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Differential Tolerance of *Agrostis tenuis* Populations Growing at two Mine Soils to Cu, Zn, and Pb

By

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With 5 figures

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Summary

The Cu, Zn and Pb tolerance of *Agrostis tenuis* SIBTH. populations found in the area of two mines in England as well as in uncontaminated areas were studied by determining the effect of these metals on the rooting of tillers. The populations proved tolerant to the particular metals present in high quantities in the soil of their original habitats as compared to the populations collected from uncontaminated soil. The populations of the Trelogan mine were tolerant only to Zn and not to Cu and Pb. On the contrary, the populations in the mine of Parys Mountain were highly tolerant to all these metals. A linear correlation in the index of tolerance between Zn and Pb in both mines was found suggesting the possibility of a physiological association of the tolerance mechanisms to these two elements.

Zusammenfassung

Unterschiedliche Toleranz von *Agrostis tenuis*-Populationen aus der Umgebung zweier Bergwerke gegen Cu, Zn und Pb

Die Cu-, Zn- und Pb-Toleranz von *Agrostis tenuis* SIBTH.-Populationen aus der Umgebung zweier Bergwerke in England sowie aus unbelasteten Gebieten wurde anhand der Wirkung dieser Metalle auf die Bewurzelung der Ausläufer untersucht. Im Vergleich zu den Proben von unbelasteten Böden erwiesen sich die Populationen gegen die Metalle, die im Boden ihres Wuchsortes in hohen Quantitäten enthalten sind, widerstandsfähig. Die Pflanzen von der Trelogan Mine waren nur gegen Zn tolerant, nicht aber gegen Cu und Pb, hingegen waren die Pflanzen von der Mine Parys Mountain gegen alle drei

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Metalle tolerant. Eine lineare Korrelation der Toleranz-Indices von Zn und Pb legt die Möglichkeit eines physiologischen Zusammenhanges der Toleranzmechanismen gegen diese beiden Metalle nahe. (Editor transl.)

Introduction

For innumerable years man has been abusing the environment by extracting minerals, which polluted the environment because of their potentially catastrophic effects (SMITH & BRADSHAW 1972). Although extraction procedures remove the bulk of the metal involved, at least 1% is usually left behind, and this may be accompanied by other metals which were present in the ore in low levels and were not worth extracting (BRADSHAW 1970). The metal levels vary widely from one part of the mine to another. However, all parts are toxic for plant growth, not only because of the presence of high levels of toxic metals, but also because of the very low levels of the major plant nutrients. Moreover, mine soils are poor-structured and hold little moisture. At any rate, it is the high concentration in heavy metals which renders these soils unsuitable for plant growth.

GREGORY & BRADSHAW (1965) PROCTOR (1971), PROCTOR & WOODELL (1971), list some of the species which have been found on serpentine soils or in areas affected by the mining of metal ores. There are close similarities in the flora of these areas. Serpentine soils are often high in Ni and Cr, and these metals seem to determine at least partially the serpentine flora. This flora have been of interest not only to ecologists but also to prospectors of metal ores. As early as 1585 it was observed that the distribution of *Minuartia verna* (L) HIERN. is closely related to Pb and Zn contaminated areas. Various species have been found to grow on mine areas and in nearly all cases these species have also been found on ordinary soil (BRADSHAW 1952, GADGIL 1969, URQUHART 1970a).

The objective of the work reported in this paper was to investigate whether the prevailing conditions are such as to reflect on the manifestations, which, despite the unfavourable conditions, have succeeded in colonizing these inhospitable habitats and also whether these habitats have indeed affected the structure of these populations .

Materials and Methods

Plant samples of *Agrostis tenuis* SIBTH. were collected from five areas of Pb and Zn mine soil of the Parys Mountain and from areas of the Trelogan Zn mine (England). For comparison, plant samples were also collected from uncontaminated soil. A period of growth in normal soil does not reduce heavy metal tolerance (BRADSHAW 1952). Thus to provide sufficient material for the experiment the plants were propagated in normal soil in plastic trays in a glasshouse for at least eight weeks prior to testing.

