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EVALUATION OF NICKEL TOLERANCE IN ALYSSUM

Riassunto — *Valutazione della tolleranza al nichel in* Alyssum. La tolleranza al nichel di alcune specie di *Alyssum* (*A. bertolonii, A. argenteum, A. nebrodense*) e di *Aurinia saxatilis*, è stata misurata in presenza di concentrazioni crescenti di nichel utilizzando sia l'accrescimento radicale, sia la resistenza protoplasmatica delle cellule epidermiche. Come suggerito da Craig (1977), il grado di tolleranza è stato espresso come ED50 calcolato con la probit analisi. Sono inoltre riportati i primi dati sulla relazione negativa tra assorbimento del nichel e del calcio nelle due specie nichel tolleranti (*A. bertolonii* e *A. argenteum*).

Abstract — Nickel tolerance of some species of *Alyssum* (*A. bertolonii, A. argenteum, A. nebrodense*) and of *Aurinia saxatilis* has been measured over a wide range of nickel concentrations by root elongation and by protoplasmatic resistance of epidermal cells.

As suggested by Craig (1977), the degree of nickel tolerance has been evaluated as ED50 using probit analysis.

First data are also reported on the negative correlation between calcium and nickel uptake in the nickel tolerant species (*A. bertolonii* and *A. argenteum*).

Key words — Tolerance; ED50; Nickel; *Alyssum*.

Every study on metal tolerance always poses the problem of the measurement of tolerance itself, particularly important not only when one compares different natural plant populations but also different species.

Several methods have been suggested to quantify plant response to toxic metals, both at cellular (REPP, 1963) and organ level. In the latter case root growth has been measured (WILKINS, 1957; JOWETT, 1958; DAVIES and SNAYDON, 1973). As roots are always sen-

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sitive to the presence of toxic elements in the external medium, this method has been used extensively, but only CRAIG (1977) has applied probit analysis (FINNEY, 1971) to plant material to statistically evaluate tolerance over a wide range of concentrations. Since this method is capable of comparing tolerance levels even between different species we have used it to measure nickel tolerance of some species of the genus *Alyssum* (*A. argenteum* All., *A. bertolonii* Desv., and *A. nebrodense* Tineo) and of *Aurinia saxatilis* L., which is a closely related species.

In addition, for *A. argenteum* and *A. bertolonii*, nickel tolerance was also tested using the protoplasmatic resistance of epidermal cells (REPP, 1963; GRIES, 1966) to evaluate the reliability of this method.

MATERIAL AND METHODS

The following species, belonging to the same systematic section (Odontarrhena), have been examined: two, endemic to serpentines, i.e. *A. argenteum* and *A. bertolonii* (seeds were collected at Bobbio (Piacenza) and Pieve S. Stefano (Arezzo) respectively); *A. nebrodense*, endemic to the Madonie (Sicily) where it grows on calcareous soil (seeds were collected at Quacella).

In addition, *Aurinia saxatilis*, closely related to the genus *Alyssum* (to which it was once attributed, but which is now recognised as a separate entity, DUDLEY, 1964a, b) has been examined. Seeds were obtained from a commercial source.

Root growth

Seeds of the above four species, as soon as they had germinated in the dark at 20°C, were transferred onto special perspex trays and placed in glass containers with 4 l of a solution of $Ca(NO_3)_2 . 4H_2O$ (1g/l), to which varying amounts of $NiSO_4.7H_2O$ were added in order to obtain a range of concentrations in which all species showed a gradual decrease of root elongation.

The concentrations used were the following:

— 0,10; 0,25; 0,5; 1; 1,5; 2 mM Ni (*Alyssum bertolonii*)
— 0,05; 0,15; 0,25; 0,5; 0,75; 1,5 mM Ni (*Alyssum argenteum*)
— 0,01; 0,025; 0,05; 0,075; 0,1 mM Ni (*Alyssum nebrodense*)
— 0,001; 0,0025; 0,005; 0,0075; 0,01 mM Ni (*Aurinia saxatilis*)

The glass containers were kept in a climatic chamber, with 12 h of artificial light and a constant temperature of 20°C ± 1. After 10 days the length of the roots was measured with a dissection microscope. The data obtained were analysed statistically by the probit method which permits an estimation of the effective metal concentration (ED50); at this level of concentration, 50% of the individual plants show a chosen response.

Protoplasmatic resistance

Epidermal cells of green stems of plants of *A. bertolonii* and *A. argenteum,* grown in pots on serpentine, were used. The method, described by REPP (1963), takes into account the number of living, damaged or dead cells in tangential leaf or stem sections when placed in solutions containing different concentrations of the metal tested.

