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THE EFFECTS OF ZINC ON THE LEAVES OF THE ZINC-TOLERANT AND NON-TOLERANT GENOTYPES OF *ANTHOXANTHUM ODORATUM*

by

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Abstract. *The removal of the leaves from the plant as well as the toxic effect of heavy metals cause, among other things, the speeding up of natural senescence as well as chlorosis.*

It is realized by experiment that the removed leaves from the tolerant genotypes of Anthoxanthum odoratum have shown a lower loss of chlorophyll as the time passed and with the steady presence of 1 mM zinc in relation to the non-tolerant genotypes. Just the opposite occurs with the absence of the toxic action of zinc. There is a hypothesis that this is probably due to the adaptation of the tolerant organism so that it requires the presence of zinc for its normal function and growth, while its absence speeds up the natural senescence as well as the breakdown of chlorophyll.

Therefore, the tolerance of various species which occupy toxic areas is characterized by a generally inherited evolutionary capacity rather than an innate natural tolerance.

INTRODUCTION

The waste or spoil from mining operations, often containing toxic levels of heavy metals, has been dumped in haphazard heaps in the vicinity of the mines. The heaps therefore offer an inhospitable environment for plant colonization (Bradshaw 1971, Wild 1970). The degree of contamination can be considerable, and although extraction procedures may have removed the bulk of the metal involved, the spoil generally contains at least 1% of the metal. This may be accompanied by other metals which were present in the ore at low levels and were not worth extracting, but which are often quite toxic (Bradshaw 1971).

In spite of the inhospitable environment for plant growth, a number of plant species are able to colonize mine sites to varying degrees and their distribution has been of interest not only to ecologists but also to prospectors of metal ores (Persson 1956, Warncke 1968, Lambinon and Auguier 1964, Ernst 1965, 1968).

The investigations of the past twenty years have shown that many plant families contain species tolerant to heavy metals (Antonovics et al. 1971). It is thought that these families contain some individuals with the genes which enable them to tolerate unusually high and normally toxic levels of heavy metals. Consequently, when a contaminated area is produced by mining activities or the natural processes of erosion, these individuals are able to colonize the area. The severe conditions prevalent at these sites exert very strong selection pressures, they prevent metal susceptible species from growing in the area and maintain and possibly improve the mechanisms operating in the tolerant species.

It has been established that the degree of tolerance is determined by the nature of the soil. If the soil has high levels of metal contamination then the species growing on that soil will have a high level of tolerance (Wu and Antonovics 1975, Karataglis in Bradshaw 1976). Metal tolerance in the majority of species is specific. Tolerance to one metal does not incur tolerance to another.

Turner (1969, 1970) and Turner and Marshall (1971, 1972) have shown that some tolerant strains of *Agrostis tenuis* take up more metal into the cell wall roots than do the non-tolerant species.

The majority of investigations undertaken are concerned with the mechanisms occurring in the roots of the plant, as this is where the first signs of metal toxicity occur. In addition, roots are a much easier part of the plant to work with than the shoot and so the shoot has been for the most part ignored.

Many heavy metals, including copper and zinc, cause chlorosis similar to that produced by iron deficiency, in addition to the usual toxicity symptoms such as stunting and necrosis. However, it has been shown that the loss of chlorophyll differs in tolerant and non-tolerant individuals in the presence of heavy metals, demonstrating that chlorophyll plays a part in the tolerance of a plant and so the estimation of chlorophyll levels should be a useful way of estimating the degree of tolerance of the plant shoot. This is the area which will be looked at more closely here so as to see what the effect of metal on the chlorophyll content is.

MATERIALS AND METHODS

Plants of *Anthoxanthum odoratum* were collected from zinc/lead mine soil of the Trelogan in North Wales. For comparison, plants of *Anthoxanthum odoratum* were collected from an uncontaminated area.

Tolerant and non-tolerant genotypes were propagated in normal soil in a glasshouse for at least eight weeks prior to testing, to provide sufficient material for the experiment.

A test for the soil pH was made and it was found fluctuating from 6.1 to 7.6. Also the levels of contaminants have been measured by a Unicam atomic absorption SP 90. After three measurements the mean of contaminants were:

Zinc	20.000	to	60.000	ppm
Lead	2.500	to	3.600	ppm
copper	100	to	500	ppm

In order to measure the effect of zinc on the level of chlorophyll in the leaves, leaf blades were cut off the plant just above the leaf node and were placed in solution, with and without metal ions respectively, contained in 300 ml plastic beakers using plastic tubes held in an 8 cm square plastic top to support the leaves. Each plastic top held ten tubes. The plants were in a cabinet illuminated continuously by fluorescent lights and the temperature was kept at 23-25° C under 80-90% humidity. Ten replicates were set up to reduce any variations in the results and so that standard errors are calculated. Two genotypes one tolerant and one non-tolerant were used so that chlorophyll extraction were carried every two days.

