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Short Communication

Effect of Bentazon, a Hill Reaction Inhibitor, on Symbiotic Nitrogen-fixing Capability and Apparent Photosynthesis[1]

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ABSTRACT

Symbiotic associations of bean plants (*Phaseolus vulgaris* L. cv. Blue Lake) and *Rhizobium phaseoli* strain 127K17 were treated with the Hill reaction inhibitor bentazon (3-isopropyl-1 H-2,1,3-benzothiadiazin-4-(3H)-one-2,2-dioxide). Plants receiving foliar and root treatments of 1.8 kilograms per hectare bentazon were assayed at 6 hour intervals for N_2-fixing capacity by measuring C_2H_2-dependent C_2H_4 production and H_2 evolution and for CO_2 exchange rates. In foliar treated plants greatest measured inhibition of CO_2 exchange rates and N_2-fixing capacity occurred 6 and 12 hours after treatment, respectively. In root-treated plants maximum inhibition of both processes was delayed by 6 hours, and was less severe than in foliar treated plants. Nitrogen-fixing capacity and CO_2 exchange rate recovered to control levels in all plants. Application of higher rates of bentazon resulted in greater inhibition of CO_2 exchange rate and N_2-fixing capacity. Inhibition of the two processes was positively correlated (r = 0.985). The results indicate that inhibition of N_2-fixing capacity was not caused by bentazon directly, but indirectly through limiting the availability of photosynthate to support root nodule activity.

Dependence of symbiotic N_2 fixation on energy derived from photosynthesis is a well established concept (2, 5, 13). Controversy exists over the effect of environmental factors which presumably could alter short term rates of N_2 fixation by affecting photosynthesis (4, 10, 12). An acropetally translocated Hill reaction inhibitor (8, 11) such as bentazon (6), when applied to resistant plants (9), may provide a convenient experimental tool for correlating short term changes in apparent photosynthesis, estimated as CER,[2] with corresponding changes in N_2 fixation. The present study was undertaken to test the validity of this approach and to determine any direct inhibition of N_2-fixing capability by bentazon.

MATERIALS AND METHODS

Bean (*Phaseolus vulgaris* L. cv. Blue Lake) plants were grown in an environmental chamber under 16/8-hr light/dark cycle at 26/21 C, 50/70% RH, and photosynthetic photon flux density of 500 $\mu E/m^2$ sec measured in the photosynthetically active range. Plants were grown in vermiculite in 600-ml plastic pots, and inoculated with *Rhizobium phaseoli* strain 127K17 (obtained originally from J. C. Burton, The Nitragin Co., Milwaukee, Wis.). Plants were watered daily with nutrient solution containing 4 mM $CaSO_4$, 2 mM K_2HPO_4, 1 mM K_2SO_4, and 1 mM $MgSO_4$. Micronutrients were according to Johnson *et al.* (7), except that 4.2 nM $CoCl_2$ was added. Plants were treated with 1.8 kg/ha of bentazon (provided by BASF Corp.) when the first trifoliate leaf was one-third expanded. Bentazon was applied to the foliage with a greenhouse belt sprayer, or equivalent amounts were dissolved in nutrient solution and added to each pot. The equivalent of 9.4 l/ha of nonphytotoxic oil (Sun 11E) was added to the foliar sprays to increase wetting and penetration; control plants were sprayed with the adjuvant solution minus the herbicide. Apparent photosynthesis, C_2H_2-dependent C_2H_4 production, and H_2 evolution assays were made on control plants at the time of treatment and on treated plants at 6-hr intervals thereafter. Plant response to different inhibitor concentrations (0.45, 0.90, 1.80, and 3.60 kg/ha) was determined 12 hr after foliar treatment. All plants were kept in the light continuously following treatment. Light and temperature conditions in the assay chamber were the same as during growth. Nitrogen-fixing capability was computed as (C_2H_2-dependent C_2H_4 production—H_2 evolution in air)/3 (3), and apparent photosynthesis, estimated as CER, was determined by differential IR gas analyses as described previously (3).

