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REGULAR ARTICLE

# Variability of Mn hyperaccumulation in the Australian rainforest tree *Gossia bidwillii* (Myrtaceae)

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**Abstract** This study examines the heterogeneity of the Mn-hyperaccumulative trait in natural stands of the Australian rainforest tree species *Gossia bidwillii* (Myrtaceae). It is the only known Mn hyperaccumulator from Australia, and has an unusual spatial distribution of Mn in its leaves. *G. bidwillii* occurs naturally on a range of Mn-containing substrates including ultramafic soils. Leaf samples were collected from individual trees and four small stands, over a longitudinal range of ~600 km. While no variation in the spatial distribution of foliar Mn was detected, considerable variation in Mn concentration was found. *G. bidwillii* was shown to accumulate Mn when growing on a variety of substrates, and dry weight (DW) foliar Mn concentrations of all trees sampled ranged between 2,740 and 27,470 μg $g^{-1}$. The majority of samples exceeded 10,000 μg $g^{-1}$, the threshold value for Mn hyperaccumulation. The overall frequency distribution of foliar Mn concentration was found to be bimodal, with a small outlier of extreme hyperaccumulators. Highest values were obtained from trees growing on a basaltic krasnozem clay, not ultramafic soil. Soil Mn concentrations were measured, and no relationship was found between foliar Mn concentrations and extractable Mn concentrations in host substrates. Some of the variation in the Mn-hyperaccumulative trait in *G. bidwillii* throughout its large natural distribution may reflect the unresolved taxonomy of this most widespread species in the genus *Gossia*. Ability to hyperaccumulate Mn may serve as an additional diagnostic tool for resolving this taxonomy.

**Keywords** Foliar Mn · *Gossia bidwillii* · Mn hyperaccumulator · PIXE/EDAX · Woody hyperaccumulator

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## Introduction

Hyperaccumulators are a small group of higher plants known for their extreme ability to accumulate certain metals and metalloids in their shoots (Baker et al. 2000). Threshold dry weight (DW) foliar concentrations for hyperaccumulation have been defined as

0.01% for Cd, 0.1% for Ni, Cu, Co and Pb, and 1.0% for Zn and Mn (Baker and Brooks 1989). The phenomenon is relatively uncommon, and has drawn interest particularly for its potential exploitability for phyto-remediation and phyto-mining. Hyperaccumulators occur predominantly on ultramafic (serpentine) soils. A considerable proportion of hyperaccumulators are herbaceous species, and the majority of hyperaccumulators reported to date sequester nickel. Practical constraints as well as economical and/or environmental priorities have steered research in this field, hence Mn hyperaccumulation has been of little initial interest.

Nine species of Mn hyperaccumulators are currently known worldwide (Reeves and Baker 2000). They include seven from New Caledonia, and latterly one each from Australia and China (Bidwell et al. 2002; Xue et al. 2004). Six families are represented amongst these predominantly woody species. *Phytolacca acinosa* Roxb. (Phytolaccaceae), the most recently described Mn hyperaccumulator, is a herbaceous plant from China. In controlled-environment studies it has been found to have a constitutive hyperaccumulative trait (Xue et al. 2005). Thus far, populational studies designed to investigate variability of the hyperaccumulative trait, its heritability and the existence of distinct ecotypes, have centred around herbaceous hyperaccumulators (Baker et al. 1994; Macnair 2002; Macnair et al. 1999; Mengoni et al. 2003; Pollard et al. 2002; Reeves and Baker 1984; Xue et al. 2005). These plants are well suited to propagation and short-term experimentation under controlled environmental conditions. The variability of Ni and Zn hyperaccumulation in the genera *Thlaspi* and *Arabidopsis* (Brassicaceae) have been most researched to date (Baker et al. 1994; Macnair 2002; Macnair et al. 1999; Reeves and Baker 1984). In contrast, there has been very little research on hyperaccumulation by woody plants. This may be attributed to their slower growth rates and more restricted distributions. Pollard et al. (2002) drew attention to the need to extend current understanding of the variation of hyperaccumulation to previously uninvestigated taxa, as well as to the trait as it occurs in nature. They cautioned against over-generalisation based on research into one or two herbaceous genera.

