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EXPERIMENTAL ECOLOGICAL GENETICS IN *PLANTAGO* II. LEAD TOLERANCE IN *PLANTAGO LANCEOLATA* AND *CYNODON DACTYLON* FROM A ROADSIDE¹

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Abstract. A population of *Plantago lanceolata* (ribwort plantain) from a roadside was found to have higher Pb tolerance than populations away from the roadside; this reflected the sharp differences in Pb content of the soil and the plants at these sites. Tolerance tests on seedlings showed that the Pb tolerance was transmitted to seed progeny. *Cynodon dactylon* (Bermuda grass) showed generally a higher Pb tolerance than *P. lanceolata*, but there was no evidence of a greater Pb tolerance of the roadside population when compared with populations from control sites. These results suggest that the Pb level found at the roadside was sufficiently high to impose selection pressure for the evolution of tolerance in a sensitive species, but no overt effect was seen in a species with a greater inherent tolerance. This provides another example of rapid and highly localized evolutionary change in plants.

Key words: *Cynodon dactylon*; lead; metal tolerance; *Plantago lanceolata*; pollution; roadsides.

INTRODUCTION

Numerous papers have pointed out the increased levels of heavy metals, particularly lead (Pb), in soils in the vicinity of roadsides (for reviews see National Academy of Sciences Report 1972, Antonovics et al. 1971, Cannon and Anderson 1971). Surprisingly there have been no studies on metal tolerance of plants from roadsides in spite of extensive evidence for the evolution of metal tolerance in plants growing on soils contaminated by mining and smelting activity (Antonovics et al. 1971, Wu and Bradshaw 1972, Wu et al. 1974). Pollution levels at roadsides, and in urban areas generally (Goodman and Roberts 1971), are frequently one or two orders of magnitude lower than levels found in mining waste, and it has been questioned whether such low levels are injurious to higher plants (National Academy of Sciences Report 1972). Populations of the liverwort, *Marchantia polymorpha*, growing in urban areas have been shown to be more tolerant to lead than rural populations (Briggs 1972). It is, therefore, clearly pertinent to ask whether evolutionary changes analogous to those in heavily contaminated areas have occurred in plant populations at roadsides.

The present study investigates Pb tolerance in *Plantago lanceolata* L. (ribwort plantain) and *Cynodon dactylon* (L.) Persoon from a roadside in the center of Durham, North Carolina, USA. Both species are widespread in North Carolina, and are commonly found along roadsides. They have been recorded in areas contaminated by mine wastes (Ernst

1968, Wild 1970, 1974) and plantain has been shown to evolve tolerance to Zn (Schwanitz and Hahn 1954).

MATERIALS AND METHODS

Plants of both species were collected from a transect perpendicular to a roadside (Main Street) with a very high traffic density in Durham, North Carolina. The transect extended into an intermittently mown field or lawn on East Campus, Duke University (Table 1). Previous investigations of this transect (Stanford et al. 1975) have shown that the soil and plants directly adjacent to the roadside contained greater levels of several metals than plants and soil sampled away from the roadside. Lead contents of plants and soil at the sites are summarized in Table 1. Lead analyses were carried out by R. Walter, R. Willis, and J. Stanford (Physics Department, Duke University), using proton induced X-ray emission analysis (see Walter et al. 1974 for details of the technique).

Lead tolerance of plantain was measured using both adults and seed derived materials. Five adult plants were collected from each site (E1, E2, E3, and C1), grown in the growth chamber, and cloned by separating the side shoots from the parent plant. The cloned material was grown for an additional 3 wk and prior to tolerance testing all the mature leaves and roots were removed from the rosette stalk. Within each clone one plant was grown in Hoagland's nutrient solution and the other in the same nutrient solution with 15.6 ppm lead added as $\text{Pb}(\text{NO}_3)_2$. There were two replicates for each sampled, individual, the treatments were fully randomized, and the culture solutions were changed every 3 days. The plants were grown in a growth

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TABLE 1. Lead content (ppm dry wt) of soil and plants along roadside transect from Main Street to East Campus, Durham, N. C.

