Cadmium Tolerance in a Metal-contaminated Population of the Grassland Moss *Rhytidiadelphus squarrosus*

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Two populations of *Rhytidiadelphus squarrosus*, from metal-contaminated and uncontaminated habitats, differed in their intra- and extracellular Cd contents but had similar cellular levels of Cu. Moss shoots were supplied with a pulse of toxic metal by incubating in Cu or Cd nitrate solutions and effects on respiration, photosynthesis and intracellular K loss were monitored with time after initial exposure. Increasing intracellular Cu levels correlated most closely with a concurrent decline in intracellular K. Photosynthesis also declined in proportion to intracellular Cu; significant Cu-induced stimulation of respiration was observed. The most significant effect of Cd treatment was a decline in photosynthesis in proportion to the intracellular concentration of Cd. Apical segments from both populations showed similar sensitivity to Cu, whereas the metal-contaminated population showed increased resistance to Cd. Sensitivity to Cd increased in the more basal portions of moss gametophores, indicating that apparent resistance of Cd might reflect shoot vitality and age effects. After laboratory growth to eliminate differences in the physiological status of apical segments, it was confirmed that the metal-contaminated population of the moss was photosynthetically more tolerant to Cd at intracellular Cd concentrations found to cause considerable photosynthetic inhibition in the uncontaminated population. The metal-contaminated population of the moss that was tolerant to Cd was not co-tolerant to Cu.

Key words: *Rhytidiadelphus squarrosus*, cadmium, copper, potassium leakage, photosynthesis, respiration, intracellular metals, moss, heavy-metal tolerance.

INTRODUCTION

Several studies have used bryophytes to monitor dispersion patterns of atmospheric pollutants and it is frequently observed that some bryophytes accumulate potentially toxic levels of metals without apparent damage (see Brown, 1984; Brown and Wells, 1990a; Herrmann, 1990; Tyler, 1990). Other studies have focused on the substratum on which bryophytes grow and there is evidence that some species show specific tolerance to metals present in their natural environment (see reviews by Brown, 1982; Shaw, 1989).

Brown and House (1978), using K leakage as a measure of membrane damage, showed a population of the hepatic *Solenostoma crenulatum* from a disused Cu mine, to be more resistant to experimentally supplied Cu than a population from a disused Pb mine. Similarly, urban populations of *Marchantia polymorpha*, were not affected by experimentally supplied Ni at concentrations inhibitory to the growth of glasshouse populations (Lepp and Hockenhull, 1983). Gemmae taken from populations of *M. polymorpha* subject to atmospheric Pb pollution were more tolerant to experimentally supplied Pb than gemmae taken from plants subject to low Pb pollution (Briggs, 1972). More recent studies have concentrated on the effects of metal stress on the protonemal phase of the life cycle (Chopra and Kapur, 1989; Shaw, 1990). These examples demonstrate the existence of heavy metal tolerance in bryophytes. In each case tolerant bryophyte populations occurred when con-

ditions of metal stress prevailed in the plants' natural habitat.

An important question when considering tolerance mechanisms is that of specificity. There are several examples (see review by Steffens, 1990) in higher plants that selection of tolerance to one metal results in increased tolerance to one or more further metals, while others have found that tolerance of one metal does not confer co-tolerance to a second metal. Phyto-chelators (plant metal-binding peptides), whilst induced by heavy metals, vary in the specificity of tolerance that they confer to plants according to species and genotype (Steffens, 1990).

For *Funaria hygrometrica* it has been shown that pretreatment of plant material with one metal (Zn) may increase tolerance to a second metal (Cd) (Shaw, 1987). However, it has not been shown whether metal-tolerant populations of bryophytes possess tolerance mechanisms specific for the metals experienced in the environment. In this paper we report a detailed comparison of Cd toxicity in 'metal-contaminated' and 'uncontaminated' populations of *R. squarrosus* and evidence is presented for the existence of a Cd-tolerant population of this moss.