In *Gossia bidwillii* (Benth.) N. Snow and Guymer, an Australian tree that hyperaccumulates Mn, primary sequestration of foliar Mn in naturally growing plants was found to occur in the photosynthetic tissues, a pattern previously undescribed in other hyperaccumulating plants (Fernando et al. 2006b). This has since been observed in naturally occurring *Virotia neurophylla* (Guillaumin) Virot (Proteaceae), the Mn-hyperaccumulating tree species endemic to New Caledonia (Fernando et al. 2006a). In both cases, the highest concentrations of foliar Mn were found in the multiple-layer palisade mesophyll. Manganese is an essential plant nutrient that plays a major role in photosynthesis, and palisade cells are important for light harvesting and photosynthesis. As has been previously suggested (Fernando et al. 2006a, b), these factors may have some bearing on the Mn sequestration pattern observed in the leaves of *G. bidwillii* and *V. neurophylla*.

*Gossia bidwillii* was recently placed in this new genus in current taxonomic treatment (Snow et al. 2003), and belonged to the genus *Austromyrtus* at the time its ability to hyperaccumulate Mn was first discovered (Bidwell et al. 2002). Snow et al. (2003) have narrowed down the former *Austromyrtus* genus by differentiating out two new genera, *Lenwebbia* and *Gossia*. All three occur in Australia and New Caledonia. *Gossia* is the most diverse, and at present comprises a total of 30 species, the majority of which were formerly placed in *Austromyrtus*. There are 24 *Gossia* species in Australia, including *G. bidwillii*, which is distributed along the eastern seaboard in sparse stands, on a variety of substrates including ultramafic soils. This species is the most widespread in the genus, exhibiting considerable morphological variation throughout its range (Snow et al. 2003). It has been suggested that additional diagnostic features may be useful in resolving *G. bidwillii* from regional variants warranting recognition as separate taxa, particularly in the North Queensland populations (G. Guymer personal communication).

The major aim here was to investigate the heterogeneity of the Mn-hyperaccumulative trait in *G. bidwillii*, both in terms of foliar Mn concentration and spatial distribution. Since this species is not ideally suited to controlled studies, an investigation of Mn hyperaccumulation in its native environment was undertaken. Host substrates were analysed in order to assess the relationship between foliar and soil Mn concentrations. In so doing, this study addresses the overall lack of knowledge on the heterogeneity of the hyperaccumulative trait in woody species, and is the

**Table 1** Summary of plant and soil samples collected, and site descriptions

| Site number | Site coordinates | Number of trees sampled | Number of soil samples from under trees | Number of soil samples from outside drip line of trees | Soil type |
|---|---|---|---|---|---|
| 1* | 27°18′ 20″S; 152°41′10″E | 11 | 3 | – | Basalt, deep lateritic red clay krasnozem |
| 2* | 27°24′39″S; 152°48′30″E | 9 | 3 | – | Basalt, deep lateritic red krasnozem clay |
| 3 | 27°31′10″S; 152°51′49″E | 14 | 14 | 14 | Stony, shallow brown clay loam |
| 4 | 22°52′30″S; 149°50′30″E | 6 | 12 | – | Serpentine, lateritic red-brown loam |
| 5 | 23°43′49″S; 151°02′11″E | 1 | 2 | – | Stony brown silty loam |
| 6 | 19°45′50″S; 147°29′50″E | 1 | 2 | – | Acid volcanic, clay loam |
| 7 | 19°50′33″S; 147°42′20″E | 2 | 2 | – | Littoral margins, alluvial contains calcareous sand to sandy loam |
| 8 | 22°45′30″S; 149°50′30″E | 3 | 4 | – | Volcanic, brown clay loam |

Leaves from three trees per population marked * were sampled and processed so as to enable PIXE/EDAX localisation studies

first such evaluation of a Mn hyperaccumulator. The natural distribution of *G. bidwillii*, combined with its ability to accumulate foliar Mn, favour its potential usefulness in the long-term management of high Mn soils. An understanding of variations in the Mn-hyperaccumulative trait could prove useful for sourcing plants and selectively breeding them for such a purpose. Data obtained here could also provide a basis on which further studies can be carried out to determine if the Mn-hyperaccumulative trait in *G. bidwillii* may be used as a taxonomic tool, as suggested by Bidwell et al. (2002).