Site	Description	Distance from roadside	Lead content ($\bar{x} \pm SE$)			
			Soil		Plants	
			Extractable ^a	Total ^b	<i>Plantago lanceolata</i> ^c	<i>Cynodon dactylon</i> ^d
E1	Roadside verge	0.5 m	365 $\pm$ 17	2,850	313 $\pm$ 28	40 $\pm$ 6
E2	Over a wall into East Campus field	4 m	4 $\pm$ 1	772	21 $\pm$ 6	10 $\pm$ 1
E3	Center of East Campus field	80 m	0.9 $\pm$ 0.3	200	6 $\pm$ 2	4 $\pm$ 0.8
C1	Control site, West Campus waste ground	----	0.4 $\pm$ 0.2	52	4 $\pm$ 0.5	----

^a Average of 3 samples 0.5 m acetic acid extractable. Sampled March 1974.

^b One sample. Sampled August 1974.

^c Average of 3 plants (5 plants, site E1), 3 leaves per plant. Sampled March 1974.

^d Average of 6 plants, ca. 4 leaves per plant. Sampled August 1974.

chamber at 21°C with a 9 h daylength. After 6 wk the plants were harvested, separated into roots and shoots (old rosette stalk plus newly produced leaves), and dried at 60°C. The index of Pb tolerance was measured as: (mean dry wt produced in solution with lead) $\div$ (mean dry wt produced in solution without Pb).

The experiment was repeated with 10 plants from E1 and E3, which were cloned by removing individual leaves from parent plants and treating with 3-indole-acetic acid (Wu and Antonovics 1975). The leaves subsequently develop roots and then young plants. Young plants of comparable size (two leaves and $\approx$ 4 cm high) were chosen for testing. The Pb tolerance test was the same as above except that leaves and roots were not removed and there were three replicates of each individual.

A number of previous studies have shown tolerance to heavy metals to be a highly heritable character (Antonovics et al. 1971). The inheritance of metal tolerance was investigated further in this study. Seeds from sites E1, E2, E3, and C1 were sown in the Phytotron. After 2 weeks tolerance was estimated for 36 seedlings from each population. For the tolerance test, elongation rate of the longest root of the seedlings was measured for 3 days in nutrient solution and for an additional 6 days in nutrient solution containing 15.6 ppm with added Pb; otherwise, the conditions of the experiment were the same as above. The index of Pb tolerance was estimated as: (increase in length per day of the longest root in solution with lead) $\div$ (increase in length per day of the longest root in solution without Pb).

Lead tolerance in Bermuda grass was estimated on 10 adults sampled from each of sites E1 and E3. The plant samples were cloned in a growth chamber for 3 mo and tolerance was estimated by measuring dry weight production in solutions with and without added Pb, simultaneously and in exactly the same

way as for plantains in the leaf shoot experiment described above.

A further study of the effect of different levels of Pb on the two species was performed by growing them together in a series of Pb concentrations in culture solution. Cloning and testing methods were the same as in the previous experiments. One individual from the roadside and one individual from the campus were used for each species.

To examine further the apparent lack of enhanced lead tolerance in Bermuda grass from the roadside, the experiment was repeated using a tiller rooting test such as is normally used for testing metal tolerance of grasses (Jowett 1964). Two other populations, one from Crandon Park, Miami, Florida and one from farmland in Cook County, Georgia were included in this test to see if the Durham populations in general had a higher Pb tolerance than populations from other areas. Five uniform tillers from each of the plants were grown in $\text{Ca}(\text{NO}_3)_2$ solution for 12 days with and without added Pb. Use of $\text{Ca}(\text{NO}_3)_2$ rather than a general culture solution also served to increase the Pb toxicity (Ernst 1968). The index of Pb tolerance was estimated as: (mean length of longest roots of five tillers in $\text{Ca}(\text{NO}_3)_2$ solution [0.5 g/liter]) $\div$ (mean length of longest roots of five tillers in $\text{Ca}(\text{NO}_3)_2$ solution [0.5 g/liter] without lead).