MATERIALS AND METHODS

Plant material

Field-grown material from two populations of *Rhytidiadelphus squarrosus* (Hedw.) Warnst of contrasting metal

FIG. 1. Intra- (A, C, E) and extracellular (B, D, F) Zn (A, B), Cd (C, D) and Cu (E, F) concentrations in sequential 2-cm segments of uncontaminated (■) and metal-contaminated (□) populations of *Rhytidiadelphus squarrosus*. * Denotes significant difference between means ($P < 0.05$).

status were collected from grassland in Burrington Combe, Avon (Ordnance Survey grid reference ST 478582, referred to as 'uncontaminated') and Glovers Field, Shipham, UK a disused zinc mine (Ordnance Survey grid reference ST 446574, referred to as 'metal-contaminated'). Analysis of the cellular locations of Zn, Cd and Cu in the two populations showed that the 'metal-contaminated' material was enriched in intracellular Cd and extracellular Zn and Cd compared to the 'uncontaminated' population (Fig. 1). Laboratory-grown plant material was obtained by placing 8-cm lengths of moss shoots from these populations in a covered propagator tray, lined with wet absorbent paper, in a growth room. Plants were then maintained under continuous fluorescent lighting at a photon flux density (PFD) of 50 to 75 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at $20 \pm 1^\circ\text{C}$ for 28 d. During this time shoots were watered with deionized distilled water (DDW) at 3-d intervals and new growth of about 3 cm occurred. Prior to use, sorted shoots were rinsed briefly in DDW (1 l per hundred 2-cm segments and allowed to equilibrate for 24 h to laboratory temperature (range 18–21 $^\circ\text{C}$). Unless stated otherwise, 2-cm apical moss segments were used for experiments and results are presented as means of five to six replicates with the standard error.

Where appropriate the significance of differences between means was determined by *t*-tests.

Metal pulse treatment

Initial exposure of moss shoots to Cd and Cu involved incubation for 30 min in 1 l of a range of concentrations of freshly-prepared solutions of Cd or Cu nitrates (AnalaR), aerated and agitated by bubbling with compressed air. On subsequent storage of moss shoots, extracellular metals, accumulated during initial exposure, move to intracellular locations (Brown and Wells, 1990b) permitting assessment of physiological effects in relation to increasing intracellular metal concentrations. To obtain a wide range of intracellular Cd concentrations, the two populations were initially treated with 10, 100 and 1000 μM Cd solutions and maintained for up to 72 h after exposure. As Cu is more toxic than Cd (Brown and Wells, 1990b) Cu was supplied at 1, 10 and 100 μM and only maintained for up to 16 h after exposure.

After initial exposure to metal solutions, moss shoots were separated on a Buchner funnel, rinsed quickly with 2 litre DDW and sorted into replicate samples of eight to ten segments. Samples were either transferred to 30-ml

FIG. 2. The time course of the effects of Cd on respiration (A, B), photosynthesis (C, D) and intracellular K concentrations (E, F) in 2-cm apical segments of populations of *Rhytidiadelphus squarrosus* from an uncontaminated site (A, C, E) or a metal-contaminated site (B, D, F) following a 30-min incubation in 10 μM (■), 100 μM (●) or 1000 μM (▲) Cd solutions. (---) Untreated control. The error bar shows the minimum significant difference between means ($P < 0.05$).

McCartney bottles for gas exchange measurements or for storage as follows: where gas exchange and cellular cation location was to be determined at intervals following metal treatment, samples were transferred to 2.5 × 5.0 cm specimen tubes in a moistened, glass-covered tray under continuous fluorescent lighting (PFD, 75 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ at 20 ± 1 °C) as described previously (Wells and Brown, 1987; Brown and Wells, 1990b).

Gas exchange

Photosynthetic and respiratory CO_2 exchange were determined at 20 ± 1 °C by injection infra-red gas analysis using an ADC225 Mk3 IRGA, essentially following the method of Snelgar, Brown and Green (1980). Samples were placed in 30-ml McCartney bottles having screw caps with a central hole and rubber seal to facilitate removal of an air sample with a syringe. Photosynthesis was determined at a PFD of 265 $\mu\text{mol m}^{-2} \text{ s}^{-1}$, which was determined to be light saturating for *R. squarrosus* under the conditions described here (Wells, 1988).