## Materials and methods

### Plant and soil samples

Eight geographic areas defined as sites 1–8 were sampled. Their coordinates, the numbers of plant and soil samples collected from each site, and individual soil types on each site are summarised in Table 1. Leaves were harvested from branchlets, and soils were sampled to a depth of 10 cm. Each individual soil sample comprised ten sub-samples. Since *G. bidwillii* is sparsely distributed, discrete populations are not clearly defined, and the term 'stand' is used to describe groups of trees. Four stands of trees (stands 1–4 on sites 1–4, respectively) were sampled. One to three trees and their soils were sampled from each of four other locations (sites 5–8).–

### ICP analyses and soil pH measurements

Leaf samples were rinsed in distilled water and oven-dried at 70°C for inductively coupled plasma optical emission spectrometry (ICP-OES) analyses. They were ground and weighed to ~0.3 g, and digested in 5 ml 70% nitric acid at 125°C for 2 h. Digests were treated with 30% hydrogen peroxide, diluted to 75 ml with distilled water, analysed and quantified against a series of aqueous standards acidified with nitric acid. Soil samples were oven dried at 70°C for ICP analyses (total and extractable Mn concentrations). For total concentrations, ~0.2 g soil was digested in 5 ml of *aqua regia* (a 1:3 mixture of 70% $HNO_3$ and 42% HCl) at 130°C for 3 h and treated as the leaf samples, using standards acidified with *aqua regia*. Using this methodology, concentrations of Mn, Ca, Fe and Mg in leaf dry matter samples, and total Mn, Al, Ca, Co, Cr, Fe, Mg and Ni in soil samples were obtained. To estimate extractable soil Mn, 5 g dry soil and 25 ml 0.05 M EDTA (pH 6.0) were shaken for 4 h, and the filtered supernatant analysed by ICP-OES. Soil pH was measured after 5 g of dried soil was shaken with 25 ml of distilled water for 2 h. Sample standard errors were calculated where replication exceeded three, and statistical treatment of the data was performed using SPSS 12.0.1 for Windows (SPSS Inc., Chicago, IL, USA). One-way analyses of variance (ANOVA) were carried out on the leaf data obtained from stands 1 to 4 and a *t*-test was performed on the

parallel sets of soil Mn data obtained from within and outside the drip lines of each tree on site 3.

PIXE/EDAX

Plants from sites 1 and 2 were sampled, prepared and analysed as described in Fernando et al. (2006a). Three trees from each site were examined.

## Results

ICP analyses and soil pH measurements

Leaf Mn concentrations for all *G. bidwillii* trees investigated in this study are summarised in Fig. 1, which has an overall bimodal shape. A few trees had extremely high-foliar Mn concentrations above 22,000 μg $g^{-1}$ (DW), while most were in the range 4,000–20,000 μg $g^{-1}$ DW foliar Mn. Almost 90% of plants sampled exceeded 10,000 μg $g^{-1}$, which defines Mn-hyperaccumulation.

Figure 2 shows the frequency distributions of foliar Mn concentrations in four small stands of trees (stands 1–4) on sites 1–4, and Table 2 shows mean foliar concentrations for all sites, which were 14,450, 17,990, 12,800 and 13,660 μg $g^{-1}$ (DW), respectively. No significant differences between the means were detected ($p = 0.41$). Foliar Mn concentrations varied considerably within each stand, with at least one individual tree exceeding 20,000 μg $g^{-1}$, and one around the notional threshold of 10,000 μg $g^{-1}$ DW. In contrast to Mn, significant differences between mean foliar concentrations for Fe ($p = 0.001$), Ca ($p = 0.01$) and Mg ($p < 0.001$) amongst stands 1–4 were detected.

