Nickel and copper tolerance and toxicity in three Tuscan populations of Silene paradoxa

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Nickel and copper tolerance and toxicity in three Tuscan populations of *Silene paradoxa*

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Three Tuscan ecotypes of *Silene paradoxa* L. were studied to evaluate the occurrence of multiple tolerance or co-tolerance mechanisms and to underline some tolerance strategies in plants naturally adapted to toxic concentrations of heavy metals. Seeds were collected from non-toxic calcareous soil, a serpentine outcrop with high nickel content and a copper mine dump. The evaluation of the toxic effects of the metals on root growth showed the copper-tolerant population as nickel co-tolerant, whereas the opposite was not the case. This suggests the occurrence of a non-reciprocal co-tolerance mechanism. The nickel-tolerant population seemed able to tolerate nickel by limiting its inhibiting effect on the peroxisomal H_2O_2 scavenging enzymes since, in the sensitive population, this inhibition revealed itself as one of the causes of nickel-induced oxidative stress. A very low copper root and shoot concentration seemed to be characteristic of the copper-tolerant population, combined with a low susceptibility to metal-induced oxidative stress.

Introduction

In many soils, heavy metals may occur at toxic levels owing to natural processes or antropogenic activities (Nriagu and Pacyna 1988, Nriagu 1991). Few heavy metal polluted soils are completely bare of vegetation and it is usually possible to find plants capable of growing and reproducing in such environments.

Naturally occurring contaminated soils are only rarely populated by species that adapted to them in short time periods (Kruckeberg 1967); soils recently contaminated by human activities are instead populated by tolerant ecotypes evolved from plants that generally live in other kinds of environments, thus furnishing a well-documented example of small-scale evolution (Wu et al. 1975, Bradshaw et al. 1990, Macnair 1993).

Some evidence has shown that heavy metal tolerance is largely metal specific since plants only tolerate the metals occurring at toxic concentrations in their environment (Verkleij et al. 1991). A multiple tolerance mechanism is characteristic of plants living in soils with toxic levels of several metal ions: every single element can be metabolically inactivated by a specific strategy. However, some metal-tolerant plants can also tolerate high and detrimental concentrations of those metals that in their environment are present at low, non-toxic levels (Baker 1987). When tolerance to one or more metals confers some degree of tolerance to another metal not present or enriched in the soil, a co-tolerance mechanism may be involved.

As far as natural toxic soils are concerned, serpentine outcrops contain high amounts of nickel, chromium and cobalt (Malpas 1992, Vergnano Gambi 1992); the high nickel content is probably the most harmful factor because cobalt has a lower relative abundance and chromium has low bioavailability (Proctor and Woodel 1975, Brooks 1987).

Nickel is a micronutrient required at very low concentrations by plants (Brown et al. 1987) and toxic amounts of this element can occur in many environments. In the xylem sap of some hyperaccumulator plants, histidine has been shown to be coordinated with nickel in vivo and to play a role both in nickel tolerance and transport to the shoot (Krämer et al. 1996). In the cytoplasm, high levels of free nickel are generally avoided by removal of the metal ions to the vacuoles and the formation of complexes with organic acids (Ernst et al. 1990). If the free nickel level remains high,

Abbreviations – APOD, ascorbate peroxidase; CAT, catalase; GPX guaiacol peroxidase; GR, glutathione reductase; MDA, malondialdehyde; POX, polyphenoloxidase; ROS, reactive oxygen species; SPX, syringaldazine peroxidase; SOD, superoxide dismutase.

it inevitably binds organic macromolecules and denatures them. Furthermore, nickel can replace iron, zinc and magnesium due to the chemical affinity with those elements, interfering with their metabolism (Woolhouse 1983).

Copper is another essential micronutrient that can frequently occur at toxic levels in the environment owing to its widespread industrial and agricultural use. The toxicity of copper derives from its ability to generate reactive oxygen species (ROS), such as hydroxyl radical, superoxide anion and H_2O_2 (Girotti 1985, Luna et al. 1994). The oxidative damage induced by metals with redox activity, such as copper and iron, can be explained by the metal-mediated formation of hydroxyl radicals from the Haber-Weiss reaction between superoxide and H_2O_2 (De Vos and Schat 1991).

