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-1H,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 N2-fixing capacity by measuring C2H2-dependent C6H6 production and H2 evolution and for CO2 exchange rates. In foliar treated plants greatest measured inhibition of CO2 exchange rates and N2-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 CO2 exchange rate recovered to control levels in all plants. Application of higher rates of bentazon resulted in greater inhibition of CO2 exchange rate and N2-fixing capacity. Inhibition of the two processes was positively correlated (r = 0.985). The results indicate that inhibition of N2-fixing capacity was not caused by bentazon directly, but indirectly through limiting the availability of photosynthate to support root nodule activity.

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 N2-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 N2-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 N2-fixation capacity 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
times between inhibition maxima of CER and N₂ 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 N₂-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 N₂-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 1).

The present results indicate that N₂ fixation, as measured by C₂H₂-dependent C₃H₄ production and H₂ 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 N₂-fixing capability supports the concept (2, 5, 13) that N₂ fixation on the associa-

![FIG. 1. Nitrogen fixation and CO₂ 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 C₂H₂-dependent C₃H₄ production and H₂ evolution data by the formula: (C₂H₂ reduced-H₂ evolved)/3.](image1)

![FIG. 2. Nitrogen fixation and CO₂ 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.](image2)

![FIG. 3. Nitrogen fixation and CO₂ 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.](image3)

![Table 1. Inhibition of CO₂ exchange rate and N₂-fixing capability in *Phaseolus vulgaris-Rhizobium phaseoli* associations 12 hr after foliar treatment with bentazon.](table)

<table>
<thead>
<tr>
<th>Reaction measured</th>
<th>CO₂ exchange capability</th>
<th>N₂-fixing capability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction measured</td>
<td>0.45 0.90 0.80 3.60</td>
<td>0 41.2 45.8 66.5 78.4</td>
</tr>
<tr>
<td>Inhibition (%)</td>
<td>45.1 69.2 81.9 100.0</td>
<td>0 41.2 45.8 66.5 78.4</td>
</tr>
</tbody>
</table>

**LITERATURE CITED**

- JOHNSON CM, FR STICK, TC BROKER, AB CARLTON 1975 Comparative chlorine requirements of different plant species. Plant Soil 83:37-353