Cell vitality was measured by the capacity of the cells to plasmolise in a solution 1 M saccharose or to deplasmolise in a solution 0,25 M saccharose.

As the cells of the species examined did not contain any pigment, after treatment they were coloured with neutral red (1:25000).

The sections were then classified (REPP, 1963) as follows:

LL = all living cells, except for 2 or 3 layers of cells at the outer margin of the section.

LL+ = occasional dead cells in the section.

L+ = 50% of dead cells.

L++ = more than 50% of dead cells, but groups of living cells still present.

++ = no living cells.

Calcium and Nickel analysis

All the plant material tested, dried at 80°C to constant weight, was wet-ashed using a nitric and perchloric acid mixture 5:2 (PINTA, 1962). The concentration of nickel and calcium was determined by atomic absorption spectrophotometry (Perkin Elmer, Mod. 370).

RESULTS

Root growth

In fig. 1 the inhibitory effect of increasing nickel concentration on root growth is shown for all the species tested. In every species when the root system reaches (at different concentrations, according to the species) an average 80% reduction of the mean control growth, no further appreciable reduction can be observed, even

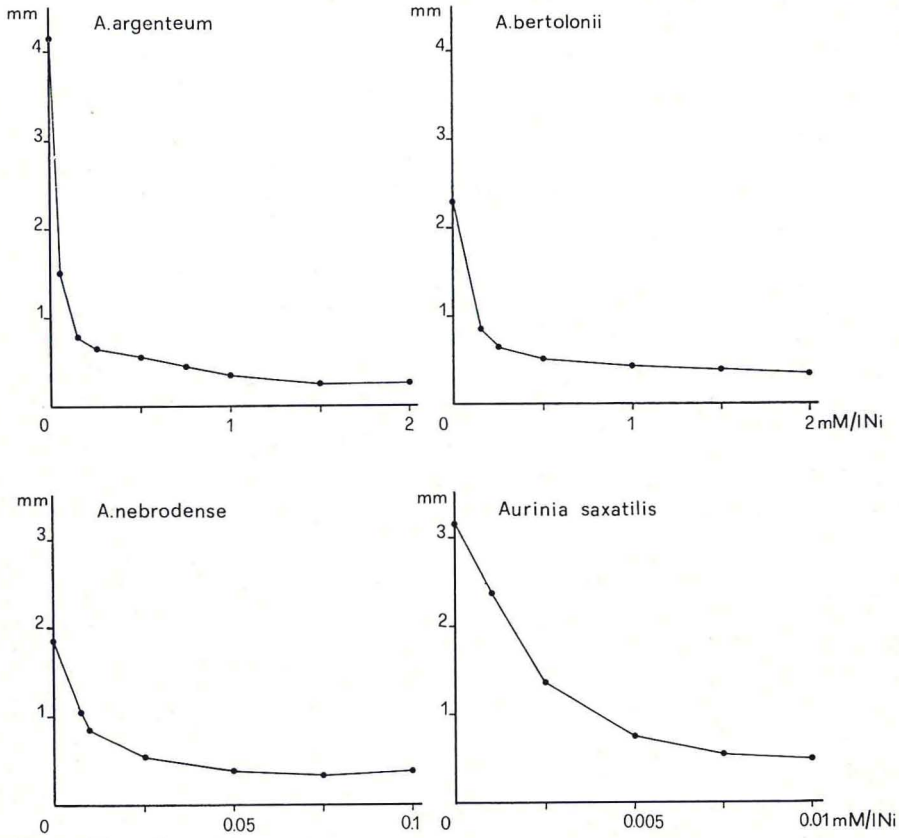

Fig. 1 - The effect of increasing nickel concentrations in 1g/l calcium nitrate solution ($Ca(NO_3)_2 . 4H_2O$) on the root elongation of seedlings ten days after germination.

when nickel concentration is increased. Therefore for the probit analysis only the roots with a growth rate of 20% of the control rate or less were considered (tab. 1). The nickel concentrations in