ROS, particularly hydroxyl radical, can damage membranes causing the initiation of the peroxidation of unsaturated fatty acids (De Vos and Schat 1991). Cells have evolved efficient strategies to counteract oxidative stress. Enzymatic and non-enzymatic reactions of free radical scavengers minimise cellular oxidations: superoxide dismutase (SOD), catalase (CAT), peroxidase and antioxidant compounds, such as α-tocopherol, ascorbate and glutathione, can play a key role in controlling cellular levels of metal-induced ROS (Weckx and Clijsters 1996).

This research was performed by studying three ecotypes of *Silene paradoxa* L., from a calcareous soil, a serpentine outcrop and a copper mine dump, respectively. The aim was to investigate the probable occurrence of multiple tolerance or co-tolerance mechanisms and to shed light on some of the tolerance strategies used by this plant, especially with regard to the involvement of antioxidative defences against heavy metal-induced oxidative stress.

Materials and methods

Plant material

Silene paradoxa L. seeds were collected from plants living on non-contaminated soil (Colle Val d'Elsa, CVD), serpentine soil (Pieve Santo Stefano, PSS) and a copper mine deposit (Fenice Capanne, FC). The seeds were sterilised with ethanol 70% for 20 s and with NaClO 2.5% for 20 min and washed three times with distilled water. The seeds were then germinated for 3 days in the dark on floating trays held in 1-dm^3 vessels containing 400 ml of continuously aerated Arnon solution (Arnon 1938) diluted 1:10, pH 5.5 ± 0.1, and with iron supplied as iron tartrate. The culture conditions were a 16-h (day) photoperiod, provided by Philips TDL 58W/33 fluorescent tubes (160 μmol m^{-2} s^{-1}), at 23 ± 1°C and a relative humidity of 60/65%.

Root elongation test

After germination, the floating trays with seedlings were placed in fresh hydroponic solutions containing various $NiSO_4$ or $CuSO_4$ concentrations. After 10 days of growth, the root length of 20 plantlets for each treatment was measured. This was chosen as a measurement of the toxic effects of metals on the populations because root elongation is particularly sensitive to the presence of metal toxins (Baker and Walker 1989).

Plant nickel and copper concentration

The plantlets, grown in the conditions of the above-mentioned experiment, were rinsed with distilled water and the root was carefully washed with $CaCl_2$ 10 m*M* at 4°C for 10 min to remove the adhering metals. The plantlets were then divided into shoots and roots, dried at 70°C for 1 day and subsequently weighed and exposed to acid digestion with HNO_3 (65% v/v) and $HClO_4$ (70% v/v). The concentration of metals was determined by atomic absorption spectrometry with a Perkin-Elmer 370 spectrophotometer.

Malondialdehyde (MDA), acid ascorbic and enzyme assay

The plantlets, after germination and 10 days of culturing, were treated for 4 days with 0.1 m*M* $NiSO_4$ or $CuSO_4$. The roots were then washed with 10 m*M* $CaCl_2$ at 4°C for 10 min. The plantlets were then divided into shoots and roots and subjected to the following analyses:

- MDA assay: lipid peroxidation was measured as the amount of MDA, determined by the thiobarbituric acid reaction (Buege and Aust 1978).
- Ascorbic acid determination: this assay quantified spectrophotometrically total ascorbate and oxidated ascorbate according to the method of Hodges et al. (1996).
- Enzyme assays: frozen cell material was ground in 2.5 ml g^{-1} fresh weight of 50 m*M* phosphate buffer, pH 7, containing 5 m*M* Na-ascorbate, 10 g l^{-1} PVP-10, 0.2 m*M* EDTA and 10 ml l^{-1} Triton X-100. After filtration with Miracloth (Calbiochem), the extract was centrifuged at 4°C for 20 min at 20000 *g*. The protein content was assayed according to the method of Bradford (Bradford 1976).

For enzyme activity determination, the following methods were used: CAT (EC 1.11.1.6) Aebi (1984); guaiacol peroxidase (GPX, EC 1.11.1.7) Pandolfini et al. (1992); ascorbate peroxidase (APOD, EC 1.11.1.11) Nakano and Asada (1981); glutathione reductase (GR, EC 1.6.4.2) Fielding and Hall (1978); SOD (EC 1.15.1.1) McCord and Fridovich (1969); polyphenoloxidase (POX, EC 1.10.3.1) Boyer (1977); syringaldazine peroxidase (SPX, EC 1.11.1.7) Pandolfini et al. (1992).

