Zinc Tolerance in *Panicum virgatum* L. (Switchgrass*)* from the Picher Mine Area

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Samples of *Panicum virgatum* L. taken from zinc-contaminated soils in the Picher mine fields, Ottawa County, OK were shown, by root growth assays, to be more tolerant to $Zn(NO_3)_2$ in aqueous solution than plants from an uncontaminated site in Payne County, OK. Among plants from the Picher area, those from soils containing the highest levels of zinc were generally more tolerant than plants from less metalliferous soils. Soil contamination in the area may provide significant environmental pressure to select for zinc-tolerant variants within this species.

INTRODUCTION

Tolerance to extreme levels of heavy metals in the soil has provided a fertile field for botanical investigation. Plants that survive in areas subject to metal contamination from mining activities usually have been found to evolve tolerant ecotypes in response to the level of metal in the soil (1). The list of species known to include such ecotypes is quite long (2,3). By contrast, only a few species have been reported to display constitutional metal tolerance without evolving tolerant ecotypes (3). One such species, *Andropogon virginicus* L., was studied by Gibson and Risser (4) in a population from the Picher mine area of northeastern Oklahoma.

Heavy metal tolerance has been investigated in the Poaceae and found to be common in this family (5). *Panicum virgatum* (Poaceae, Paniceae), commonly called switchgrass, is capable of growth on a variety of soil types. It is present throughout the Picher mine area on soils likely to be metalliferous based both on proximity to sources of contamination and on absence of other vegetation. Previous studies on this species have shown ecotypic variability in terms of culm height, flowering culm height, leaf size, pubescence, and glaucousness (6). It is the focus of the study now reported to investigate whether zinc-tolerant individuals of *P. virgatum* occur in areas subject to heavy metal contamination of the soil.

STUDY AREA

The Picher mine field is located in Ottawa County, OK. During the late 1920's and early 1940's, lead and zinc mining reached peak levels (7). Mine waste materials containing unextractable, low-grade metals were accumulated in large heaps. Leachate from tailing heaps, aerial deposition of dust, and seepage from flooded mine shafts are the most likely sources of zinc that contaminates the upper layers of soil

The herbaceous flora of the areas surrounding the heaps is composed of disturbance indicator species such as *Andropogon virginicus, Ambrosia trifida* L., and *Specularia perfoliata* (L.) A. DC., as well as climax prairie species such as *Panicum virgatum* and *Sporobolus asper* (Michx.) Kunth (8). Although these species are present in the area, the vegetation is patchy, and much soil is barren of vegetation.

MATERIALS AND METHODS

Plants were collected from four sites in the Picher mining area during March 1987. Each site was located on the banks of Tar or Lytle Creek, or in one case along a ditch draining into Tar Creek. The pH of the water at the collection sites was measured in the field with an Orion 231 pH meter. At each site, the rhizomes and culm bases from one clump (presumably a single genotype) of *P. virgatum* were collected. A plant was also collected from a population northwest of Stillwater, OK, to serve as a control. Plants were transferred to a greenhouse and

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placed in potting soil to propagate test material.

Soil collected in the field from the area directly surrounding the roots was subjected to zinc content analysis. Samples were oven dried and passed through a Newark #10-mesh sieve (0.34 inch opening). Ten grams of soil was heated in 20% nitric acid for 7 hr and the mixture filtered. The filtrates were analyzed by atomic absorption spectrophotometry to determine the content of zinc in the soil. This technique estimates total zinc content of the soil, and the values derived may be only qualitatively related to zinc bioavailability to plants.

To test for tolerance, tillers were harvested from the greenhouse materials. Individual, rootless tillers were suspended in 25 × 150 mm test tubes containing Hoagland's nutrient solution and placed under 24 hr illumination. When a tiller had produced a root of 1 cm, the nutrient solution was replaced with either distilled H_2O or one of two solutions of $Zn(NO_3)_2$; these contained either 0.45 mg/L Zn (low) or 0.91 mg/L Zn (high). Four replicates of each treatment were used in testing tillers from the five plants. After 5 days exposure to the treatment, the length of the root was measured.

In other studies of root growth in metal solutions, various media have been employed, including complete nutrient solutions and $Ca(NO_3)_2$ solutions. Such media are important when prolonged experiments are conducted, but unexpected interactions among solutes may also occur (3, 9). In this study, all tillers were initially rooted in Hoagland's solution, but testing was carried out in solutions of $Zn(NO_3)_2$ only. Preliminary studies had shown that no visible deficiency symptoms occurred over the 5-day period employed for testing.

