The Influence of Soil Zinc on Nodulation, Mycorrhizae, and Ozone-Sensitivity of Pinto Bean

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ABSTRACT

The effect of soil zinc amendments on nodulation, vesicular-arbuscular mycorrhizae, and ozone sensitivity of pinto bean was studied. Zinc amendments of 45 and 135 μg/g decreased both nodulation and mycorrhizae when compared to levels in nonamended soil. Ozone sensitivity of plants increased as available soil zinc increased.

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The consequence of repeated application of metals to soil, whether from industrial sources (8, 12), sewage sludge (2, 9), or other sources prompted this study. Zinc (Zn) was selected because it is found in large amounts in sewage sludge (2, 9) and industrial wastes (4, 12). Further, Zn is an essential element for plants and interacts with several essential micronutrients and macronutrients (13). Because the effects of Zn on soil microorganisms are poorly documented, its effect on the nodulation and development of vesicular-arbuscular mycorrhizae (VAM) was investigated. Elevated levels of atmospheric ozone and soil Zn may occur simultaneously; thus it is also appropriate to determine whether a relationship exists between the two which affects plants.

MATERIALS AND METHODS.—*Phaseolus vulgaris* L. 'Pinto 111' seeds were planted 4 per pot in 10 cm plastic pots containing approximately 400 g of soil. The pots were maintained in a controlled environment growth chamber with a 12 hour photoperiod temperature of 25 ± 2 C, 60 ± 4% relative humidity, and fluorescent-incandescent light energy between 400 nm and 700 nm of 160 microeinsteins/m^2/sec; nycto period conditions were 16 ± 2 C and 75 ± 4% relative humidity. One week after planting seedlings were thinned to 2 uniform plants per pot.

The soil used in this study was a Hagerstown loam, pH 6.5, cation exchange capacity 14.9 collected in an area of permanent pasture. The exchangeable K, Mg, and Ca levels were 0.48, 1.8, 10.5 meq per 100 g soil, respectively. The available nutrients as determined by the soil-test method of Baker (3) were as follows: Fe 0.54 μg/g, Mn 1.20 μg/g, Cu 0.078 μg/g. The soil Zn levels were adjusted by atomizing suitable concentrations of Zn sulfate solution into the soil as it was mixed in a soil mixer. The Zn additions were equivalent to 0 (check), 18, 45, or 135 μg/g Zn on a dry soil weight basis. Analysis of the soils by the baker method showed corresponding available Zn levels of 0.60 (check), 0.66, 1.55, and 4.18 μg/g. The levels represent a gradation from background (0.60 μg/g) to phytotoxic (4.18 μg/g) concentration. Zinc levels in Pennsylvania are within this range, although levels as high as 8 percent (4) have been reported near Zn smelters.

Fifteen days after planting, half of the plants at each Zn level were exposed to 582 μg/m^3 (0.30 μg/g) ozone, as measured by 1% KI (10), for 4 hours at 22 ± 2 C, 70 ± 2% relative humidity, and 300 microeinsteins/m^2/second light energy. Ozone injury to primary leaves was evaluated 3 days after ozonation. Percent tissue injured was estimated using a continuous scale of 0 to 100, with 0 representing no visible symptoms and 100 representing complete necrosis. Roots of ozonated plants were not examined.

The remaining plants were maintained under similar conditions in activated-charcoal-filtered air during

TABLE 1. Influence of various concentrations of soil zinc on growth, nodulation, mycorrhizal development, and ozone sensitivity of pinto bean

Zinc concentration (μg/g)		Oven-dry shoot wt.	Fresh root wt.	Nodules/ g root	V.A.M.[a] infection	Ozone injury
Added	Available	(g)	(g)	(no.)	(%)	(%)
0.0	0.60	0.74[be]x	2.77[b]x	3.25[b]xy	35.7[c]x	21[d]x
18.0	0.66	0.69 x	2.43 x	5.58 x	27.2 xy	23 xy
45.0	1.55	0.62 x	2.16 x	1.33 y	18.6 y	28 xy
135.0	4.18	0.70 x	2.35 x	1.96 xy	21.2 y	32 y

[a]V.A.M. = vesicular-arbuscular mycorrhizae.
[b]Each value is the mean of 24 observations in two experiments.
[c]Each value is the mean of 180 observations in two experiments.
[d]Each value is the mean of 48 observations in four experiments.
[e]Values followed by the same letter in the same column are not significantly different, $P = 0.01$.

ozonation and for an additional 2 weeks. The effect of Zn on nodulation by *Rhizobium* sp. and VAM formation by *Endogone* sp. was determined for the nonozonated plants at this time, 4 weeks after planting. Inoculum for establishing the symbiotic relationships was furnished by components of the natural soil population. Plants were harvested, and the oven-dry weight of the shoots determined. The soil was removed from the plant roots by gently washing the root system under running tap water. Roots were patted dry on paper towels and weighed. The number of root nodules was determined and the number of nodules per gram of root fresh weight was computed. To determine mycorrhizae infections, samples of fresh root tissue 1.0 cm long were collected from the center of the root ball and cleared in 10% KOH and stained in lactophenol trypan blue according to the method of Phillips, et al. (14).

The average percent mycorrhizae infection of 30 root samples was determined for each pair of plants replicated three times in two experiments.

Data for all experiments were subjected to analysis of variance using a complete randomized block design. Means were separated by Duncan's modified (Bayesian) least significant difference test (15).

RESULTS AND DISCUSSION.—There was no significant effect of Zn on either oven-dry shoot weight or fresh root weight (Table 1). The number of nodules per gram of root was greatest in those plants growing in soils having the two lowest rates of Zn (0.60 and 0.66 μg/g available). Nodulation was reduced in plants grown in soils amended with the two highest rates of Zn. The development of mycorrhizal infections in the roots of the plants was also reduced at the higher rates of added Zn. The reduction in nodulation and development of VAM at the higher zinc levels ultimately could result in reduced quality or quantity of the final products over a longer period of time than used in this experiment.

The percent ozone injury on foliage of plants grown in soil amended with 18 or 45 μg/g Zn (0.66 or 1.55 μg/g available Zn, respectively) increased with increasing Zn concentration, but the differences were not significant (Table 1) when compared with check plants grown in the nonamended soil (0.60 μg/g available Zn). The ozone injury to plants grown in soil amended with 135 μg/g Zn (4.18 μg/g available Zn) was significantly greater than the check plants.

Various nutrients such as sulfur (1) phosphorous (11), and copper and iron (5) are known to influence plant sensitivity to ozone. In this study, ozone injury increased with increasing available soil Zn. This is consistent with the results recently reported by Czuba and Ormrod in work with cress and lettuce (6, 7). The effect of Zn on ozone sensitivity reported herein demonstrates the need to be aware of the micronutrient status of plants grown in areas subject to oxidant air pollutants.

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