All spectrophotometric analyses were performed in a 551S Perkin-Elmer UV/Visible spectrophotometer.

All the chemical reagents were from Sigma Chemical Co. (St Louis, MO, USA).

Statistics

All treatments were performed in triplicate and repeated in at least two independent experiments. The significance of differences was analysed by one-factorial ANOVA followed by a multiple *t*-test.

Results

Root elongation test

The results showed that the CVD was significantly the population most sensitive to nickel, whereas the other two were equally more tolerant (Fig. 1a). In the sensitive population, the nickel induced a significant inhibition of the root growth even at the lowest concentrations used.

The presence of copper ions in the culture medium produced the lowest toxic effect on the FC population in terms of root growth reduction, whereas the other two populations were equally more sensitive to this ion (Fig. 1b).

Plant nickel and copper concentration

In all the populations, the shoot nickel concentration after 10 days of treatment was proportional to the nickel medium concentration; the maximum value reached was similar for CVD and FC (about 1 mg g^{-1} dry weight), whereas the population of PSS showed the highest values (up to 2.7 mg g^{-1} dry weight for the highest $NiSO_4$ concentration used) (Fig. 2a).

In the roots of the CVD and FC plantlets, nickel was taken up following the same saturation trend (Fig. 2b) though reaching different maximum values (about 3 mg g^{-1} dry weight for CVD and about 1.9 mg g^{-1} dry weight for FC). The PSS population showed a nickel concentration proportional to the medium metal concentration without any detectable saturation trend for the concentrations used (Fig. 2b) and the maximum tissue concentration was up to 3.5 mg g^{-1} dry weight.

The copper concentration in the shoots differed greatly between FC and the other two populations; the highest copper concentration reached was about 0.3 mg g^{-1} dry weight, whereas in CVD and PSS it was about 0.9 mg g^{-1} dry weight (Fig. 2c).

Root copper concentration followed the same saturation trend in all the populations, though different highest values were reached (about 1.7, 2.1 and 3.0 mg g^{-1} dry weight, respectively, for FC, PSS and CVD populations) (Fig. 2d).

MDA content

The three populations showed different degrees of susceptibility to nickel- and copper-induced lipid peroxidation. The CVD plantlets showed a considerable increase in MDA concentration in the shoots after nickel and especially copper treatment (Fig. 3a). In the roots, only copper was able to induce a slight, but significant, increase in the MDA concentration (Fig. 3b). In the PSS plantlets, only copper treatment induced lipid peroxidation in both roots and shoots (Fig. 3c,d). Copper was able to induce lipid peroxidation in the shoots of FC plantlets, but at a rate that was the lowest among the three populations (Fig. 3e). In the roots, neither nickel nor copper treatment caused significant changes in MDA content (Fig. 3f).

Ascorbate content

The populations demonstrated the same behaviour in terms of ascorbate content variations after nickel or copper treatments (Table 1). In the roots, the presence of nickel ions in the culture medium increased the reduced ascorbate content; conversely, copper did not affect it. The total root ascorbate content was, however, increased by nickel treatment and, particularly, by copper treatment. In the shoots, copper and, to a lesser extent, nickel induced a significant increase in reduced as well as total ascorbate content.

The activity of SOD, some of the ascorbate-glutathione cycle enzymes (APOD, GR) and CAT

The activity of SOD was not affected by nickel treatment, whereas copper provoked a great induction of this enzyme in both the CVD and the PSS populations.

CAT activity decreased during nickel treatment in the shoots of the CVD population (Table 2); copper affected this enzyme by increasing activity in roots. Shoot CAT inhibition brought about by nickel was not present in the PSS population; rather, it increased. Copper also increased this activity in this population. In the FC population, no