	M.C.r.	Ni nM/l	N	NR	R%	ED_{50} NimM/l	$LogED_{50}$	f.l.	χ^2	b (±S.E.)
Alyssum argenteum	41.6	0.05	61	5	8.2	0.145	1.162	1.23	1.45 (P > 70%)	2.71 (±0.14)
		0.15	60	34	56.7					
		0.25	62	45	72.6			1.09		
		0.50	58	53	91.4					
		0.75	41	40	97.6					
Alyssum bertolonii	23	0.25	150	39	26.0	0.615	1.789	1.87	3.78 (P > 20%)	1.42 (±0.06)
		0.50	132	68	51.5					
		1.00	129	79	61.2			1.71		
		1.50	66	44	66.7					
		2.00	56	44	78.6					
Alyssum nebrodense	19	0.01	73	2	2.7	0.057	1.755	1.85	4.14 (P > 20%)	2.73 (±0.09)
		0.025	72	9	12.5					
		0.05	64	35	54.7			1.66		
		0.075	78	47	60.3					
		0.1	68	51	75.0					
Aurinia saxatilis	31.6	0.001	34	1	2.9	0.0053	1.727	1.80	1.56 (P > 80%)	2.82 (±0.12)
		0.0025	47	8	17.2					
		0.005	46	19	41.3			1.65		
		0.0075	33	24	72.7					
		0.01	34	26	76.5					

TABLE 1 - Probit analysis of root tolerance to nickel.
M.C.r. = Mean control rate; N = No of roots measured; NR = No of roots with a growth rate of 20% of the mean control rate or less; R% = Percentage of NR values; f.l. = fiducial limits of $LogED_{50}$; b = regression coefficient.

which the response was too near either to 0% or to 100%, were excluded from the analysis since they were not relevant for the ED50 calculation. The ED50 values show that *A. bertolonii* is the most tolerant species, being about four times more tolerant than *A. argenteum,* about ten times more than *A. nebrodense* and about one hundred times more than *Aurinia saxatilis.* In spite of the great differences between the observed values, the comparison must be limited only to the ED50 values, because the regression line obtained for *A. bertolonii* is not parallel to that of the other three species.

This lack of parallelism among the regression lines implies that the tolerance distribution shows a different variance and consequently the ratio between tolerance estimates must be considered as dependent on the specific response level examined (FINNEY, 1971).

As the regression lines of the three species, *A. argenteum, A. nebrodense* and *Aurinia saxatilis,* are parallel, the tolerance differences are constant at every level of response (FINNEY, 1971). In addition, as the slope of the regression line for *A. bertolonii* is very shallow, a unit increase of nickel concentration induces a percentage response variation lower than that observed in the other spe-

cies. As the regression coefficient is the reciprocal of the standard deviation, the lower nickel sensitivity of *A. bertolonii* implies also a greater variation in tolerance distribution.

Comparing *Zea mays* hybrids with genetically variable populations of *Panicum* and *Chloris*, CRAIG (1977) notes that a lower variation in tolerance might be related with a higher genetic stability among the individuals: on this basis, one could expect that the three species characterised by significantly parallel regression lines, possess a lower adaption capacity to different nickel concentrations.

Protoplasmatic resistance

The results of the protoplasmatic resistance tests (tab. 2) indicate that if we take the nickel tolerance threshold as corresponding to about 70% of undamaged sections, this threshold is reached in *A. bertolonii* at a concentration 0.5 mM Ni, and in *A. argenteum* at 0.0001 mM Ni. Hence, even by this method, the greater nickel resistance of *A. bertolonii* is confirmed.

	Alyssum bertolonii					Alyssum argenteum				
	LL	LL+	L+	L++	++	LL	LL+	L+	L++	++
Ni mM/l										
0.00001						93.3	6.6			
0.0001						74.1	18.5	-	3.7	
0.001						50.0	40.5	9.5	-	-
0.01						32.2	46.8	16.1	3.2	1.6
0.1	97.5	2.5				34.0	46.0	14.0	6.0	-
0.5	73.2	26.8				30.8	42.3	7.7	-	19.2
1	15.4	66.7	7.7	5.1	5.1	3.4	37.9	19.0	27.6	12.1
5	22.0	40.7	11.9	16.9	8.5		25.0	50.0	20.8	4.2
10	13.1	39.5	13.2	28.9	5.3		17.5	35.0	20.0	27.5
50		18.2	31.8	31.8	18.2		-	-	-	-
100			21.6	67.6	10.8			14.1	31.2	54.7
500				54.5	45.5			9.1	45.4	45.5
1000				13.8	86.2					100

TABLE 2 - Nickel tolerance in *A. bertolonii* and *A. argenteum*. Values as percentages of sections with different vitality.

At nickel concentrations higher than the tolerance threshold, in both species the number of dead cells gradually increases. The protoplasmatic resistance test seems therefore very reliable, as in

two closely related species, both nickel tolerant and nickel accumulating, it can show appreciable differences. Of course these nickel tolerance thresholds cannot be compared with those obtained through the probit analysis, as the response criteria are quite different.

