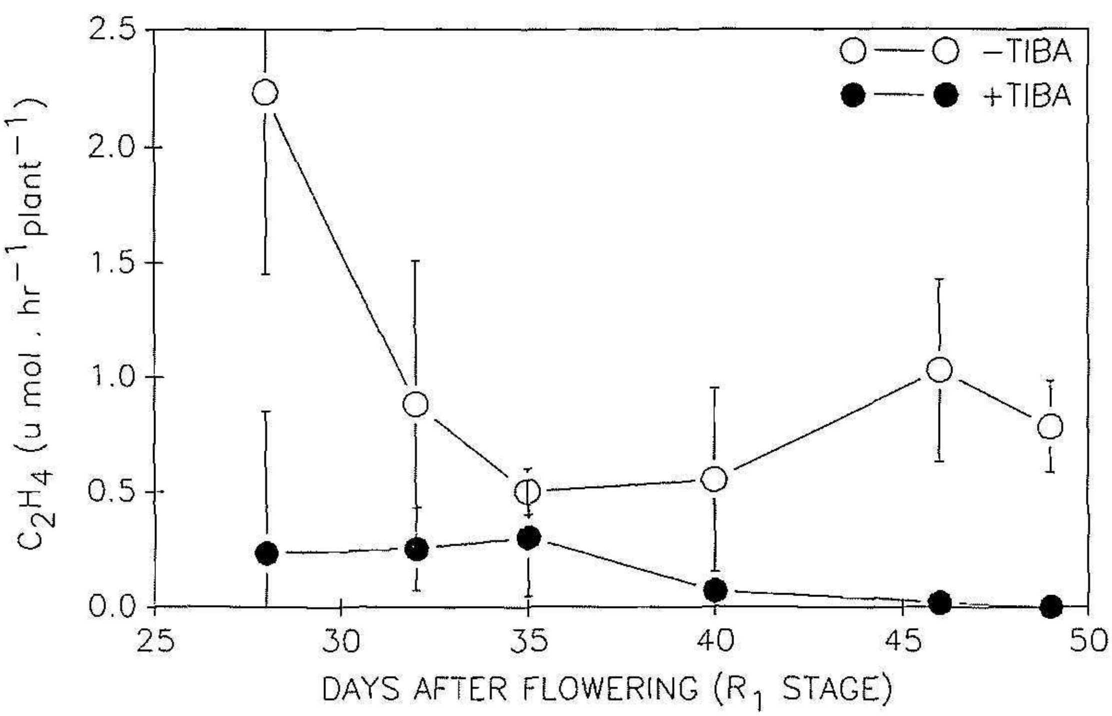

FIG. 2.—Effect of TIBA on postflowering nitrogenase activity of the crown root nodules of field grown *Phaseolus vulgaris* L. plants. Each point in the figure represents the mean of four replicate samples ± – SD.

Burton and Curly (5) observed that field grown symbiotic soybean plants sprayed foliarly with low concentrations of TIBA sustained root nodule weight, size distribution, and total nodule number equal to that of the control. Leaf nitrate reductase activity suggests that nitrate assimilation decreased about 10 days earlier in TIBA-treated plants, although similar levels occurred until 40 days after flowering (fig. 3). It appears that TIBA-treated dry bean plants have a combined disadvantage in that the duration of nitrate, as well as symbiotic nitrogen incorporation, is reduced. Rapid decay of leaf chlorophyll in the TIBA-treated plants after 40 days (fig. 4) also suggests premature senescence.

DISCUSSION

The developing ovules of the protein-rich seed legumes are strong attractants for nitrogen. Creegan and Van Berkum (6) suggest that a large amount of nitrogen reaching soybean seeds is derived from direct flow of either symbiotically fixed or fertilizer derived nitrogen, without prior incorporation into leaves. An early decay of root nodule nitrogenase and leaf nitrate reductase activities (fig. 2, 3) suggest a reduction of direct nitrogen flow to the ovules, and thus a likely increase of leaf protein hydrolisis. This could partially explain premature senescence of TIBA plants (fig. 4).