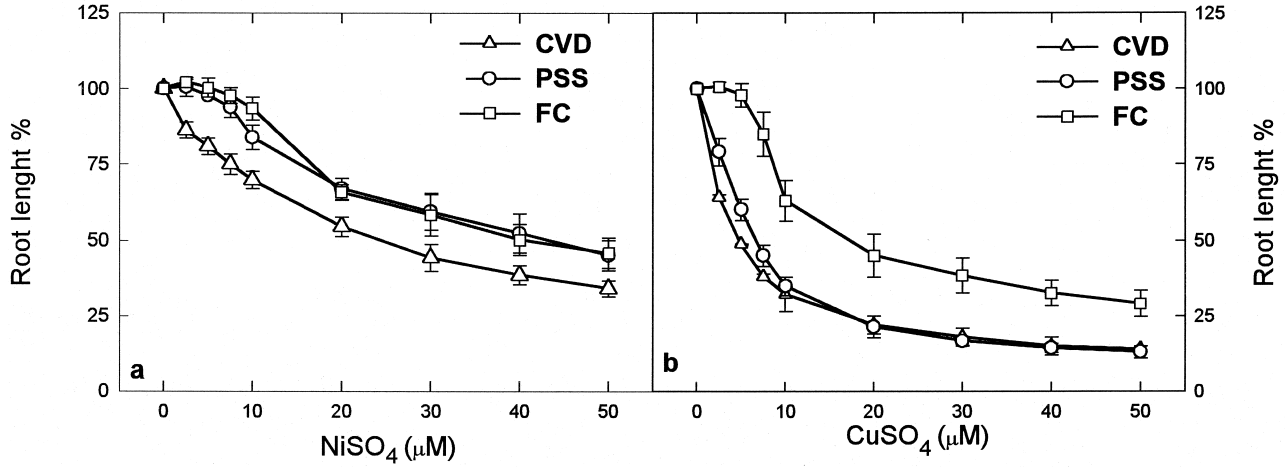

Fig. 1. Root elongation test of the ecotypes CVD (sensitive), PSS (nickel-tolerant) and FC (copper-tolerant) of *S. paradoxa* treated with different concentrations of $NiSO_4$ and $CuSO_4$. Values are expressed as percentage of the control. Values are mean ± SE.