Cation location and analysis

The cellular location of cations in untreated, Cd-treated and Cu-treated moss shoots was determined using modifica-

tions of a sequential elution technique described by Brown and Wells (1988). Eight to 10 2-cm segments were shaken three times for 20 min in 10 ml of either 20 mM NiCl_2 (untreated and Cd-treated shoots) or 500 mM $\text{Pb}(\text{NO}_3)_2$ (Cu-treated shoots) to displace extracellular ions. In each case the three fractions were pooled and made up to volume (50 or 100 ml) for analysis.

Moss samples were then soaked overnight in 10 ml of 1 M HNO_3 to displace intracellular cations. After removal of a known volume for appropriate dilution and analysis, samples were washed thoroughly in distilled water, oven dried at 80 °C and weighed. Electron microscopy studies have shown that 1 M HNO_3 treatment removes most of the protoplast of *R. squarrosus* and the post-acid dry weight may be taken as an estimate of the mass of cell wall material in moss segments (Wells, 1988). The cell wall:protoplast dry weight ratio was determined by taking the dry weights of untreated moss segments before and after treatment with 1 M HNO_3 . This ratio was used to estimate the mass of protoplasts within moss segments, allowing results for intracellular cation analyses to be expressed on a protoplast dry weight basis. This is essential when comparing populations of plant material in which it is possible that field-grown segments of particular lengths may have different age structures and morphological characteristics (Wells and Brown, 1987). The necessity for using these estimates has

FIG. 3. The time course of the effects of Cu on respiration (A, B), photosynthesis (C, D) and intracellular K concentrations (E, F) in 2-cm apical segments of populations of *Rhytidadelphus squarrosus* from an uncontaminated site (A, C, E) or a metal-contaminated site (B, D, F) following a 30 min incubation in 1 μM (■), 10 μM (●) or 100 μM (▲) Cu solutions. (—) Untreated control. The error bar shows the minimum significant difference between means ($P < 0.05$).

been discussed elsewhere and shown to be appropriate for the experiments presented in this paper (Brown and Wells, 1988, 1990a; Wells, 1988).

Extracellular and intracellular fractions were analysed for K, Cu, Cd and Zn using a Varian Techtron AA6 atomic absorption spectrophotometer. All elements were determined in an air/acetylene flame using the manufacturer's recommended instrument parameters. In all cases, LaCl_3 and CsCl were added to sample and standard solutions to a final concentration of 1 g l^{-1} of La and Cs as suppressants of refractory compound formation and ionization, respectively.

RESULTS

Zn, Cd and Cu distribution in uncontaminated and metal-contaminated populations of *R. squarrosus*

Evidence that the two populations of *R. squarrosus* differed in their heavy metal status is shown in Fig. 1. The intracellular Zn or Cu levels of two populations did not differ significantly ($P > 0.05$, Fig. 1A, E) but subapical segments of the metal-contaminated population had signifi-

cantly more ($P < 0.05$) intracellular Cd than the uncontaminated population (Fig. 1C). Extracellular Cd and Zn increased in progressively older segments of the metal-contaminated population (Fig. 1B, D) whereas the converse was true for the uncontaminated population. Extracellular Cu distribution was not significantly different ($P > 0.05$) between the two populations of *R. squarrosus* (Fig. 1F). On average, the total concentrations of Zn and Cd in the metal-contaminated population were respectively 3.7 and 3.0 times higher than in the metal-uncontaminated population whereas Cu concentrations were not significantly different between the two populations (Fig. 1).

Effects of Cd and Cu treatment

Treatment with Cd had no significant ($P > 0.05$) effect on respiration rate in either population, compared to controls incubated in DDW (Fig. 2A, B). Cadmium-treated shoots of both populations showed reduced photosynthesis with time after exposure compared to DDW-incubated controls (Fig. 2C, D). The uncontaminated population of *R. squarrosus* showed progressively lower photosynthetic rates

FIG. 4. Photosynthesis as a function of intracellular Cd concentration in 2-cm apical segments of populations of *Rhytidiadelphus squarrosus* from an uncontaminated site (—, ■) or a metal-contaminated site (---, □) during transfer of a pulse of supplied Cd from the cell wall to the cell interior. Regression statistics are shown in Table 1.

with higher concentrations of supplied Cd. Intracellular K concentrations declined slightly, but insignificantly ($P > 0.05$), with time after Cd treatment (Fig. 2E, F).