Chlorophyll determination

A weighed sample of leaf material was ground in a pestle and mortar in a solution of 80% aqueous acetone. The solution was then centrifuged at 3000 rpm for about five minutes so as to precipitate out all the leaf fibre and chloroplast fragments leaving a clear, bright green solution of chlorophyll.

The solution was then made up to 10 ml with 80% acetone and the chlorophyll concentration was determined by measuring the absorption of the extract in a Unicam spectrophotometer at 663 and 645 nm. The two readings obtained and the specific absorption coefficients for chlorophylls *a* and *b* as determined by simultaneous equations are used.

$$D663 = 82.04 \ Ca + 9.27 \ Cb$$

$$D645 = 16.75 \ Ca + 45.6 \ Cb$$

where Ca and Cb are grams per litre of chlorophylls *a* and *b* respectively and D is the density value at the respective wavelength as obtained from the spectrophotometer.

By solving for Ca and Cb the total chlorophyll C can be determined.

$$C = Ca + Cb.$$

$$C = 0.0202\ D645 + 0.00802\ D663\ \text{g/litre or}$$

$$C = 20.2\ D645 + 8.02\ D663\ \text{mg/litre.}$$

RESULTS

The results are expressed in milligrams of chlorophyll per gram fresh weight of leaf material. In the results obtained, the levels of chlorophyll in the 0.0 mM concentration are different in the two genotypes, so to allow for this, the chlorophyll concentration is also expressed as a percentage of the 0.0 mM zinc control.

The ten leaves of each genotype for each day were extracted separately and an average chlorophyll concentration and its standard error were calculated. The chlorophyll contents were expressed as a percentage of the O time control. The logs of the percentage figures were plotted and a line of best fit was calculated.

A comparison of the slopes for tolerant and non-tolerant genotype produced from the percentage data clearly show that there is a difference in the natural senescence rates of the detached leaves. The results show that the tolerant leaf loses more chlorophyll over the ten day period than the non-tolerant leaf. The tolerant loses about 80% of its chlorophyll in this period whereas the non-tolerant loses only 60%, a difference of 20%, which is sufficiently significant.

When 1.0 mM zinc is present in the solution it can be seen by a comparison with the respective controls that the non-tolerant leaf loses 13% more chlorophyll than this without zinc, due to the effect of the zinc and that the loss is very similar to that obtained for the tolerant control. However, when tolerant genotype in the presence of zinc is compared with its control it is found that it has lost as much chlorophyll as the non-tolerant genotype which is about 13%.

The loss of chlorophyll has been quantified by calculating the significance of the regression lines. The most significant are the ones obtained for the tolerant and non-tolerant+1mM Zn genotypes, showing that these have the greatest loss of chlorophyll. The line obtained for the tolerant+1 mM Zn is significant at the 0.05 probability level indicating the loss of chlorophyll as slightly less and the line obtained for non-tolerant genotype is not significant at either level showing this has the lowest level of chlorophyll loss.

DISCUSSION

The observations reported by Wainwright and Woolhouse (1973) that the effect of toxic metals on the chlorophyll content of grass leaves is greater in the non-tolerant genotypes than in the tolerant genotypes has to some extent been supported here. The results given in Tables 1 and 2 and their related graphs indicate that zinc has no effect on the chlorophyll content of the tolerant genotype but it does cause some of the chlorophyll loss which occurs in the nontolerant genotype.

TABLE I

Chlorophyll concentration expressed per gr fresh weight and its standard error.

mg chlorophyll/g fresh weight, showing stadard errors.

DAY	Tolerant	Tolerant+1mM Zn	Non-tolerant	Non-tolerant+1mM Zn
0	2.18±0.16	2.18±0.16	2.09±0.13	2.09±0.13
2	1.01±0.14	0.95±0.09	0.98±0.08	1.06±0.12
4	0.99±0.06	0.79±0.05	1.10±0.10	0.88±0.05
6	0.95±0.20	0.52±0.07	0.92±0.08	0.68±0.10
8	0.53±0.06	0.69±0.12	0.96±0.18	0.47±0.07
10	0.43±0.06	0.70±0.14	0.80±0.08	0.53±0.12

TABLE II

Chlorophyll contents expressed as percentage of the 0 time and the logs of them.