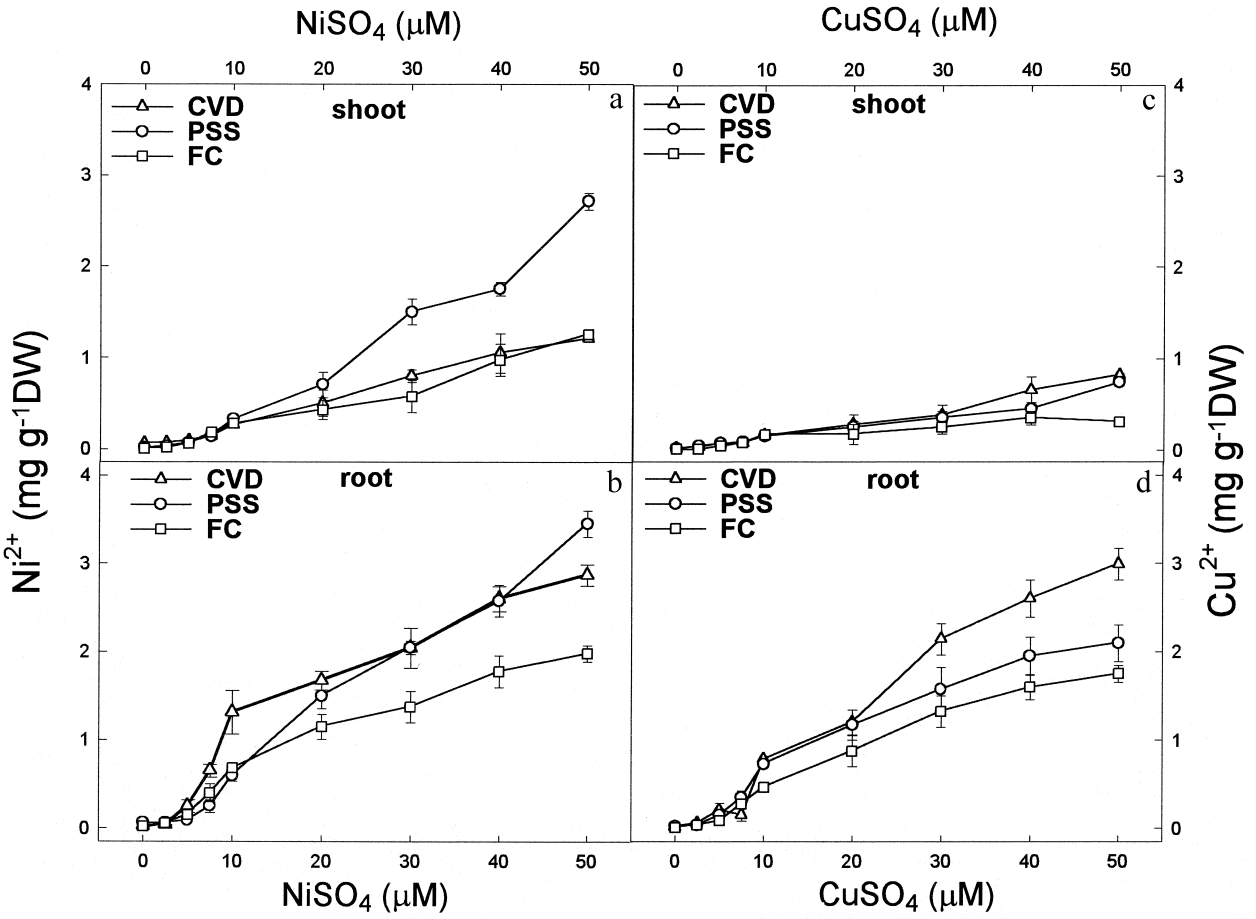

Fig. 2. Nickel and copper concentration in the ecotypes CVD (sensitive), PSS (nickel-tolerant) and FC (copper-tolerant) of *S. paradoxa* treated with different concentrations of $NiSO_4$ and $CuSO_4$. Values are mean ± SE.

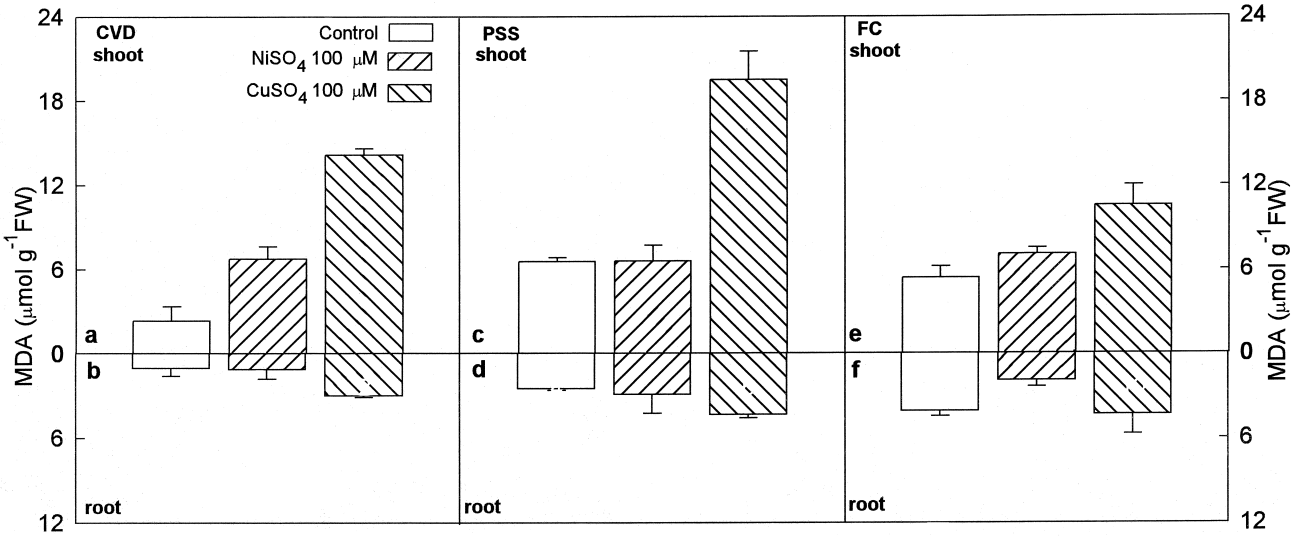

Fig. 3. MDA concentration in roots and shoots of the ecotypes CVD (sensitive), PSS (nickel-tolerant) and FC (copper-tolerant) of *S. paradoxa* treated with 100 μ*M* $NiSO_4$ or $CuSO_4$ for 4 days. Values are mean ± SE.

significant variation of activity was registered for either the nickel and copper treatments.

Concerning APOD activity, the presence of nickel was able to induce it in the roots of the sensitive population and in the shoots of the nickel-tolerant population. Copper produced a similar effect and, moreover, it was able to induce the activity of this enzyme in the shoots of CVD as well. The activity of GR was induced by copper and nickel in both the CVD and PSS populations. FC did not show any significant induction.