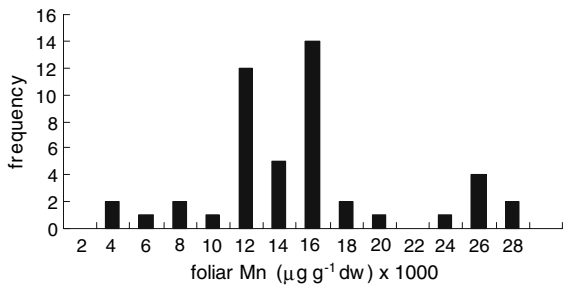


**Fig. 1** Frequency distribution of foliar Mn concentrations of all *G. bidwillii* trees studied

Table 3 summarises soil data for sites 1–8, and includes two sets of samples from site 3, which were from inside (3A) and outside (3B) the drip line of each tree, i.e. directly under each tree canopy and away from it. The mean total and extractable soil Mn concentrations were found to be higher directly under the trees, than away from them. However, the *t*-test showed no significant difference between mean total soil Mn concentrations ($p = 0.12$), while the corre-

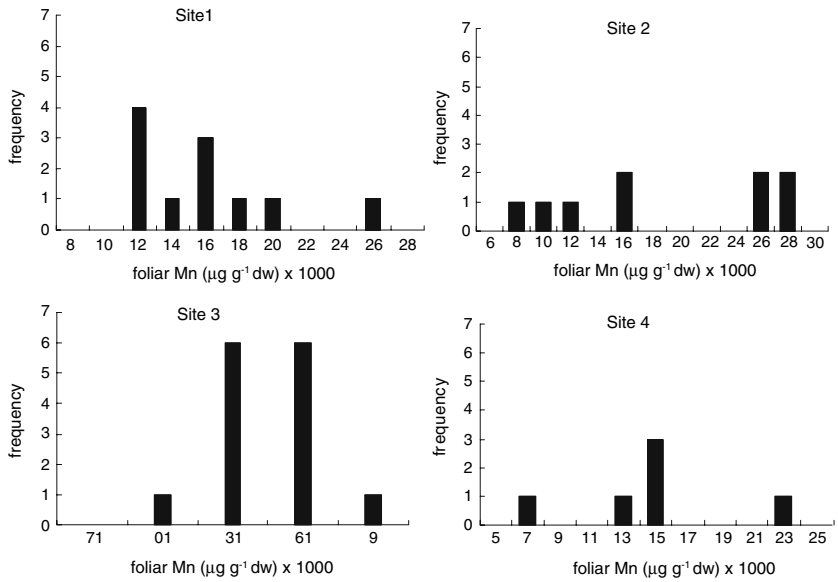


**Fig. 2** Frequency distributions of foliar Mn concentrations in four individual stands of *G. bidwillii*

**Table 2** Mean concentrations of Ca, Fe, Mg and Mn in *G. bidwillii* leaves, collected from sites 1 to 8

| Sites | Mean foliar concentrations (μg $g^{-1}$ dw) | | | |
|---|---|---|---|---|
| | Ca | Fe | Mg | Mn |
| | Se | Se | Se | Se |
| 1 | 12,000 | 93 | 7,430 | 14,540 |
| | 798 | 4.9 | 341 | 13,90 |
| 2 | 10,570 | 106 | 2,800 | 17,990 |
| | 451 | 10.4 | 145 | 24,90 |
| 3 | 8,870 | 60 | 2,630 | 12,800 |
| | 393 | 7.8 | 183 | 584 |
| 4 | 9,450 | 85 | 3,240 | 13,660 |
| | 1,350 | 8.4 | 812 | 2,240 |
| 5 | 14,670 | 104 | 4,280 | 12,310 |
| 6 | 11,680 | 74 | 3,730 | 14,150 |
| 7 | 8,790 | 73 | 6,100 | 3,130 |
| 8 | 12,920 | 61 | 5,260 | 16,720 |

sponding mean extractable Mn concentrations were significantly different ($p = 0.02$). This table also shows mean total concentrations (μg $g^{-1}$) of Al, Ca, Co, Cr, Fe, Mg, Mn and Ni, as well as mean extractable Mn, mean total Mg : Ca concentration ratios, and pH for sites 1–8. Soil from the serpentine site (no. 4) typically contained very high total Cr, Ni and Mg concentrations, the highest total Co concentration, and was the only site where the mean soil Mg : Ca concentration ratio exceeds 1. The soil pH of all sites varied between 5.7 and 6.6.