Following Cu treatment, respiration of the two populations during the first 4 h of storage was not significantly different ($P > 0.05$) from controls (Fig. 3A, B). After storage for between 4 and 16 h, respiration was considerably stimulated following 10 or 100 μM Cu treatments (Fig. 3A, B). Photosynthetic rates (Fig. 3C, D) and intracellular K (Fig. 3E, F) declined with both concentration and time after exposure to Cu, although the effects were not always significant ($P > 0.05$). Treatment with 100 μM Cu significantly lowered photosynthetic rates ($P < 0.05$) after 4 h in the uncontaminated population but after 8 h in the metal-contaminated moss shoots (Fig. 3C, D). By contrast, intracellular K loss was significant ($P < 0.05$) only after 8 h in the uncontaminated population but after 4 h in the metal-contaminated moss shoots (Fig. 3E, F).

Physiological response in relation to intracellular Cd or Cu uptake

Although Cd was supplied at the same concentrations to each population, metal-contaminated moss shoots accumulated less intracellular Cd than uncontaminated moss shoots (Fig. 4). Regression analysis indicated that the uncontaminated population displayed a significantly greater ($P < 0.01$) decline in photosynthetic rate per unit intracellular Cd than the metal-contaminated population (Fig. 4, Table 1).

As with Cd, the metal-contaminated population of *R. squarrosus* accumulated less intracellular Cu than the uncontaminated population (Fig. 5). Although variability was high, regression analysis indicated a significant ($P < 0.01$) relationship between intracellular Cu uptake and respiration (Fig. 5A, Table 2). Photosynthetic rates and intracellular K concentrations declined in proportion to intracellular Cu levels in both populations (Fig. 5B, C). There were no significant differences between the two populations of *R. squarrosus* in relation to the physiological parameters measured in response to Cu treatment (Table 2).

Effect of shoot age and laboratory growth on Cd tolerance in *R. squarrosus*

Sequential shoot segments of both populations showed progressively increased sensitivity to Cd, the effect being relatively greater for the metal-contaminated population (Fig. 6). Consequently, in segments between 2 and 6 cm from the apex, no significant difference ($P > 0.05$) in the response to intracellular Cd levels could be detected between the two populations of *R. squarrosus* (Fig. 6B, C, Table 1).

For this experiment, photosynthetic capacities of untreated plant material from the metal-contaminated population were about 15% higher in apical segments (Fig. 7). However, photosynthetic rates were substantially less in segments between 4 and 8 cm from the apex in the metal-contaminated population than in the metal-uncontaminated population (Fig. 7).

After a period of laboratory growth, apical 2-cm segments from metal-contaminated and uncontaminated populations

TABLE 1. Summary of regression statistics on the relationship between photosynthesis and intracellular Cd concentration in two populations of *Rhytidiadelphus squarrosus*

Figure referred to	Segment	Population	Slope	F-ratio	Intercept
Fig. 4	0–2 cm	–M	–0.714 ^a	13.61**	(87.4) ^{ab}
		+M	–0.259 ^b	148.06**	(90.3) ^a
Fig. 6A	0–2 cm	–M	–0.658 ^{ad}	13.33**	(81.3) ^b
		+M	–0.413 ^c	21.37**	93.8 ^a
Fig. 6B	2–4 cm	–M	–0.470 ^{cd}	15.84**	(67.7) ^c
		+M	–0.446 ^c	14.99**	92.8 ^a
Fig. 6C	4–6 cm	–M	–0.301 ^{bu}	5.35*	(56.0) ^c
		+M	–0.343 ^{bc}	2.86 ns	(64.0) ^c
Fig. 8	Laboratory-grown	–M	–0.509 ^d	50.42**	(80.6) ^b
		+M	–0.214 ^b	10.23*	106.0 ^d

Significance levels, F-ratio; * $P > 0.05$, ** $P > 0.01$; ns, not significant. Moss shoots were from uncontaminated (–M) or metal-contaminated (+M) populations, *t*-tests showed that figures in the same column followed by the same letter are not significantly different ($P > 0.05$) and that intercepts in parentheses were significantly less than 100% ($P < 0.05$).