Activity of peroxidases (GPX, SPX) and of POX

The CVD and PSS populations showed the same behaviour toward nickel or copper stress in terms of GPX induction

Table 1. Changes of reduced ascorbate and total ascorbate content (nmol g^{-1} FW) in roots and shoots of three populations of *S. paradoxa* after 4 days of treatment with 100 μM $NiSO_4$ or $CuSO_4$. AA, reduced ascorbate; DHA, oxidized ascorbate. $^aP<0.001$, $^bP<0.01$, $^cP<0.05$. Values are mean ± SE.

Population	Root			Shoot		
	Control	$NiSO_4$	$CuSO_4$	Control	$NiSO_4$	$CuSO_4$
AA						
CVD	218 ± 55	419 ± 33^b	187 ± 41	338 ± 25	486 ± 5^b	666 ± 22^b
PSS	240 ± 33	355 ± 41^b	190 ± 23	274 ± 22	534 ± 87^c	632 ± 5^b
FC	450 ± 64	542 ± 55	430 ± 5	633 ± 58	596 ± 70	1001 ± 5^c
DHA + AA						
CVD	297 ± 9	453 ± 22^b	3274 ± 41^b	959 ± 19	2093 ± 111^b	2375 ± 81^b
PSS	363 ± 39	674 ± 28^c	1366 ± 321^b	1579 ± 176	2369 ± 54^b	4458 ± 47^a
FC	590 ± 47	984 ± 89^c	9319 ± 235^a	1659 ± 91	2059 ± 173^b	2848 ± 106^c

Table 2. Changes of the specific activity of SOD (U mg^{-1} protein min^{-1}), CAT, APOD and GR ($\Delta\varepsilon$ mg^{-1} protein min^{-1}) in roots and shoots of three populations of *S. paradoxa* after 4 days of treatment with 100 μM $NiSO_4$ or $CuSO_4$. $^aP<0.001$, $^bP<0.01$, $^cP<0.05$. Values are mean ± SE.

Population	Root			Shoot		
	Control	$NiSO_4$	$CuSO_4$	Control	$NiSO_4$	$CuSO_4$
SOD						
CVD	0.23 ± 0.03	0.27 ± 0.06	0.57 ± 0.05^a	0.32 ± 0.02	0.29 ± 0.01	0.84 ± 0.03^a
PSS	0.59 ± 0.01	0.53 ± 0.04	0.63 ± 0.01^b	0.46 ± 0.03	0.53 ± 0.05	0.55 ± 0.04^c
FC	0.40 ± 0.04	0.48 ± 0.03	0.55 ± 0.09	0.32 ± 0.04	0.31 ± 0.03	0.41 ± 0.10
CAT						
CVD	2.35 ± 0.21	2.20 ± 0.25	4.50 ± 0.42^b	5.90 ± 0.19	3.96 ± 0.11^c	5.00 ± 0.33
PSS	3.28 ± 0.23	2.86 ± 0.13	4.55 ± 0.14^b	5.56 ± 0.55	7.82 ± 0.54^b	9.41 ± 0.60^b
FC	4.96 ± 0.57	4.48 ± 0.02	5.41 ± 0.41	3.99 ± 0.32	3.31 ± 0.44	4.53 ± 0.75
APOD						
CVD	0.54 ± 0.02	3.92 ± 0.33^a	1.23 ± 0.02^b	1.31 ± 0.04	1.24 ± 0.02	2.03 ± 0.20^b
PSS	4.51 ± 0.06	3.99 ± 0.29	3.90 ± 0.37	1.04 ± 0.11	2.45 ± 0.23^a	2.74 ± 0.32^a
FC	4.16 ± 0.09	3.19 ± 0.49	4.16 ± 0.05	4.32 ± 0.07	3.87 ± 0.14	4.26 ± 0.31
GR						
CVD	1.31 ± 0.09	2.20 ± 0.17^b	4.53 ± 0.35^b	1.30 ± 0.11	1.94 ± 0.06^a	2.10 ± 0.20^a
PSS	1.51 ± 0.06	1.94 ± 0.03^a	7.66 ± 0.60^a	2.28 ± 0.34	3.69 ± 0.43^c	3.60 ± 0.58^c
FC	1.20 ± 0.11	2.09 ± 0.04	1.52 ± 0.14	4.40 ± 0.13	4.91 ± 0.85	4.02 ± 0.42

Table 3. Changes of the specific activity of GPX, SPX and POX ($\Delta\varepsilon$ mg^{-1} protein min^{-1}) in roots and shoots of three populations of *S. paradoxa* after 4 days of treatment with 100 μM $NiSO_4$ or $CuSO_4$. $^aP<0.001$, $^bP<0.01$, $^cP<0.05$. Values are mean ± SE.