% chlorophyll content - log% content

DAY	Tolerant		Tolerant+1mM Zn		Non-tolerant		Non-tolerant+1m	
	%	log	%	log	%	log	%	log
0	100	2.0000	100	2.0000	100	2.0000	100	2.0000
2	46.3	1.6659	43.6	1.6393	46.9	1.6720	50.7	1.7052
4	45.4	1.6572	36.2	1.5592	52.6	1.7212	42.1	1.6244
6	43.6	1.6393	23.9	1.3775	44.0	1.6437	32.5	1.5124
8	24.3	1.3858	31.7	1.5004	45.9	1.6621	22.5	1.3519
10	19.7	1.2949	32.1	1.5006	38.3	1.5830	25.4	1.4041

Chlorophyll is continuously produced in the leaves and broken down to various rates, something which depends on the age of the leaf. From the time, however, that the leaf is detached from the plant, the process of scenescence already begins. If the leaf were not detached,

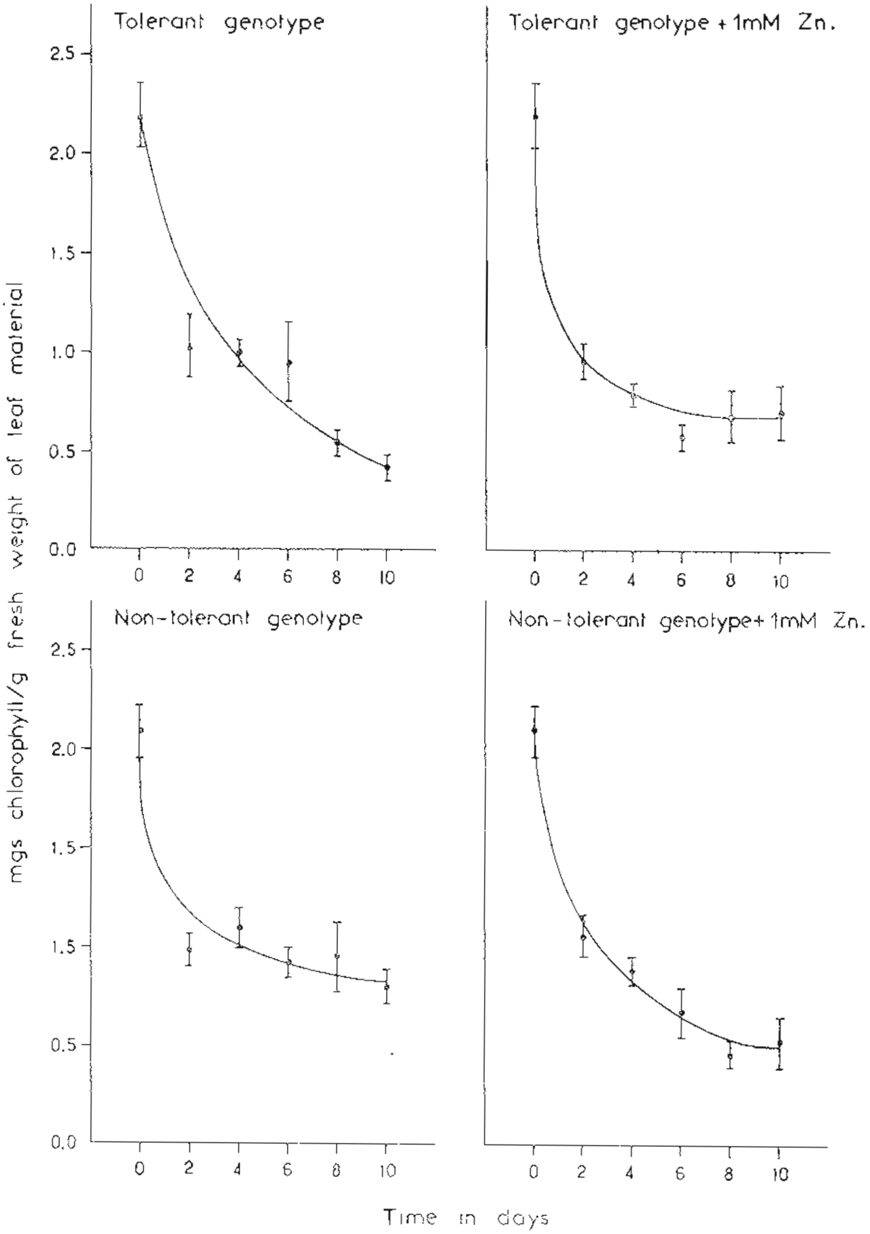

Fig. 1 Rate of chlorophyll loss from leaves in a period 10days, showing also standard errors.