The relationship between foliar and substrate extractable Mn is shown in Fig. 3. Although the lowest foliar and soil Mn concentrations correlated, no overall significant correlation between these two variables was detected.

## PIXE/EDAX

In order to test for heterogeneity in the spatial pattern of foliar Mn distribution, leaves from three randomly chosen trees from sites 1 and 2 were analysed using PIXE/EDAX. Consistent with previous findings for this species (Fernando et al. 2006a, b), the highest localised concentrations of foliar Mn occured in the photosynthetic tissues, most often the palisade mesophyll cells. Manganese was also found in the spongy

**Table 3** Mean total soil concentrations; extractable Mn; Mg/Ca ratios of total mean concentrations, and pH for all sites 1–8

| Sites | Mean total soil concentrations (μg $g^{-1}$ dw) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Al | Ca | Co | Cr | Fe | Mg | Mn | Ni | Mean soil extractable Mn (μg $g^{-1}$ dw) | Ratio of mean total concentrations Mg/Ca | Mean pH |
| | Se | Se | Se | Se | Se | Se | Se | Se | Se | Se | Se |
| 1 | 114,990 | 10,610 | 29 | 49 | 149,230 | 6,890 | 4,880 | 50 | 4,220 | 0.77 | 6.18 |
| 2 | 98,880 | 6,850 | 4.0 | 22 | 58,620 | 3,390 | 2,590 | 17 | 1,730 | 0.50 | 6.00 |
| 3A | 30,610 | 8,260 | ND | ND | 52,470 | 2,960 | 3,850 | ND | 2,420 | 0.59 | 5.97 |
| | 1,960 | 2,050 | | | 4,450 | 267 | 792 | | 432 | 0.16 | 0.10 |
| 3B | 38,530 | 4,630 | ND | ND | 64,760 | 3,060 | 2,410 | ND | 1,210 | 0.81 | 5.74 |
| | 1,380 | 716 | | | 5,250 | 310 | 394 | | 138 | 0.09 | 0.13 |
| 4 | 43,640 | 14,120 | 79 | 1,890 | 201,830 | 35,630 | 4,240 | 1,850 | 3,510 | 2.41 | 6.76 |
| | 2,260 | 849 | 13 | 171 | 11,070 | 11,280 | 786 | 209 | 405 | 0.80 | 0.08 |
| 5 | 59,050 | 8,350 | 7.3 | 14 | 147,630 | 7,170 | 2,000 | 8.1 | 574 | 0.91 | 6.27 |
| 6 | 25,730 | 3,350 | 9.8 | 12 | 21,660 | 1,370 | 1,510 | 7.8 | 1,150 | 0.41 | 6.35 |
| 7 | 8,170 | 6,510 | 7.0 | 3.2 | 13,160 | 1,040 | 764 | 1.8 | 554 | 0.16 | 6.37 |
| | 344 | 1,070 | 0.54 | 0.63 | 1,440 | 167 | 160 | 0.11 | 154 | 0.01 | 0.12 |
| 8 | 89,890 | 27,120 | 16 | 163 | 79,920 | 12,770 | 2,860 | 49 | 2,360 | 0.48 | 6.61 |
| | 5,160 | 1,110 | 3.3 | 9.0 | 3,880 | 1,830 | 450 | 1.5 | 373 | 0.08 | 0.10 |

There are two collections for site 3, labelled 3A under each tree sampled, and 3B away from each tree

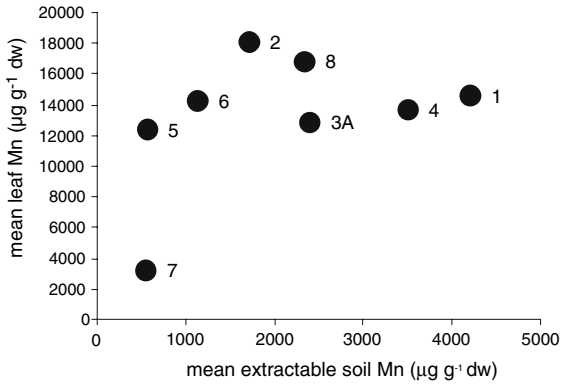


**Fig. 3** Mean foliar Mn concentrations versus soil extractable Mn concentrations (µg $g^{-1}$ dw) for *G. bidwillii* from sites 1 to 8

mesophyll of all samples, while relatively low levels of sequestration in the dermal tissues were detected in some samples. Maps of K distribution showed the presence of K in all samples, indicating that the sample preparation methodology used was effective in preserving cell solutes.