RESULTS AND DISCUSSION

Foliar treatment of bean plants with bentazon resulted in a rapid decrease in apparent photosynthesis (Fig. 1). At no time in the course of these experiments did the inhibitor produce any visible symptoms in the plants. A minimum value of CER was measured 6 hr after spraying, but approximately pretreatment levels of CER were recorded again within 24 hr (Fig. 1). Nitrogen-fixing capacity also declined significantly, but the decline lagged behind that of CER by 6 hr in attaining its minimum value. Recovery in N_2-fixation capability commenced only after positive CER had been regained.

Addition of bentazon to the rooting medium, at a rate equivalent to that used in the foliar treatment, also resulted in decreased CER and N_2-fixation capacity (Fig. 2). Inhibition of both traits was not so severe, and occurrence of the minima was delayed by 6 hr when compared to data from foliar treated plants. The minimum in N_2-fixing capability in the root-treated plants again paralleled that of CER with the 6-hr delay.

Lower levels of inhibition in root-treated plants may indicate a loss of active ingredient through adsorption to, or leaching from, the Vermiculite (1). If the rate of inhibitor uptake by roots is reduced in a tolerant plant, activity would be expected to be lower as the detoxification mechanism can maintain the inhibitor available at the active site at a lower level. The delay in maximum response of the measured processes in root-treated plants relative to that observed in foliar treated plants probably reflects time of absorption and translocation to the active sites in the chloroplasts. A similar delay in activity has been previously reported for root *versus* foliar uptake of bentazon by rice (11). The identical delay

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[2] Abbreviation: CER: CO_2 exchange rate.

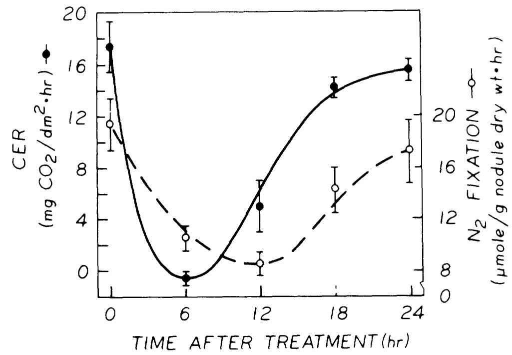

FIG. 1. Nitrogen fixation and CO_2 exchange rate (CER) in *P. vulgaris-R. phaseoli* associations treated with 1.8 kg/ha bentazon as a foliar spray. Means ± SE were computed from four replicate plants. Nitrogen fixation rates were computed from C_2H_2-dependent C_2H_4 production and H_2 evolution data by the formula: (C_2H_2 reduced-H_2 evolved)/3.

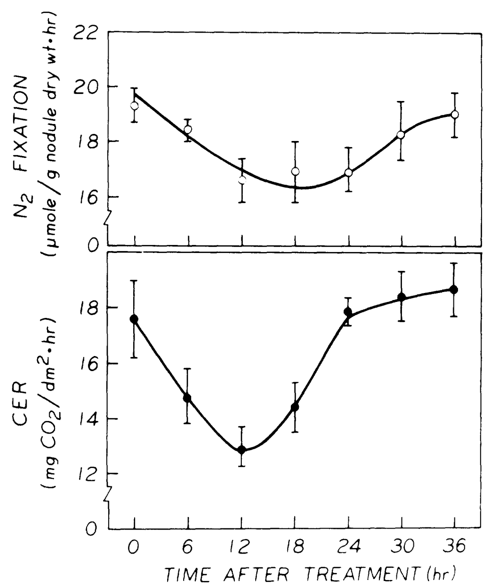

FIG. 2. Nitrogen fixation and CO_2 exchange rate (CER) in *P. vulgaris-R. phaseoli* associations treated with 1.8 kg/ha bentazon as a root drench. Means ± SE were computed from four replicate plants. Nitrogen fixation rates were computed as in Figure 1.