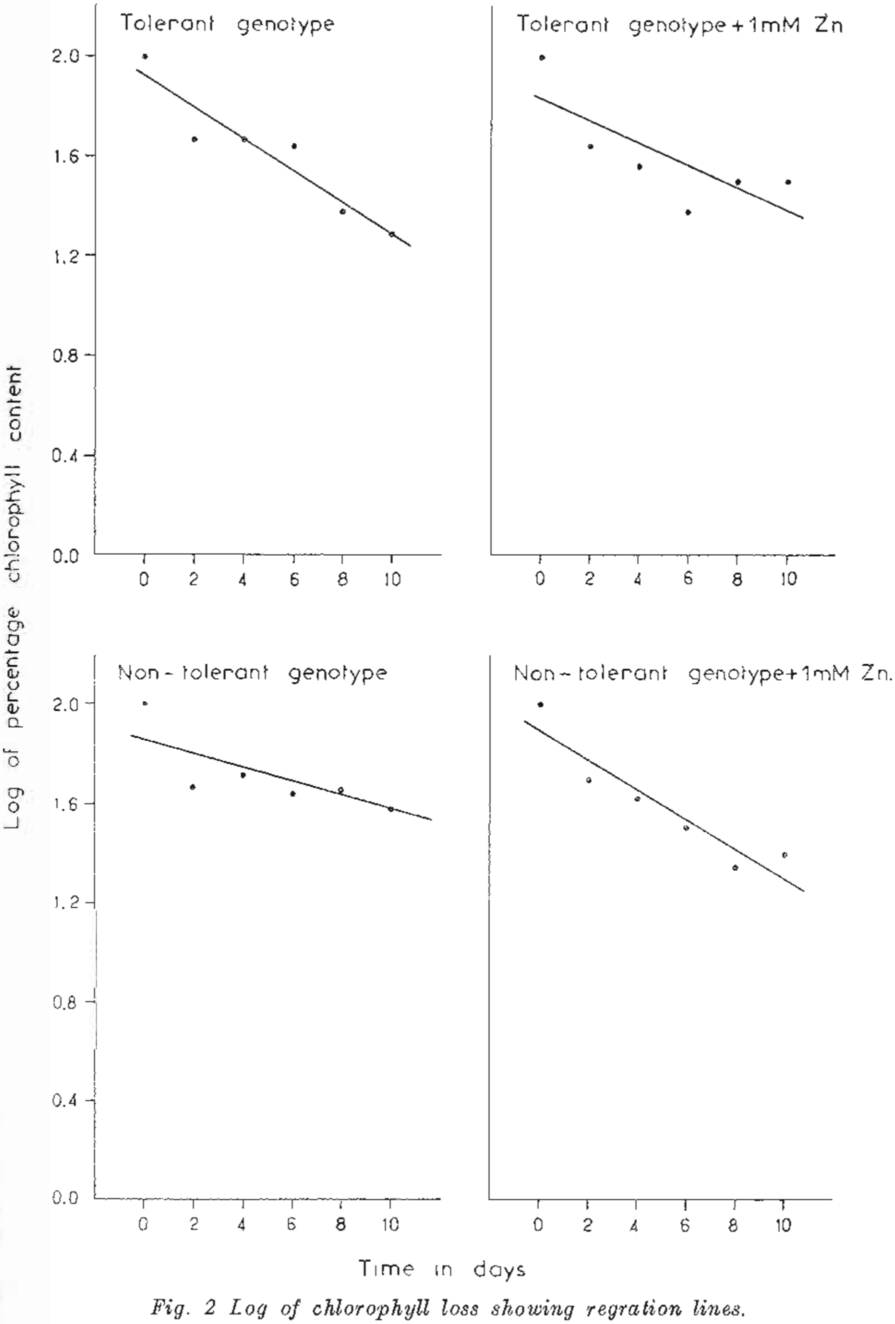

Fig. 2 Log of chlorophyll loss showing regration lines.

this process of scenescence would be prevented for quite a long time owing to the existing balance of the different substances produced by the plant. However, on detaching the leaf from the plant, we remove this control and so the process of scenescence, which participates in the break down of chlorophyll and proteins as well as many other physiological metabolisms in the cells and cell walls, already starts to take place.