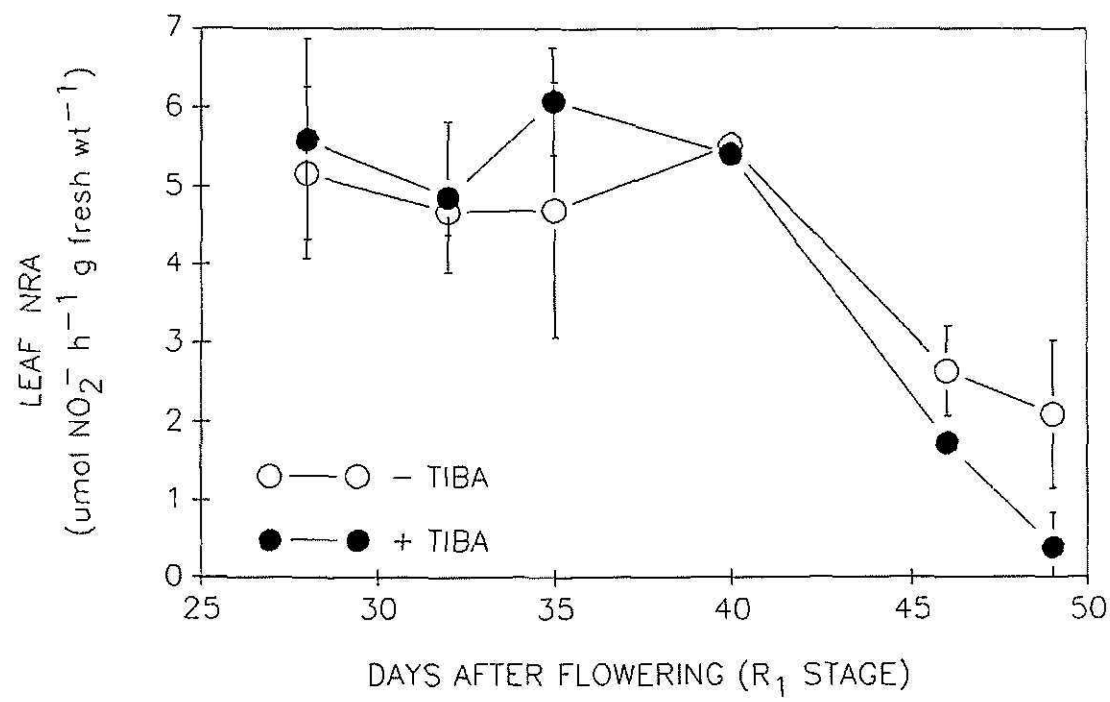

FIG. 3.—Effect of TIBA on postflowering *in vivo* nitrate reductase activity in uppermost fully expanded trifoliate leaf of field grown *Phaseolus vulgaris* L. plants. Each point in the figure represents the mean of four replicate samples ± – SD.

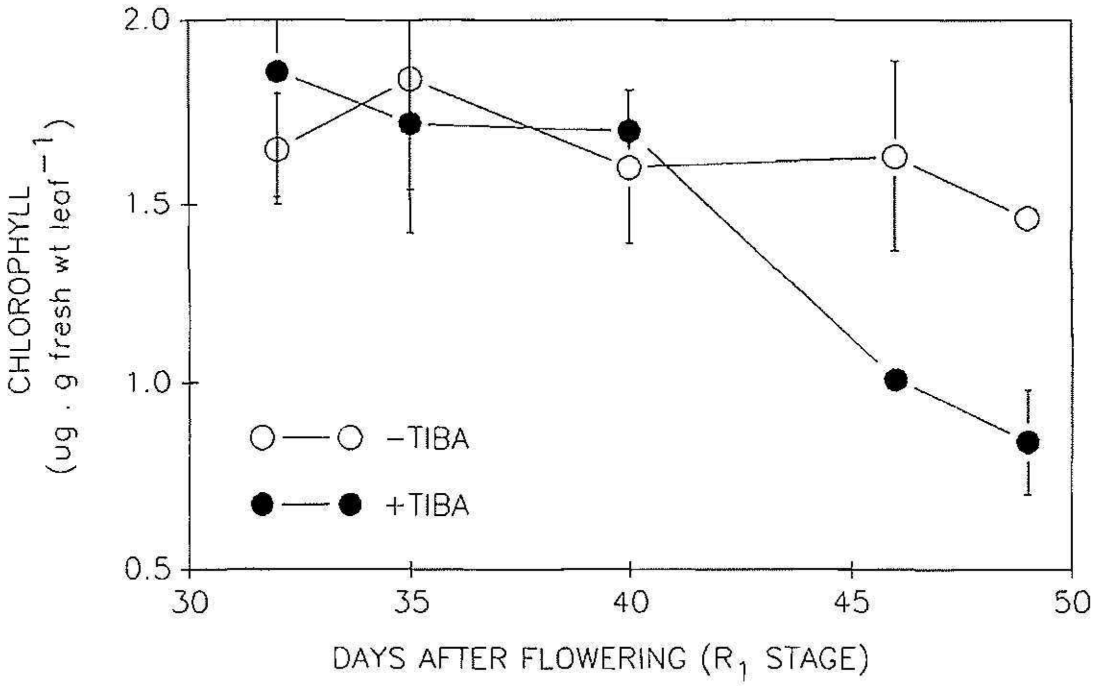

FIG. 4.—Effect of TIBA on postflowering chlorophyll concentration in uppermost fully expanded trifoliate leaf of field grown *Phaseolus vulgaris* L. plants. Each point in the figure represnts the mean of four replicate samples ± − SD.

The rate of 50 g TIBA/ha, which produced yield increases in soybean (3, 12), induced morphological differences including increased pod number, but no seed yield increases in the indeterminate dry bean genotype Puebla 152-B. This finding suggests that nitrogen fixation alone may not be sufficient for optimal development of the increased number of pods attributed to TIBA. Burton and Curly (5) found that with TIBA, inoculated soybean plants showed yield increases only when receiving a side dressing of fertilizer nitrogen. Since this experiment clearly showed that TIBA caused a shift from vegetative toward reproductive development, further evaluation of TIBA application may be necessary under higher fertilizer nitrogen levels. Such studies could reveal a yet untapped economic potential associated with TIBA-induced morphological alterations of the bean plant.

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