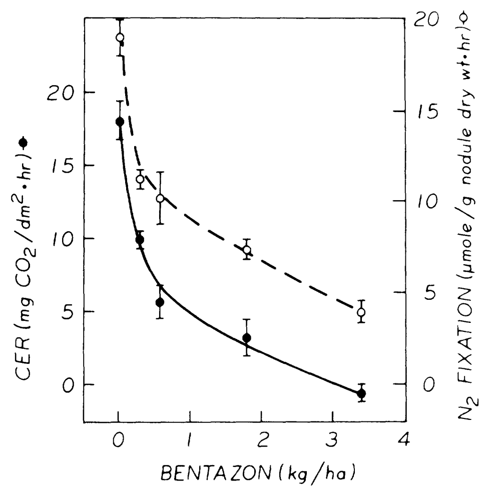

FIG. 3. Nitrogen fixation and CO_2 exchange rate (CER) in *P. vulgaris-R. phaseoli* associations 12 hr after foliar treatment with various concentrations of bentazon. Means ± SE were computed from four replicate plants. Nitrogen fixation rates were computed as in Figure 1.

Table I. Inhibition of CO_2 exchange rate and N_2-fixing capability in Phaseolus vulgaris-Rhizobium phaseoli associations 12 hr after foliar treatment with bentazon.

Data represent a linear function (y = 0.79 x) with correlation coefficient r = 0.985. The untreated control values were: CO_2 exchange rate 18.2 ± 1.3 ($mg CO_2/dm^2 \cdot hr$); N_2-fixing capability, 19.0 ± 1.2 ($\mu mole$/g nodule dry weight·hr). Percent inhibition was calculated as (1-treatment/control) x 100; treatment and control values represent the means of four replicates. Nitrogen-fixing capability was computed from C_2H_2-dependent C_2H_4 production and H_2-evolution data by the formula: (C_2H_2 reduced-H_2 evolved)/3.

	Bentazon (kg/ha)				
Reaction measured	0	0.45	0.90	0.80	3.60
	Inhibition (%)				
CO_2 exchange capability	0	45.1	69.2	81.9	100.0
N_2-fixing capability	0	41.2	45.8	66.5	78.4

times between inhibition maxima of CER and N_2 fixation capacity in root and foliar treated plants indicate that bentazon does not affect nodule activity directly, but probably acts indirectly by restricting the availability of photosynthate. Direct action on nodule activity by bentazon should have resulted in a rapid, CER-independent decrease in N_2-fixing capability in the root-treated plants. The minimal amount and slow rate of basipetal translocation of bentazon in legumes (8) support this interpretation. When photosynthate availability to root nodules presumably was varied through different rates of inhibitor application to foliage, declines in N_2-fixing capability paralleled decreases in CER (Fig. 3). A direct, linear correlation (r = 0.985) existed between the inhibition of the two traits by bentazon applied at different concentrations (Table I).

The present results indicate that N_2 fixation, as measured by C_2H_2-dependent C_2H_4 production and H_2 evolution, in 18-day-old blue lake beans is dependent on recently translocated photosynthate, but do not rule out the possibility of utilization of stored photosynthate in other *Rhizobium*-legume symbioses. The close correlation between the inhibition of CER and N_2-fixing capability supports the concept (2, 5, 13) that N_2 fixation on the association level depends directly on availability of photosynthate. The rapid response of N_2-fixation capacity to inhibitor concentration-dependent changes in CER may provide a useful technique for studying the relationships between N_2-fixation and photosynthesis. This technique is dependent upon the inherent capacity of the bean plant to metabolize and inactivate (6, 8, 9) the inhibitor. Other photosynthetic inhibitors which cannot be inactivated, such as DCMU, would not permit this experimental approach because photosynthesis, and thus CER, would not recover.

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