Nickel and Calcium uptake

Calcium and nickel concentrations have been assessed (fig. 2) using seedlings grown for 10 days in the calcium nitrate solution (1g/l) with nickel additions (0.01-2 mM Ni).

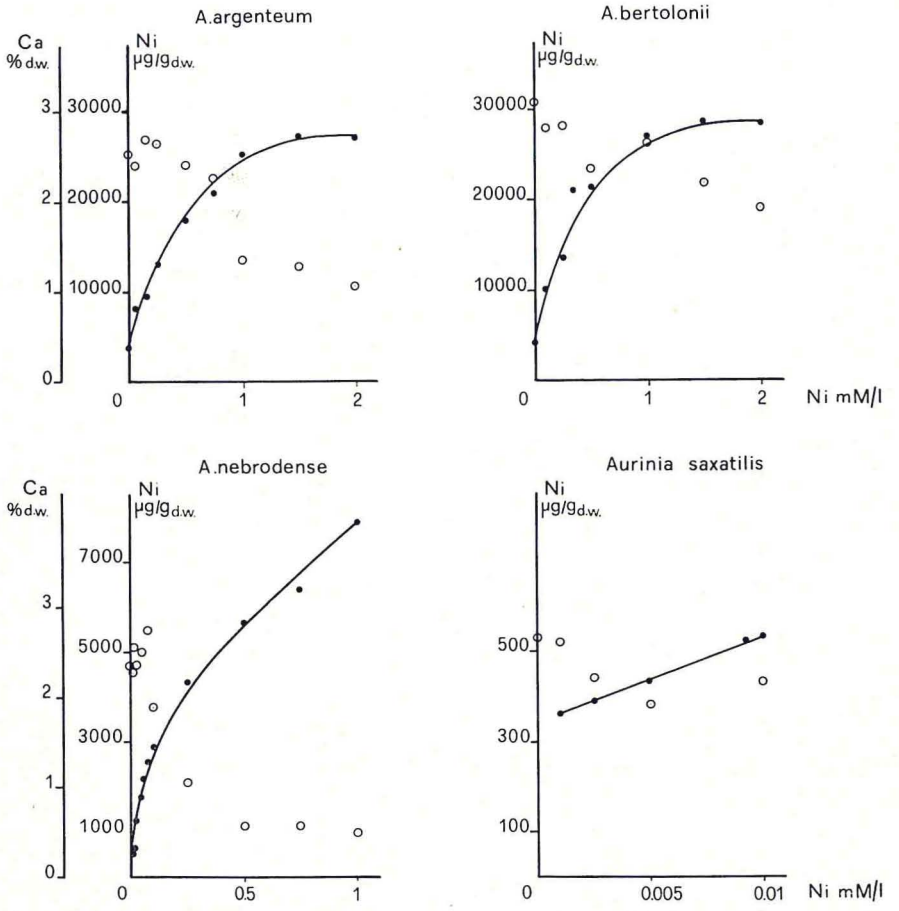

Fig. 2 - Uptake of Ni^{2+} (●) and Ca^{2+} (O), on a dry weight basis, by two nickel accumulators (*A. bertolonii* and *A. argenteum*) and two non-accumulators (*A. nebrodense* and *Aurinia saxatilis*).

Both nickel accumulating species *A. argenteum* and *A. bertolonii* reach an exceptional nickel concentration in the whole seedling (ca 25.000 μg/g dw) even with the solution 1 mM Ni; higher nickel concentrations (1.5 and 2 mM) do not induce a further significant increase in nickel accumulation, so that nickel uptake is no longer proportional to the external ion concentration. The same uptake pattern has been observed, in other experimental conditions, by MORRISON et al. (1980) in leaves of several *Alyssum* species. In the case of *A. nebrodense,* which belongs to Section Odontarrhena (Meyer) Kock, as *A. argenteum* and *A. bertolonii,* but is not endemic to serpentines, the seedlings reach a nickel concentration of 8000 μg/g, demonstrating a high nickel accumulating capacity (nickel is mainly located in the cotyledons).

Instead MORRISON et al. (1980) had observed that *A. serpyllifolium* Desf. (Section Odontarrhena, not endemic to serpentines) grown in a soil to which 350 μg/g Ni were added, accumulated nickel in leaves only up to 60 μg/g dw. In both species the pattern of nickel uptake is proportional to the concentration.

Aurinia saxatilis, used by us to compare the behaviour of a species closely related to the genus *Alyssum,* shows a more limited (500 μg/g) and almost linear nickel uptake, like *Alyssum montanum* L. (Section Alyssum) used by MORRISON et al. for the same purpose.