Sensitivity to the presence of metal ions was evaluated by recording inhibition of root growth. The technique followed was based upon the work of BRADSHAW (1952), WILKINS (1957, 1960), JOWETT (1958, 1964) as modified by McNEILLY & BRADSHAW (1968). Stock solutions of $Ca(NO_3)_2.4H_2O$, $CuSO_4.5H_2O$, $ZnSO_4.7H_2O$ and $Pb(NO_3)_2$ were prepared. Then we prepared the different solutions by appropriate dilution of the stock to give a working strength of 0.5 g/l $Ca(NO_3)_2$, 0.25 ppm Cu, 3.75 ppm Zn and 6.0 ppm Pb.

Tillers from a single tuft of each population were used for the test. The roots were removed from tillers leaving a basal node capable for root production. The tillers were placed in solutions, with and without metal ions, contained in 300 cm^3 plastic beakers using plastic tubes held in an 8 cm square plastic top to support the tillers. Each plastic top held 10 tubes. The plants were in a cabinet illuminated continuously by fluorescent lamps. The temperature was kept at 23—25° C and the humidity at 80—90%.

The solutions were changed every 2 days to provide aeration and to maintain the desired metal concentration. After 10 days the longest roots of each tiller for the different solutions were measured and the index of tolerance was calculated by the following formula:

$$\text{Index of Tolerance} = \frac{\text{mean lenght of longest root in solution with metal}}{\text{mean length of longest root in solution without metal}} \times 100$$

Results

The various degrees of tolerance to different toxic metals among different populations are characteristic (Figs. 1, 3).

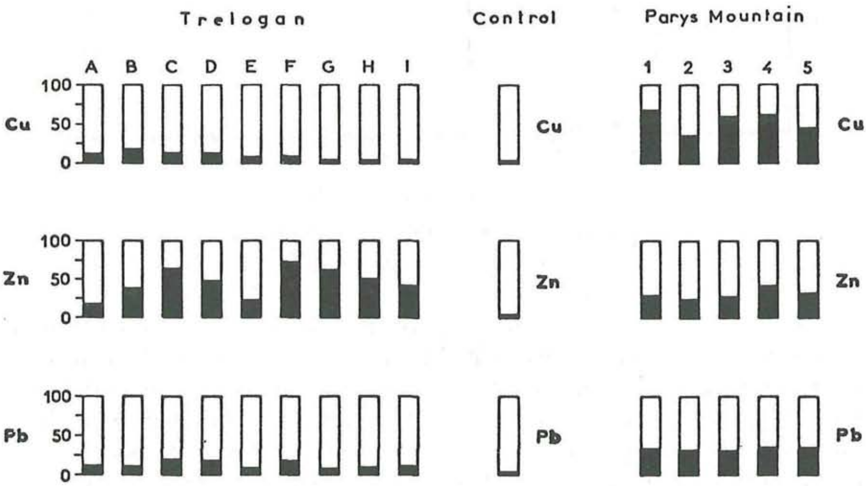

Fig. 1. The root length of populations of *Agrostis tenuis* in various toxic solutions, expressed as percent of their growth. The populations originated from Trelogan (A—I), Parys Mountain (1—5) and Garway (control)

The relation between root growth and various levels of concentration of Zn for a tolerant genotype in comparison to a pasture population is given in Fig. 2. When we used only $Ca(NO_3)_2$ as solution, the root of plants with a high index of tolerance was longer than the root of plants in a pasture population. When, however, 3.75 ppm Zn was added to the solution of $Ca(NO_3)_2$, the root length among plants of the pasture population was