RESULTS

Soil analysis data and brief site descriptions are recorded in Table 1. There was a large difference, as expected, in zinc content between soils from Picher sites and the Stillwater control site, and considerable variation among the Picher sites.

Mean root lengths of tillers in distilled water were similar (Fig. 1). In the low-zinc treatment, differences in root growth were evident. The control plant displayed inhibition, while the mine plants showed a variety of responses. The roots of control tillers were shorter still at the high zinc concentration. Mine plants became more similar to each other in root growth in the high treatment and had roots longer than those of the control plant. The root length data were used to compute a tolerance index for each plant in the zinc treatments, where

$$\text{Tolerance Index} = \frac{\text{mean root length in Zn solution}}{\text{mean root length in pure } H_2O} \times 100$$

TABLE 1. Description of collection sites

Site	Water pH	Soil Zn (μg/g)	Location
A	7.6	103	Stream by USDA facility NW of Stillwater (control)
B	5.5	3875	Confluence of Lytle Creek and Tar Creek, SW of Picher
C	2.9	8053	Roadside east of Site B, near seep from mine shaft
D	7.3	15632	Tar Creek at intersection with section rd.; 1.3 km W of Picher
E	7.1	16139	Lytle Creek at intersection with section rd; 3.2 km N and 100 m W of U.S. 66—69 junction

Tolerance indices and mean root lengths are presented in Table 2.

A two-way analysis of variance revealed statistically significant differences in root lengths among collection sites, among zinc test concentrations, and in the site × zinc interaction (Table 3). The last of these terms is the one of interest here, because it indicates that the plants from different collection sites responded unequally to zinc.

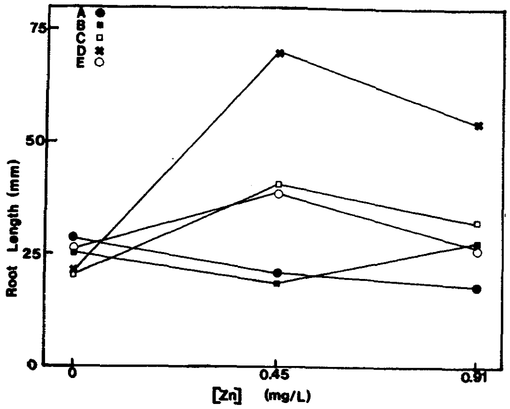

FIGURE 1. Mean root lengths of *P. virgatum* tillers after 5 days growth in distilled H_2O and in Zn solutions of two concentrations. Collection sites as indicated in upper left corner.

One-way ANOVAs for each zinc level were computed to locate specific differences between plants' growth. At 0 Zn, no significant difference was found between plants. In 0.45 mg/L Zn, differences in root growth were highly significant ($\rho <$ 0.0001). Comparisons of site means at this concentration were made using Tukey's Studentized Range Test (an *a posteriori* or unplanned comparison procedure), with α = 0.05. This test indicated that site D plants had significantly longer roots than all the other plants, and that those from C were significantly longer than those from B (Table 2). In 0.91 mg/L Zn, site means differed at the level ρ = 0.07. Although not a low enough probability for conventional rejection of the null hypothesis of equality of means, this value is at least suggestive of significant differences. Tukey's Test, with α set at 0.07, suggested (tentatively, because of the elevated probability level) that site D plants had significantly longer roots than site A (control) plants (Table 2).

DISCUSSION

The data obtained in this study show that plants from zinc-contaminated sites are more zinc-tolerant than the plant from the noncontaminated site. The plant from site D also showed a greater tolerance than all other plants from the Picher area, which is consistent with soil test data indicating very high zinc levels at site D.

The tolerance index for the Stillwater

TABLE 2. Mean root lengths (± S.E. of mean) and tolerance indices for *Panicum virgatum*. For each column, means with same letters are not significantly different according to Tukey's Test (α=0.005 for 0 and 0.45 mg/L; α= 0.07 for 0.91 mg/L).