One of the first symptoms of metal toxicity in plants is the appearence of chlorosis. Heavy-metal induced chlorosis is similar in appearence to that due to iron deficiency and can be cured by applying iron to the plant. Iron is involved in the formation of delta-aminolevulinic acid, a precursor of protoporphyrin. The role of metal toxicity is not so clear. An antagonism may exist between them and iron for uptake or it may indirectly affect chlorophyll synthesis by interfering with general metabolism (A. Reilly and C. Reilly 1973).

On comparing the behaviour of the two genotypes of *Anthoxanthum odoratum* which were used in our experiments, we can say that the loss of chlorophyll occurring in the leaves of the tolerant genotypes in case there is presence of Zn is probably due to the process of natural scenescence rather than to the toxic effect of Zn. This may occur because of the leaf adaptation which requires the presence of Zn for its normal function, while its absence causes a shock to the organism.

On the contrary, the loss of chlorophyll in the leaves of non-tolerant genotypes is greater when Zn is absent. Indeed, it has been noticed that as the toxic action increases, the overall absorption of Fe from the plant is reduced resulting directly to the decrease of Fe concentration in chloloplasts. (A. Reilly and C. Reilly 1973). This accummulation is probably essential for chlorophyll synthesis and this toxic metal interferes indirectly with this process. Zinc may interfere with the permeability of the chloroplast membrane to limit entry to forms of transport iron (T. Hutchinson 1968; A. Reilly and C. Reilly 1973) into the organelle.

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ΠΕΡΙΛΗΨΙΣ

ΕΠΙΔΡΑΣΕΙΣ ΤΟΥ ΨΕΥΔΑΡΓΥΡΟΥ ΕΠΙ ΤΩΝ ΦΥΛΛΩΝ ΑΝΘΕΚΤΙΚΩΝ ΚΑΙ ΜΗ ΑΝΘΕΚΤΙΚΩΝ ΓΕΝΟΤΥΠΩΝ ΤΟΥ *ANTHOXANTHUM ODORATUM*

Ὑπὸ
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Ἡ ἀπομάκρυνσις τῶν φύλλων ἀπὸ τὸ φυτὸ καθὼς καὶ ἡ τοξικὴ ἐπίδρασις τῶν βαρέων μετάλλων προκαλοῦν μεταξὺ τῶν ἄλλων, ἐπιτάχυνσιν τοῦ φυσικοῦ γήρατος καθὼς ἐπίσης καὶ χλώρωσιν.

Ἐκ τῶν πειραματικῶν δεδομένων γίνεται ἀντιληπτὸν ὅτι τὰ ἀποκοπέντα φύλλα τῶν ἀνθεκτικῶν γενοτύπων τοῦ *Anthoxanthum odoratum* παρουσίασαν μικροτέραν ἀπώλειαν χλωροφύλλης μὲ τὴν πάροδον τοῦ χρόνου καὶ τὴν σταθερὰν παρουσίαν τοῦ 1 mM ψευδαργύρου, ἐν σχέσει πρὸς τοὺς μὴ ἀνθεκτικοὺς γενοτύπους. Τὸ ἀντίθετον ἀκριβῶς συμβαίνει μὲ τὴν ἀπουσίαν τῆς τοξικῆς δράσεως τοῦ ψευδαργύρου. Διατυποῦται ἡ ὑπόθεσις ὅτι πιθανῶς τοῦτο ὀφείλεται εἰς τὴν προσαρμογὴν τοῦ ἀνθεκτικοῦ ὀργανισμοῦ ὥστε νὰ ἀπαιτῇ τὴν παρουσίαν τοῦ ψευδαργύρου διὰ τὴν κανονικὴν λειτουργίαν καὶ ἀνάπτυξιν, ἐνῶ ἡ ἔλλειψις αὐτοῦ ἐπιταχύνει τὸ φυσικὸν γῆρας καθὼς καὶ τὴν διάσπασιν τῆς χλωροφύλλης.

Ἡ ἐκδήλωσις συνεπῶς τῆς ἀνθεκτικότητος τῶν διαφόρων εἰδῶν, τὰ ὁποῖα καταλαμβάνουν τοξικὲς περιοχές, χαρακτηρίζεται ἀπὸ μίαν γενικῶς κληρονομουμένην ἐξελικτικὴν ἱκανότητα μᾶλλον, παρὰ ἀπὸ ἔμφυτον φυσιολογικὴν ἀνθεκτικότητα.