Population	Root			Shoot		
	Control	$NiSO_4$	$CuSO_4$	Control	$NiSO_4$	$CuSO_4$
GPX						
CVD	9.7 ± 0.3	17.4 ± 1.4^a	58.2 ± 0.1^a	1.5 ± 0.2	2.7 ± 0.7	14.5 ± 0.3^a
PSS	57.6 ± 2.7	78.8 ± 2.0^a	85.0 ± 1.5^a	3.6 ± 0.3	4.2 ± 0.2	11.3 ± 1.8^b
FC	57.8 ± 1.2	50.1 ± 1.4^b	84.0 ± 2.0^a	7.7 ± 1.3	5.0 ± 0.1^c	13.9 ± 0.4^c
SPX						
CVD	75.4 ± 3.5	66.4 ± 6.7	177.2 ± 1.8^a	25.1 ± 1.3	24.3 ± 1.8	87.5 ± 3.5^a
PSS	179.7 ± 6.9	172.8 ± 1.4	232.0 ± 19.0^c	27.5 ± 1.2	32.6 ± 0.5^c	93.7 ± 4.0^a
FC	112.9 ± 1.6	134.7 ± 8.6^c	259.9 ± 2.5^a	46.3 ± 0.8	46.1 ± 1.7	70.2 ± 2.2^a
POX						
CVD	0.22 ± 0.03	0.55 ± 0.12^c	1.32 ± 0.06^a	0.22 ± 0.08	0.22 ± 0.04	0.42 ± 0.02^c
PSS	0.52 ± 0.06	1.25 ± 0.08^a	0.85 ± 0.15^c	0.27 ± 0.03	1.02 ± 0.14^a	0.52 ± 0.10^c
FC	0.53 ± 0.07	0.46 ± 0.02	0.57 ± 0.05	1.05 ± 0.18	0.87 ± 0.16	1.10 ± 0.12

(Table 3). This enzyme was induced in the roots and shoots by copper and only in the roots by nickel. The FC population showed a decrease in GPX activity after nickel stress and an increase in the presence of copper. SPX activity was induced in all the populations by copper treatment; nickel instead increased it only in the shoots of the PSS population and the roots of the FC population. POX activity was induced in the roots of the CVD population and both in the roots and shoots of the PSS population. Copper also induced POX in the CVD and PSS populations.

Discussion

S. paradoxa L. (Caryophyllaceae) has evolved several populations capable of surviving on many contaminated soils in Central Italy (Chiarucci et al. 1995). So, this plant shows a high adaptation capability to extreme nutritional conditions since it generally lives on non-contaminated soils (Pichi-Sermolli 1948).

Three Tuscan ecotypes of *S. paradoxa* living on a calcareous soil, a serpentine outcrop and a copper mine dump, respectively, were compared in order to investigate the presence of multiple tolerance or co-tolerance mechanisms and some physiological strategies of metal tolerance.

As a result of the root elongation test, the population from the copper mine soil, characterised by a very low nickel concentration (M. Falsini 1999. Thesis, University of Firenze, Firenze, Italy), proved to be nickel-tolerant as well, whereas the nickel-tolerant population was not copper-tolerant. This confirms the hypothesis that metal co-tolerance does not necessarily represent a two-way relationship (Baker and Walker 1989). Similar results were obtained by Verkleij and Bast-Cramer (1985) for a population of *Silene cucubalus* from copper-enriched soils, which showed zinc co-tolerance, but the converse was not the case.

The results regarding the plant metal concentration suggested different strategies for tolerating metals with different characteristics, such as nickel and copper: in roots and shoots, the nickel-tolerant population showed the highest nickel concentrations, whereas the copper-tolerant population had the lowest copper concentrations. So, in these natural ecotypes of *S. paradoxa*, the ability to tolerate metals seemed to rely on avoidance (Baker, 1981) only for copper.