FIG. 5. Respiration (A), photosynthesis (B) and intracellular K (C) as a function of intracellular Cu concentration in 2-cm apical segments of populations of *Rhytidiadelphus squarrosus* from an uncontaminated site (—, ■) or a metal-contaminated site (---, □) during transfer of a pulse of supplied Cu from the cell wall to the cell interior. Regression statistics are shown in Table 2.

of *R. squarrosus* did not differ significantly in gas exchange rates, intracellular K concentrations, dry weights per unit length, or cell wall: protoplast weight ratios (Table 3). Untreated samples of laboratory grown apices had no detectable Cd. Despite the similarity in morphological and

TABLE 2. Summary of regression statistics on the relationship between intracellular Cu concentration and respiration, photosynthesis or intracellular K in two populations of *Rhytidiadelphus squarrosus*

Physiological parameter	Population	Slope	F-ratio
Respiration	—M	0.559 ^a	37.53**
	+M	0.879 ^a	26.55**
Photosynthesis	—M	−0.655 ^b	150.20**
	+M	−0.589 ^b	56.34**
Intracellular K	—M	−0.333 ^c	77.16**
	+M	−0.382 ^c	62.29**

Moss shoots were from uncontaminated (—M) or metal-contaminated (+M) populations; *t*-tests showed that figures in the same column followed by the same letter are not significantly different ($P > 0.05$).

physiological characteristics, newly-grown apical segments derived from the metal-contaminated population continued to display lower photosynthetic sensitivity to Cd than those derived from the uncontaminated population (Fig. 8, Table 1).

DISCUSSION

In general, bryophytes growing on metal-rich substrata show an increase in the concentration of metal from apex to base (Brown, 1982). Metals derived from atmospheric deposition tend to decrease from apex to base (Brown, 1982) although partial redistribution of heavy metals may occur from apex to base after deposition (Brown and Brown, 1990). The increase in total Zn and Cd in more basal segments of 'metal-contaminated' *R. squarrosus* was evidence that these metals were derived from the substratum in the disused Zn mine. In contrast, the Zn and Cd concentrations of the metal-uncontaminated population of *R. squarrosus* tended to decrease from apex to base, suggesting an atmospheric input. No particular source could be suggested for the copper content of moss shoots as the distribution of Cu showed little variation in sequential shoot segments of either population. Soil samples at the two collection sites reported here were not analysed but Brown and Wells (1990a) point out that it is the intracellular metal concentration experienced by moss cells which is of the greatest physiological significance.

Brown and Wells (1990b) showed that in relatively short-term assays of toxicity, Zn has little effect on the physiology of *R. squarrosus* but that Cd and Cu are toxic. Sidhu (1992) showed that Zn toxicity may best be addressed in longer term studies using shoot elongation as the parameter for assessment. Zinc was present at the highest total concentrations in the apical segments used in the experiments reported here with *R. squarrosus* at both sites (Fig. 1), which could have resulted in analytical difficulties. As the two populations had similar intracellular levels of Zn, their response to experimentally-supplied Zn was not investigated. In contrast to the situation with Zn, the cellular

FIG. 6. Photosynthesis as a function of intracellular Cd uptake in populations of *Rhytidadelphus squarrosus* from an uncontaminated site (—, ■) or a metal-contaminated site (---, □) during transfer of a pulse of supplied Cd from the cell wall to the cell interior of segments initially 0 to 2 (A), 2 to 4 (B) or 4 to 6 cm (C) from the apex. Regression statistics are shown in Table 1.

location of experimentally supplied Cd and Cu could be readily determined after exposure and their effects on *R. squarrosus* could be detected without having to supply the

FIG. 7. Photosynthesis in untreated sequential 2-cm segments of metal-contaminated (□) and uncontaminated (■) populations of *Rhytidadelphus squarrosus*. * Denotes significant difference between means ($P < 0.05$).