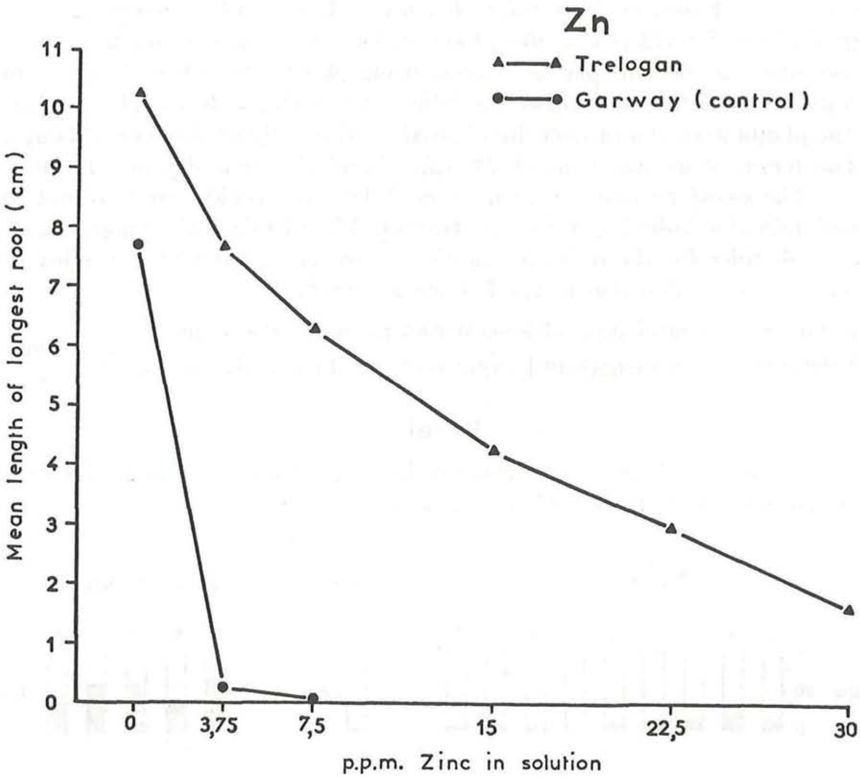

Fig. 2. The effect of zinc concentration on length of longest root of two different genotypes of *Agrostis tenuis*

shortened. As a result, the root length in the concentration of 3.75 ppm Zn among pasture population was equal to 3% of the respective length, when no toxic metal was used. On the contrary the root length of individuals with a tolerant genotype developed at 3.75 ppm Zn roots which were about 75% shorter as compared to plants growing in a normal solution.

At the next level of concentration the index of tolerance approached zero and the root length was approximately 0.6% of the respective length when no toxic metals were added. The root length of the tolerant population in contrast remained at high levels retaining 62% of the length in the normal solution. At the subsequent levels, the genotype of the Trelogan displayed tolerance even at 30 ppm at which root length was 16% of that in the normal solution (Fig. 2). Consequently, the variations were characteristic when the concentration of 3.75 ppm was used, and therefore we have chosen it as the most suitable concentration for the control of rooting experiment. The distribution of the index of tolerance for Cu, Zn and Pb regarding populations collected from different mines in comparison to the pasture population is given in Fig. 1. The results show that the index of tolerance for Cu among populations of Trelogan is low, as is the case with the pasture population. The tolerance of the populations of the Parys Mountain was uniformly high and ranged from 35% to 67%.

Two out of the Trelogan mine (A and E) populations studied in the Zn treatments showed an index of tolerance below 25%, while the remaining were considerably more tolerant, the index ranging within 37% and 73%. The populations collected from the mine of the Parys Mountain were of medium tolerance to Zn, because all but one (i. e. N° 4) showed an index of tolerance within 25% and 30%. As regards Pb, the Trelogan populations displayed low index of tolerance (below 20%) in contrast to the populations of Parys Mountain, whose index of tolerance was medium (30—35%).

Discussion

The indices of tolerance of the various populations differ characteristically in the presence of different metals. The Trelogan populations for instance, do not display any tolerance to Cu and Pb. However, they do show a high tolerance to Zn, ranging within wide limits.

The wide variability among populations and the lack of uniformity in their distribution may be due firstly, to the fact that the populations were collected from various sites of the mine, containing different quantities of toxic metals, each population having been adapted to the special conditions of its own environment (Wilkins 1960, Jowett 1964, Gregory & Bradshaw 1965). Secondly, perhaps these populations resulted from the splitting of characters of a population which was heterozygous for this character (Urquhart 1970b, Gartside & McNeilly 1974). Therefore there will be an escalation of the characters from very low to very high tolerance, depending on the tolerance of their parents. There is also a possibility that both explanations may be valid at the same time.