To underline some effects and responses to toxic concentrations of these elements, the relation between heavy metals and oxidative stress was evaluated, taking into account that there may also be other mechanisms involved in metal tolerance, such as metal vacuolar localisation, metal speciation (Rengel 1997, Ernst 1998) and the production of metal chelators. For example, Murphy and Taiz (1995) indicated metallothionein (MT2) expression as a primary determinant of ecotypic differences in the copper tolerance in *Arabidopsis*, whereas, for nickel tolerance, Krämer et al. (1996) suggested that free histidine may play a crucial role in hyperaccumulator species of *Alyssum*.

In the sensitive population, copper was able to induce lipid peroxidation to a greater extent than nickel, in agreement with the fact that different metals have different capabilities for inducing oxidative stress (Gallego et al. 1996) owing to their different action mechanisms (De Vos and Schat 1991, Baccouch et al. 1998). Only the copper-tolerant population did not suffer both copper and nickel toxicity in terms of MDA production, whereas the same did not happen in the nickel-tolerant population, thus confirming the above-mentioned possibility of a non-reciprocal co-tolerance mechanism.

In the sensitive population, the high copper-induced peroxidation rate was followed by an increase in SOD activity, which can generally be explained by an increase in superoxide anion levels (Chongpraditnum et al. 1992) or as result of the effect of ROS on gene regulation (Kurepa et al. 1997). The nickel-induced lipid peroxidation was not followed by SOD induction. As a consequence, the formation of ROS following nickel stress may occur, but not through superoxide production or, in any case, not through the copper-induced mechanism. In fact, nickel was able to provoke CAT inhibition preventing the active scavenging of H_2O_2, which is normally formed during peroxisomal metabolism. Higher levels of H_2O_2 could in part explain the nickel-induced oxidative stress.

Regarding the ascorbate-glutathione cycle, nickel and, more pronouncedly, copper were able to activate the tested enzymes in the sensitive population in both shoots and roots. This suggests that even a redox-inactive metal, such as nickel, is able to enhance the ascorbate-glutathione cycle as part of the defence against oxidative stress, as was already shown by Gupta et al. (1999) for copper, a redox-active metal. The involvement of this cycle in response to copper and nickel stress was also suggested by the increase of the total and reduced ascorbate content induced by these metals.

Peroxidases were investigated in relation to their ability to prevent oxidative damage to plasma membranes, acting as peroxide radical scavengers (Van Assche and Clijsters 1990), and, in fact, in the sensitive population, the activity of GPX and of SPX was increased by copper stress in both roots and shoots. Nickel did not affect peroxidase activity, meaning that this type of response was not involved in the nickel stress in this population.

To complete the investigation on the enzymes that can induce lignification changes in relation to heavy metal stress, POX activity was evaluated. The sensitive population showed an induction of POX activity in roots and shoots after nickel or copper treatment, probably to act synergistically with acid peroxidases in the cell wall stiffening.

The nickel-tolerant population activated the same enzymes during nickel and copper stress as the sensitive population. The difference in the effect of nickel on CAT activity might account for nickel tolerance: nickel did not induce CAT inhibition in the nickel-tolerant population, thus allowing the active scavenging of H_2O_2 that prevents oxidative stress to continue. The stability of this enzyme during nickel stress could be the consequence of a low biochemical sensitivity to heavy metal ions in situ or of an increased and effective turnover. This characteristic was not able to confer copper co-tolerance because this ion is redox-active and can directly generate ROS.

The copper-tolerant population did not show any striking variation in enzyme activities during nickel and copper

stress. The copper-activated enzymes were only GPX, as in the other populations, and SPX in the roots. This latter induction might limit the copper-induced damage already at the root level since the cell wall stiffening, caused by SPX activity, and the correlated lower growth rate are well known as a mechanical adaptation to stress conditions (Gaspar et al. 1985).

Ascorbate is considered the primary antioxidant cellular compound (Noctor and Foyer 1998). Only in the copper-tolerant population did the metal treatments not affect the reduced ascorbate levels, thus confirming the limitation of the oxidative damage. This could be an effect of the highest production of total ascorbate in the roots of this population after nickel and copper treatment. Nickel-induced CAT inhibition did not take place in this population either.

Mengoni et al. (2000, 2001) produced some convincing evidence that the copper-tolerant population came from its neighbouring serpentine populations, thus providing a sound explanation for the occurrence of the nickel co-tolerance.

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