RESULTS

Roadside samples of ribwort plantain had a higher Pb tolerance than samples from the campus and from the control site (Fig. 1). There was a very sharp reduction in Pb tolerance away from the roadside which reflected the reduction in Pb content of the soil and the plants at these sites (Table 1).

The results of Pb tolerance testing from the cloned individuals of ribwort plantain (Fig. 2) again showed that the roadside population had a greater tolerance

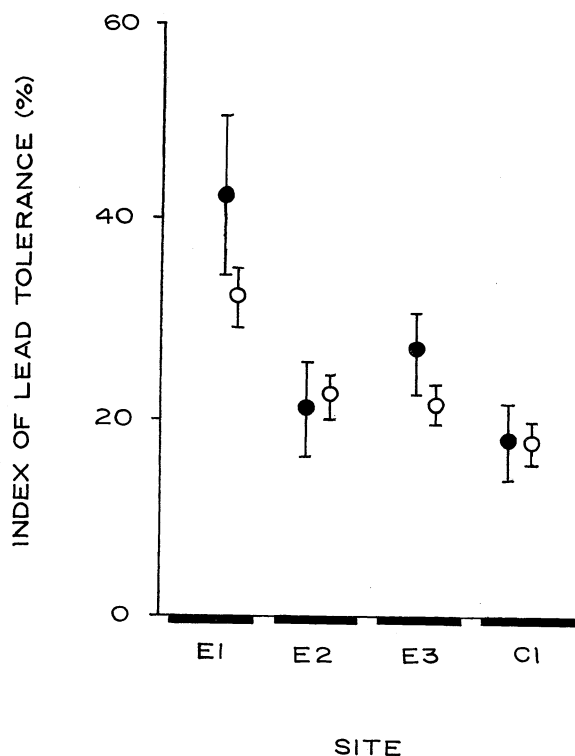


FIG. 1. Lead tolerance of adult (●) and seed (○) samples taken from populations of *Plantago lanceolata* from different sites in Durham, N.C. Lead contents of the soil and of the two species are indicated in Table 1. Sites E1, E2, and E3 represent a transect from the roadside into the lawn of East Campus and are at 0.5 m, 4 m, and 80 m from the edge of Main Street, Durham. The control site, C1, was situated on West Campus away from any heavily used roads. Bars on the graph represent SE of the means.

than the campus population: correlation between the tolerance estimated from the root dry weight and shoot dry weight was significant ($r = 0.78$; $n = 20$; $p < 0.01$).

Seedlings from the roadside plantain population had a higher average tolerance than seedlings from the three populations not heavily contaminated by Pb (Fig. 1). In addition the roadside population had eight seedlings with a high tolerance ($> 50\%$) whereas only one such individual was found in E2, and none in E3 and C1. This result indicates that the characteristics of Pb tolerance in the roadside population are transmitted to the seed progeny.

Bermuda grass populations had generally a higher Pb tolerance than the plantain populations but there was no evidence of a greater Pb tolerance of the roadside Bermuda grass population when compared with the campus population (Fig. 2).

Further study of the effect of different levels of Pb on the two species showed that whereas root