FIG. 8. Photosynthesis as a function of intracellular Cd uptake in 2-cm apical segments of laboratory-grown shoots of populations of *Rhytidadelphus squarrosus* from an uncontaminated site (—, ■) or a metal-contaminated site (---, □) during transfer of a pulse of supplied Cd from the cell wall to the cell interior. Regression statistics are shown in Table 1.

metals at very high concentrations (Brown and Wells, 1990b). It was therefore possible to compare Cd and Cu toxicity in the aerial shoots of populations of *R. squarrosus*

TABLE 3. Gas exchange rates, intracellular K concentrations, dry weights and cell wall: protoplast weight (CW:P) ratios in laboratory-grown 2-cm apical segments of two populations of *Rhytidadelphus squarrosus* of contrasting metal status

Population	CO ₂ exchange (μg g ⁻¹ min ⁻¹)		Intracellular K concentration (μmol g ⁻¹)	Dry weights of ten apical segments (mg)	CW:P ratio
	Photosynthesis	Respiration			
Uncontaminated	56 ^a	26 ^a	324 ^a	10.1 ^a	3.57 ^a
Metal-contaminated	58 ^a	22 ^a	328 ^a	10.6 ^a	3.35 ^a

Figures in the same column followed by the same letter are not significantly different using *t*-tests ($P > 0.05$).

containing similar, naturally occurring, intracellular Cu levels but contrasting intracellular Cd levels.

The results indicate that care must be taken in interpretation according to the toxic metal being studied and the physiological parameter chosen to assess toxicity. It has been suggested that an early and significant effect of toxic metals on cryptograms is membrane damage, assessed by loss of intracellular soluble K, leading to a general decline in protoplast vitality (Puckett, 1976; Nieboer *et al.*, 1979). Our results show that measuring intracellular K loss may be appropriate for assessing Cu toxicity in *R. squarrosus*, at least in short-term experiments, where K loss was proportional to the intracellular concentration of Cu. However, it has been demonstrated for *R. squarrosus* that according to the metal used, substantial reductions in photosynthetic ability can be achieved in the absence of significant K loss (Brown and Wells, 1990b). This was the case for Cd-treated shoots of *R. squarrosus* and it must be concluded that intracellular K loss is neither appropriate for determining Cd toxicity in *R. squarrosus*, nor useful for making inter-population comparisons of the physiological effects of Cd in this bryophyte. Comparative measures of intracellular K loss may be more appropriate for studies of desiccation effects in bryophytes where a general decline in protoplast vitality may be expected in response to water stress (Brown, 1982, 1984).

The effects of Cu on respiration in *R. squarrosus* reported here demonstrate the benefits of following the time course of response to treatment with toxic metals. Previously we reported a slight stimulation in respiration immediately following treatment with millimolar concentrations of Cu followed by a depression in CO₂ output 24 h after treatment (Brown and Wells, 1990b) comparable with results for *Funaria hygrometrica* (Sommer and Winkler, 1982). In the current study, considerable Cu-induced stimulation of respiration was observed, demonstrating that transitory effects on physiological processes may not be observed without frequent assessments following exposure to toxic metals. It has been suggested that a similar stimulation of respiration in *R. squarrosus* by Hg may be due to accelerated electron transport uncoupled from phosphorylation (Brown and Whitehead, 1986). Future pulse-chase experiments of the type reported here, in conjunction with studies of the cellular compartmentalization of the supplied metal, may reveal significantly more about the mechanisms of toxicity than straightforward dose-response studies.

Wells and Brown (1987) reported that morphologically distinct types of *R. squarrosus* from the same uncontaminated site reported here show a differential response to Cd treatment. A morphological type that appeared less sensitive to Cd also took up less intracellular Cd uptake than a second type. Both showed a similar photosynthetic response to the same intracellular levels of Cd (Brown and Wells, 1990b). In the current study it was therefore necessary to compare the physiological effects of Cd and Cu upon uncontaminated and metal-contaminated populations of *R. squarrosus* in relation to the intracellular concentrations of Cu or Cd experienced by moss shoots.

It has been reported that a near-linear relationship exists between the photosynthetic capacity of *R. squarrosus* and

the intracellular concentration of Cd or Zn supplied in experiments, regardless of the duration of exposure (Brown and Wells, 1990b; Brown and Sidhu, 1992). Here, we have used linear regression to test for interpopulation differences in response to intracellular Cu and Cd levels. For Cu, linear regression on experimental results seemed appropriate and it was concluded that both populations were equally sensitive to the same intracellular Cu concentration.

