Short Communication

The Inhibition of Soybean Metabolism by Cadmium and Lead

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ABSTRACT

Lead (300 μM) and cadmium (18 μM) inhibit pod fresh weight in soybeans (Glycine max L.) by 35%. Eighteen micromolar cadmium caused a 30% decline in nitrogenase activity by day 52 (the day on which maximum activity was measured) and a 71% inhibition by day 59. The heavy metals depressed photosynthetic rates; when photosynthesis was depressed by 60%, as measured on the day of peak photosynthesis activity, carbohydrate did not accumulate in the nodules. The reduction of pod fresh weight correlated with the effect of lead and cadmium on several other aspects of plant metabolism (shoot, root, leaf, and nodule dry weight; nodule ammonia, protein and carbohydrate content).

Preliminary studies of lead in soils (3, 4, 7, 8, 10) have, as might be expected, been concerned with biological magnification upon entrance of these heavy metals into the human terminal food chain (2–4, 10, 11). There is another potentially economically important aspect of this problem: How do the high concentrations of lead and cadmium that one finds along roadways affect crop production? Lead is known to inhibit plant growth (14). Pb and Cd also inhibit photosynthesis by isolated chloroplasts (9), and activities of isolated corn mitochondria (5, 6).

In this paper we report the effects of Pb and Cd on nodule formation, photosynthesis, and production of soybean plants grown in media containing various concentrations of the two heavy metals.

MATERIALS AND METHODS

Soybean seeds (Glycine max L. Merr. var. Beeson) were inoculated with a commercial strain of Rhizobium japonicum and cultured in a glasshouse in pots of sand-vermiculite (1:1, v/v). All experiments were done between 3 June 1973 and 30 August 1973. Plants were irrigated daily to saturation with nitrogen-free nutrient solution (1.6 mM MgSO₄, 0.8 mM KH₂PO₄, 4.0 mM KCl, 6.3 mM CaCl₂, 0.1 mM MnCl₂, 0.4 μM Na₂MoO₄, 0.3 μM CuSO₄, 0.7 μM ZnSO₄, 46.2 μM H₂BO₃, 4.9 μM Fe₂C₂H₂O₄, adjusted to pH 7.0 with 1 N KOH). Following the 2nd or 29th day after sowing, Pb and Cd were added at the indicated concentrations for the daily watering of the various experimental plants. The low binding capacity of the sand-vermiculite mixture, together with the daily watering regimen, afforded ion activities which were very close to the ion concentrations. As a precaution against accumulation of the heavy metals, all plants were excessively irrigated with glass-distilled water every 7th day. Plants of each treatment were killed at the indicated intervals for measuring the photosynthetic rate (1), and nodular nitrogenase activity (as in ref. 13, except that 20 ml bottles were used). Ammonia (Nesslerization), soluble carbohydrate (anthrone), and soluble protein (Lowry-Folin) were determined (12) by assaying the 20,000g supernatant solution of thoroughly homogenized nodules. Dry weights (overnight at 85 C) of nodules, roots, stems, and leaves were determined. Fresh weight of pods were determined on the terminal dates of the experiments.

RESULTS AND DISCUSSION

Pod fresh weight was more severely affected by Cd than Pb (Fig. 1). Cd at 18 μM had approximately the same effect as 300 μM Pb. At these concentrations, the reduction in legume fresh weight was approximately 35%. Whereas 50 μM Pb had no effect on legume fresh weight, concentrations of Cd as low as 18 μM had an appreciable effect on legume fresh weight.

The reduction of legume fresh weight correlated with the effect of these heavy metals on several aspects of plant metabolism (Table I). Of all parameters measured, Cd most dramatically affected nitrogenase activity (acetylene reduction), with 18 μM causing a 71% decline. The effect of this nitrogenase inhibition is clearly reflected in the accumulation of leaf dry weight; other aspects of metabolism were variably affected (Table I). The inhibition of photosynthesis at 18 μM Cd resulted in a much lower nodule carbohydrate concentration, and this
may have been at least partially responsible for the inhibition of nitrogenase.

Time course experiments, in which the metals were present from emergence (Figs. 2 and 3), indicated that the condition of the plant on the terminal day of the experiment (Table I) was reflected throughout the growth period. For example, cadmium at 18 μM depressed nitrogenase activity 30% at day 52, the day on which maximum activity was measured (Fig. 2A). Beginning heavy metal treatment on day 30 had a less severe effect on nitrogenase activity, with maximum activities being postponed approximately 6 days (Fig. 2, B and D).

The effects of Cd and Pb on photosynthesis were concentration-dependent (Fig. 3). Generally, Cd was more effective than Pb in inhibition of photosynthesis, especially when the metals were introduced at emergence, and photosynthetic rates were determined on the day of peak photosynthetic activity (Fig. 3, A and C, day 30). When photosynthesis was depressed by approximately 60%, as measured on the day of peak photosynthetic activity (450 μM Cd, 500 μM Pb), carbohydrate did not accumulate in the nodules (Fig. 3, A and C, insets).

CONCLUSIONS

Lead and Cd inhibited plant metabolism generally, as shown by their reduction of shoot and root growth and pod fresh weight. Key processes affected were the interdependent photosynthesis and nitrogen fixation. Lead was less effective than Cd, and addition of the metals at emergence, which more closely simulated the natural field condition, had a greater effect than addition at day 30.

Environmental concentrations of Pb and Cd have increased in recent decades because of several industrial processes. These experiments indicate that these heavy metals could affect plant growth and yield in agricultural areas near busy highways and

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Table I. Effect of Lead and Cadmium on Various Aspects of Soybean Metabolism

Plants were irrigated daily with nutrient solution containing the various concentrations of lead and cadmium. On day 59 (see Fig. 1) the plants were harvested and assayed (measured).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nodule Acetylene Reduction (nmole/plant·0.5 hr)</th>
<th>Legume (pod)</th>
<th>Root</th>
<th>Shoot</th>
<th>Leaves</th>
<th>Photosynthesis</th>
<th>Nodule NH₃⁺</th>
<th>Nodule Protein</th>
<th>Nodule Carbohydrate</th>
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<tr>
<td></td>
<td>g dry wt/plant</td>
<td>g fresh wt/plant</td>
<td>g dry wt/plant</td>
<td>wt CO₂/g dry leaf/hr</td>
<td>μg/plant</td>
<td>mg/plant</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cd Pb μM</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>0.95</td>
<td>3.07</td>
<td>4.45</td>
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<td>3.10</td>
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FIG. 2. Effect of Pb and Cd on nodule weight/plant and on acetylene reduction (nitrogenase activity) in soybean. A and C: The heavy metals were added beginning at seedling emergence; B and D: the heavy metals were added beginning at 30 days after seedling emergence. Notice that the vertical axis is entirely linear, but scaled differently in two sections. Inset axes are all as those in A.
Fig. 3. Effect of Pb and Cd on photosynthesis and nodule carbohydrate content of soybean plants. A and C: The heavy metals were added beginning at seedling emergence; B and D: the heavy metals were added beginning at 30 days after seedling emergence. Results are expressed in terms of leaf dry weight. Inset axes are all as those in A.

LITERATURE CITED