The linear relationship found between the indices of tolerance to Pb and those to Zn, suggests a close association in the physiological mechanism of the tolerance of the two metals. Thus populations, evidencing an increase of their index of tolerance to one metal may show also an increase to the other. Considering that mining in Trelogan concerns only Zn and that Pb and Zn coexist usually in the minerals, it is reasonable to deduce that each metal affects independently the tolerance of these populations. The population of Parys Mountain in contrast, display high indices of tolerance to the three metals at the same time, the index regarding Cu being particularly high.

The correlation of both indices of tolerance to Pb and Zn shows that the increase of the index of tolerance to Zn involves an increase of the respective index of tolerance to Pb (Figs. 4, 5).

Consequently, the populations of both mines responded similarly to Pb and Zn suggesting that the physiological mechanisms of tolerance to these two elements are related.

Finally, by comparing the performance of the populations of both mines, we notice that the populations of Trelogan display unilateral tolerance to Zn. The roots of one of the tolerant genotypes grow very well even in a concentration of 30 ppm while the control population has already died in concentrations of 7.5 ppm. By way of contrast the populations of Parys Mountain show tolerance to more than one metal. Among the populations studied, N° 4 was by 33% more tolerant to the three metals simultaneously. This does not mean necessarily that the high index of tolerance to one metal can affect the tolerance to the others (Jowett 1958, Broker 1963, Gregory & Bradshaw 1965, Bradshaw 1970, Waley, Khan & Bradshaw 1974).

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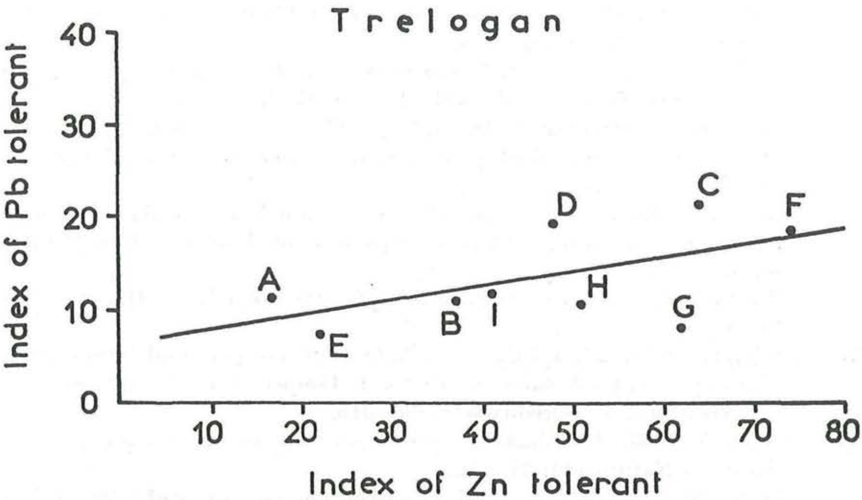

Fig. 4. The index of tolerance to lead of several populations of *Agrostis tenuis* plotted against their index of tolerance to zinc. Fitted regression: y = 6.65+ +0.146x