For Cd, intercepts significantly less ($P < 0.05$) than 100 % (Table 1), indicated a decline in photosynthetic rates attributable to incubation in Cd solutions, rather than to the intracellular concentration of Cd. Nevertheless, in apical 0–2 cm moss segments, the metal-contaminated population of *R. squarrosus* consistently showed a lower decline in photosynthetic rate per unit intracellular Cd uptake than the uncontaminated population. Thus, it was possible to conclude that the metal-contaminated population of *R. squarrosus* was more tolerant to Cd than the uncontaminated population. However, variation in tolerance to Cd with age of moss shoots indicated that the apparent Cd tolerance of apical segments of the metal-polluted population (Figs 4 and 6A) might simply reflect the age profile or shoot vitality of the various segments. Indeed, comparison of CO₂ exchange rates and/or intracellular K concentrations in Figs 2, 3 and 7 demonstrates that material from the two populations differed both between and within experiments. This is an inherent difficulty arising from the use of material collected in the field (Wells and Brown, 1987), which may be overcome by growing moss shoots in the laboratory.

On the basis of criteria outlined by Wells and Brown (1990) it was concluded that 2-cm segments from laboratory-grown apices did not differ significantly in age profile or vitality (Table 3). Laboratory-grown segments of the metal-contaminated population of *R. squarrosus* retained their tolerance to Cd even when levels of the metal were substantially less than could be detected in field-grown segments of either population. We conclude that Cd tolerance in the metal-contaminated population of *R. squarrosus* was a specific, genotypic response to Cd rather than a phenotypic adaptation that could be induced by metal stress in any population of this moss. It is probable that the increased sensitivity to Cd in progressively older segments in both populations of *R. squarrosus* reflected loss of a general low-level capacity to tolerate metal stress in more senescent tissues. This could be due, for example, to reduced protein turnover or membrane integrity, which might otherwise have partially protected the photosynthetic apparatus of *R. squarrosus*.

Phytochelatin is commonly implicated in heavy metal tolerance mechanisms in higher plants, although it is not clear to what extent they can account for differential tolerance between plant populations (see review by Steffens, 1990). It seems that heavy metals are the primary inducers of phytochelatin production in plants but that increased levels of phytochelatin within the plant may confer both specific and non-specific tolerance to metals, according to species and genotype. If phytochelatin were involved in Cd tolerance in *R. squarrosus*, it might be expected that Cd tolerance would be reduced in laboratory-grown apical segments, which had undetectable levels of Cd. Our results

suggest that Cd tolerance in *R. squarrosus* may not be an inducible phenomenon. However, it is possible that in *R. squarrosus*, newly-grown shoots from the metal-contaminated population were able to respond to much lower levels of Cd than could be detected. The fact that Cd is known to be the most potent inducer of phytochelatin production in higher plants (Steffens, 1990) lends support to this hypothesis. Alternatively, apical segments, depleted in Cd, could have received some biochemical message from subapical segments that suffered Cd stress, maintaining an inducible response to Cd in apical segments.

Shaw (1987) found that metal tolerance induced in metal-pretreated protonemal filaments of *Funaria hygrometrica* was carried over to new growth on media without high levels of toxic metals. However, Shaw (1987) did not analyse *F. hygrometrica* for metal content and it is uncertain whether a carry-over of toxic metals maintained the stimulus for tolerance. Whatever the biochemical basis for tolerance, it is clear that further research is required to elucidate mechanisms and determine the threshold levels of toxic metals required to induce or maintain metal tolerance in bryophytes.

The results of the current study confirm that valid inter-population comparisons of photosynthetic responses to metal stress may be made by relating CO₂ exchange to intracellular metal concentration, but that consideration should be given to morphological and physiological differences between populations. We have shown that the gametophyte of a metal-contaminated population of *R. squarrosus*, exposed to high levels of Cd, was photosynthetically tolerant of Cd but was not co-tolerant of Cu. The methods we have used are appropriate to relatively short-term, laboratory-based studies, but may be adapted, at least for screening purposes, for field populations. Thus, Brown and Sidhu (1992) have shown that for *R. squarrosus*, reductions in photosynthetic capacity in the field evaluated over a 4-week period show a linear relationship to intracellular accumulation of experimentally-supplied Zn similar to that reported here for short-term uptake of Cu and Cd, and reported previously for Zn (Brown and Wells, 1990b).

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