In all the species of the Section Odontarrhena calcium uptake, which does not show any difference in the treatments at low nickel concentration, gradually decreases as nickel uptake increases, so that, at high nickel concentrations, nickel and calcium uptake have opposite trends.

Between the two elements there is a negative and highly significant correlation in *A. argenteum* ($r = -0.86$; $P < 0.01$), *A. bertolonii* ($r = -0.82$, $P < 0.05$) and *A. nebrodense* ($r = -0.91$, $P < 0.01$). *A. nebrodense* reveals, also in this case, a close relationship to *A. argenteum* and *A. bertolonii.* In *Aurinia saxatilis* calcium and nickel do not show a linear correlation ($r = -0.70$, $P > 20.05$).

CONCLUSIONS

Even in metal tolerant species and ecotypes toxic metal levels inhibit root growth. All the species of *Alyssum* show a gradual root growth inhibition with increasing nickel concentration in the ex-

ternal medium. The nickel tolerance threshold is species specific: *A. nebrodense* shows a tolerance threshold about ten times, and *Aurinia saxatilis* about one hundred times, lower than that of *A. bertolonii,* and of *A. argenteum.* In cases of such different behaviour, it is difficult, as pointed out by WILKINS (1978), to evaluate tolerance quantitatively using only tolerance indexes, or comparing regression coefficients as suggested by DAVIES and SNAYDON (1973), even when there is a linear relation between root growth and concentration of metal in solution. The use of the probit analysis, as suggested by CRAIG (1977), was particularly valuable in our case, as we also had a sufficient number of seedlings (min. 34; max 150; generally more than 50), which were obtained from germinated seeds.

The toxic effect induced by nickel (growth rate 20% of the control or less) gives a ED50 value for *A. bertolonii* corresponding to a concentration of 0.615 mM/l (~ 36 ppm), of 0.145 mM/l (~ 8 ppm) for *A. argenteum,* and of 0.057 mM/l (~ 3 ppm) for *A. nebrodense. Aurinia saxatilis* has the lowest ED50 value: 0.0053 mM/l (~ 0.3 ppm). Comparing these results with those obtained by Craig for *Loudetia simplex* populations from serpentine and non serpentine areas, we can observe that the ED50 value of *A. saxatilis* is very similar to the one found for the non tolerant clone of *Loudetia simplex* (~ 0.4 ppm Ni), while the *Alyssum* species show a higher tolerance threshold compared to the *Loudetia* tolerant clone (~ 1.1 ppm Ni); the comparison has only an indicative value as the percentage reduction in root length considered is not the same. The higher nickel tolerance of *A. bertolonii* is confirmed also by the protoplasmatic tolerance test. In addition this species shows a lower sensitivity to an increase in the external nickel concentration, which could denote a higher adaptive capacity to toxic conditions. In both *A. bertolonii* and *A. argenteum* the high tolerance threshold could be associated with their nickel accumulating capacity and therefore with the efficiency with which the metal is bound and isolated in non toxic forms. Such a mechanism, which tends towards saturation in presence of high nickel concentrations, is apparently not operative in *A. nebrodense*; infact, though belonging to the same systematic section and though showing a tolerance threshold which is not particularly low, when nickel concentrations are increased, this species absorbs the metal till the seedlings become necrotic.

Aurinia saxatilis also has a similar behaviour. Although it is impossible to forecast the adaption capacity of a plant to a particular soil only on tolerance data (WILKINS, 1978), we cannot exclude that the tolerance level of *A. nebrodense* could be compatible with nickel concentrations of 1-3 ppm in serpentine soil solutions, as observed by ANDERSON et al. (1973).

Comparing our results with those obtained by MORRISON et al. (1980), it is evident that not all species of the Section Odontarrhena of the genus *Alyssum* behave in the same way. *A. serpyllifolium* seems less adaptable to high nickel concentrations in contrast to *A. nebrodense*. This would suggest that certain species within the section have evolved a metabolism which allows a greater nickel tolerance. A biochemical aspect of such different behaviour could stem from the production of great amounts of malic acid (BROOKS et al., 1981). Also interesting is the negative correlation which we have observed between nickel and calcium in tolerant species. Calcium might be important in regulating nickel uptake or in preventing it reaching levels wich might interfere with tolerance regulating mechanisms. The adaptation of special taxa to metal rich soils (DUVIGNEAUD et DENAEYER DESMET, 1973), for example, could depend upon enhanced organic acid synthesis, greater calcium uptake etc., which, being typical of certain ecotypes, must be under genetic control.

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