	0 mg/L Zn	0.45 mg/L Zn		0.91 mg/L Zn	
Site	Root Length (mm)	Root Length (mm)	Tolerance Index (%)	Root Length (mm)	Tolerance Index (%)
A	28.6±2.0 a	21.1±5.6 ab	74.0	18.3±2.4 a	64.0
B	25.4±6.2 a	19.3±3.1 b	74.7	27.7±4.1 ab	108.8
C	20.3±2.1 a	40.8±1.7 a	201.0	32.5±5.5 ab	160.2
D	21.1±1.4 a	70.4±5.6 c	334.3	54.8±15.1 b	260.5
E	26.6±4.0 a	38.9±8.5 ab	146.5	26.4±6.3 ab	99.2

TABLE 3. Analysis of variance for root lengths.

Source	df	*SS*	*F*	*p*
Site	4	4669.41	8.41	< 0.0001
Zinc	2	1553.27	5.59	0.0075
Site × Zinc	8	4087.86	3.68	0.0030
Total	14	10310.54		

plant was less than 100 in both treatments, indicating inhibition by $Zn(NO_3)_2$. For the plants from contaminated sites, tolerance indices were in some cases considerably greater than 100, indicating stimulation of root growth. The cause of this stimulation could easily be nitrate ions from the zinc salt employed, although other causes, such as a stimulatory effect of zinc, cannot be ruled out. Future work might well use a different zinc salt, such as zinc acetate. In any case, the doubly controlled design used here, with both control plants from an uncontaminated site and control treatments without zinc, indicates differential inhibition response among plants.

Of prime importance to this study are the zinc concentrations used for tolerance testing. These concentrations were chosen after a survey of the literature to find concentrations used in similar studies of zinc tolerance (e.g. 10, 11). Had too low a concentration been used, differences in lengths might not have been evident. On the other hand, overly high concentrations could have caused inhibition of all plants. Our results verify that the range chosen was acceptable. Mine plants were tolerant of the low concentration, and began to show inhibition in the high-zinc treatment. (Plant from site B showed a small but nonsignificant deviation from this trend.) All mine plants were less inhibited at high zinc levels than was the control. It is important to note that zinc concentrations used in these experiments in no way represent zinc levels or bioavailability in metalliferous soils. The test concentrations merely serve as a benchmark for comparative analysis.

The tolerance found in this preliminary study is most readily explained as the result of ecotypic variation in response to the selective pressure of toxic soil metals. The tolerance detected here was present in tillers propagated under common conditions in uncontaminated media; thus, it is constant in cultivation, but progeny testing would be required to ascertain its genetic basis. To confirm the ecotypic explanation, wider population samples will be needed.

The only other study that has specifically looked for metal tolerance in the Picher mining area found that *Andropogon virginicus* from this area had an inherently high tolerance for zinc, and did not evolve local ecotypes (4). Thus, the differential tolerances found in the current study represent a contrasting situation for *P. virgatum*. A further novel aspect of the current research is that plants received much of their zinc from flowing stream water contaminated by upstream point sources (such as drainage from mine shafts). Studies of such riparian vegetation provide the opportunity to examine the evolution of tolerance in response to linear gradients of metal concentration, and may provide ideal systems for studies of clinal adaptation patterns.

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REFERENCES

1. A.D. Bradshaw, T.S. McNeilly, and R.P.G. Gregory, in: *Ecology and the Industrial Society, British Ecological Society Symposium* 5, 1965, pp. 327-343.
2. J. Antonovics, A.D. Bradshaw, and R.D. Turner, Adv. Ecol. Res. 7: 1-85 (1971).
3. A.J.M. Baker, New Phytol. 106 (Suppl.):93-111 (1987).
4. D.J. Gibson and P.G. Risser, New Phytol. 92: 589-599 (1982).

5. R.P.G. Gregory and A.D. Bradshaw, New Phytol. 64: 131-143 (1965).
6. C. McMillan, Am. J. Bot. 51: 1119-1128 (1964).
7. A. Gibson, *Wilderness Bonanza: The Tri-State District of Missouri, Kansas, and Oklahoma,* Univ. of Oklahoma Press, Norman, OK, 1972.
8. D.J. Gibson, Southwest. Nat. 27: 425-436 (1982).
9. D.A. Wilkins, New Phytol. 80: 623-633 (1978).
10. A.J. Pollard, New Phytol. 86: 109-117 (1980).
11. L. Wu and J. Antonovics, New Phytol. 75: 231-237 (1975).