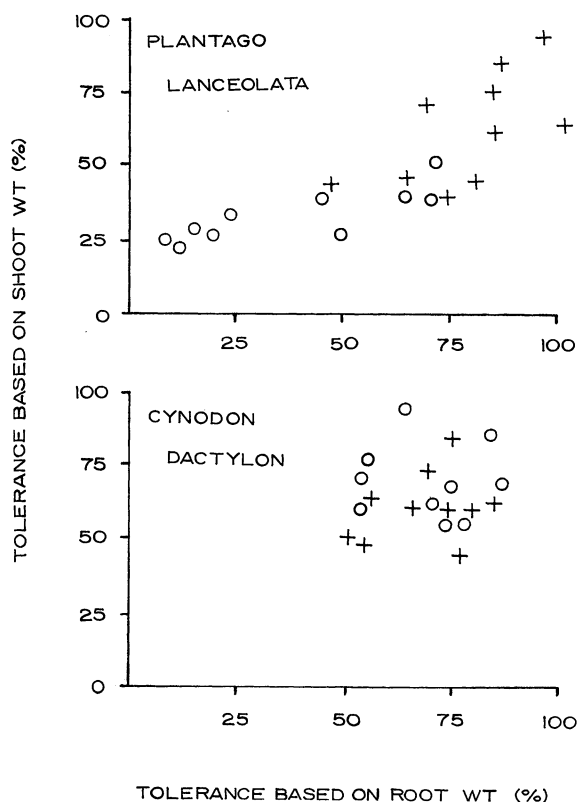


FIG. 2. Lead tolerance of individuals of *Plantago lanceolata* and *Cynodon dactylon* taken from site E1, roadside (+) and site E3, campus lawn (○).

growth was seriously inhibited (55%–91%) in plantain at 15.6 ppm lead, there was much less effect on Bermuda grass (17%–20% inhibition). At 31.2 ppm there was complete inhibition of root growth in plantain, but only partial inhibition (58%–70%) of root growth in Bermuda grass.

Results of the tiller rooting test showed that, even under this higher Pb toxicity, there were no significant differences between roadside (mean tolerance = $5.9 \pm 4\%$) and pasture (mean tolerance = $6.1 \pm 0.4\%$) populations. The control populations from Miami (mean tolerance = $6.3 \pm 0.2\%$) and Georgia (mean tolerance = $5.8 \pm 0.3\%$) did not differ significantly from the Durham populations.

DISCUSSION

It is clear from these results that the effects of Pb pollution are species dependent: there is evidence for evolution of tolerance to Pb in plantain yet no evidence for such evolution in Bermuda grass. Plantain has been shown to evolve tolerance to Zn (Schwanitz and Hahn 1954) on Zn contaminated mine soil and has been recorded in mine areas polluted with high Pb levels (Wild 1970); Pb tolerance

has not been, previously demonstrated in this species. Bermuda grass has been recorded in soil containing high levels of As, Ni, and Cu (Ernst 1968, Wild 1974). It appears that the differential evolutionary response of the two species may be related to their sensitivity to Pb. The Pb levels used in the rooting test of the different Bermuda grass populations were of the same order of magnitude as those used in Pb tolerance testing of other grasses from metal mines (Jowett 1964); in comparison with tolerant grasses from such metal mines, the roadside Bermuda grass shows an extremely low tolerance. This suggests that the Pb level found at the Durham roadside was sufficiently high to impose selection pressure for the evolution of tolerance in a sensitive species such as plantain but had no overt effect on a species with a greater inherent tolerance (cf. McNaughton et al. 1974). Plantain, where it is found on mine sites, is generally confined to areas of either low metal concentration or high organic matter, or both, and therefore low effective toxicity (Antonovics 1972).

The evolutionary response of organisms to man-made environmental effects is a frequent and well-documented occurrence. Lead pollution at roadsides has only been present for about 40 yr; and pollution levels fall off rapidly away from roadsides. The present study therefore provides another example of evolutionary change that is particularly rapid and localized.

It was stated in a 1972 National Academy of Sciences report, *Airborne Lead in Perspective* that, "... there seems to be no reliable evidence that lead injures plants in nature. In fact extensive investigations summarized by Bradshaw et al. (1965) show that some species of plants have adapted to habitats near mining operations that contain lead, zinc, and copper in amounts that are toxic to nonadapted populations of the same species." There is clearly an internal contradiction in this statement since it is precisely the injurious effects of Pb that result in natural selection for more tolerant genotypes. That such selection can occur at roadsides where lead levels are moderate compared with mine sites implies that lead may be injurious to many plant species but such injurious effects are not evident because the plants undergo concomitant evolutionary responses.

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