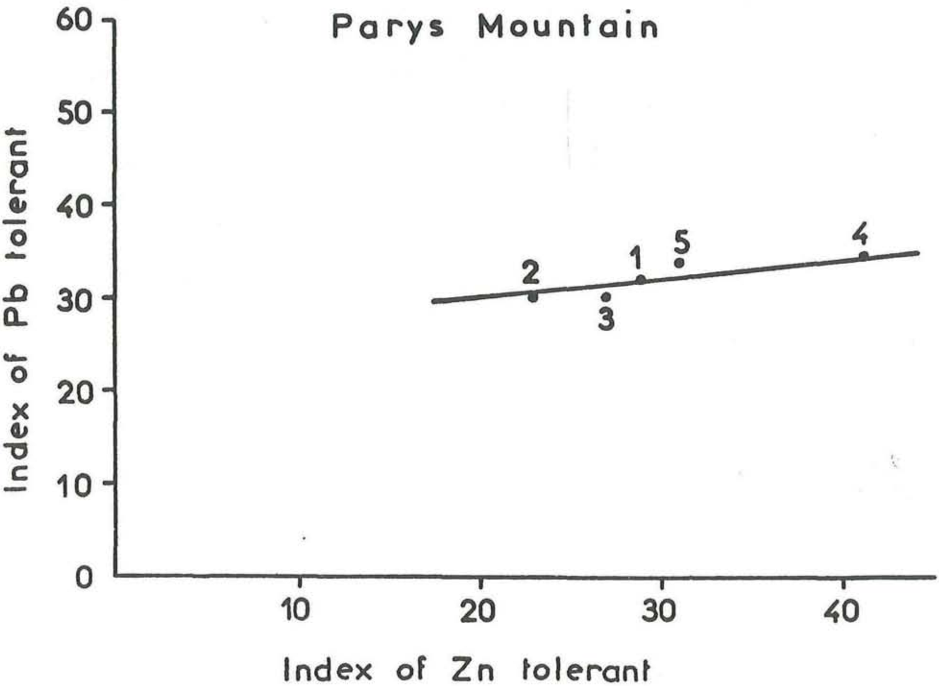

Fig. 5. The index of tolerance to lead of several populations of *Agrostis tenuis* plotted against their index of tolerance to zinc. Fitted regression: y=23.74+0.27 x

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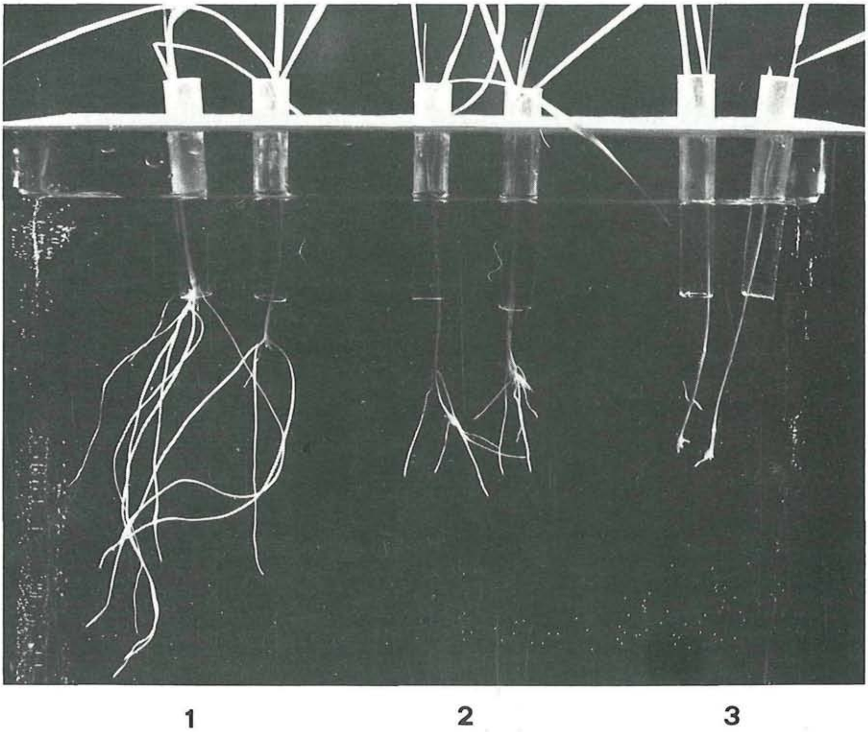

Fig. 3. Root growth of *Agrostis tenuis* in different toxic metals (from population I of Trelogan). 1. Normal solution $Ca(NO_3)_2$ 0.5 g/l. 2. Normal solution $Ca(N_3)O_2$ 0.5 g/l +3.75 ppm Zn. 3. Normal solution $Ca(NO_3)_2$ 0.5 g/l +0.25 ppm Cu