## Discussion

This study examined the variability of the Mn-hyperaccumulative trait in naturally growing *G. bidwillii* trees. Since the stands were so sparse, it was possible to sample all trees for foliar Mn concentration. It is interesting to note that highest values were observed in individual trees growing on basaltic substrates (sites 1 and 2), and not on serpentine (site 4). As can be seen in Fig. 1, the overall shape of the frequency distribution is disjunct, with a small outlying cluster of extreme hyperaccumulators, which largely came from site 2. Approximately 90% of all plants tested exceeded the threshold for Mn hyperaccumulation. This finding is consistent with other work on herbaceous hyperaccumulators, which have found hyperaccumulation to be a species-wide phenomenon (Macnair 2002; Pollard et al. 2002). The spread of all values across a tenfold range indicates that in the field, the hyperaccumulative trait in *G. bidwillii* is highly polymorphic. This is in common with naturally occurring hyperaccumulators in the herbaceous genera *Thlaspi*, *Alyssum* and *Arabidopsis* (Baker et al. 1994; Macnair 2002; Mengoni et al. 2003).

A frequency distribution of foliar Ni concentrations in 170 *Alyssum* taxa growing in their natural habitats was found to be bimodal (Pollard et al. 2002), and demonstrated that in that genus, a small number of species are Ni-hyperaccumulating. Although this is not directly comparable to Fig. 1, which demonstrates the existence of a sub-group of very highly Mn-hyperaccumulating *G. bidwillii* trees, these data show that outliers of extreme behaviour exist at the genus level in *Alyssum*, and at a species level in *G. bidwillii*. Thus far it is well documented that a distinct feature of the phenomenon of hyperaccumulation, which perhaps sets it apart from hypertolerance, is the absence of a relationship between shoot concentrations and bioavailable host-substrate concentrations of the hyperaccumulated element (Macnair 2002; Pollard et al. 2002). These authors suggest that species-wide variation of hyperaccumulation is more likely due to genotypic differences than to host substrate concentrations. In this study, no relationship was found between foliar Mn and soil-extractable Mn concentrations, highlighting the plants' ability to hyperaccumulate Mn over a range of bioavalable concentrations. If *G. bidwillii* were to be used to remediate Mn-contaminated soils, the major disadvantages are likely to be its slow growth rate and consequent slow rate of Mn accumulation in the shoot tissues. This reasoning is based on the authors' experience (unpublished data) with the Mn-accumulating tree, *Macadamia integrifolia* Maiden and Betche (Proteaceae). However, in the long-term, these factors would be offset by large shoot biomass of tree species such as *G. bidwillii*.

Site 7 contained the lowest soil Mn concentration, and trees sampled here had a mean DW foliar Mn concentration of 3,127 µg $g^{-1}$, which is similar to that reported by Bidwell et al. (2002) for the non-Mn-hyperaccumulator *Gossia acmenoides*. It is possible that in the present investigation *G. bidwillii* on site 7 and other plants below the Mn-hyperaccumulation threshold may have been of a different taxon, not recognised under the current Snow et al. (2003) treatment. The usefulness of the Mn-hyperaccumulative trait as a taxonomic tool could be initially investigated by combining plant and soil data gathered here, with similar information on other closely related species in these habitats, and possibly followed up with molecular systematics studies. This course of investigation may be useful in gaining more detailed resolution of the genus *Gossia* from others similar to it,

particularly in the northern habitats where there is a greater variation of the taxon.

Three of the four stands of *G. bidwillii* examined showed a high degree of polymorphism of the Mn-hyperaccumulative trait within each group. The exception was the densest stand on site 3 (Fig. 2). Although no significant difference was detected between their mean foliar Mn concentrations, variation amongst trees within each stand ranged from the threshold of Mn hyperaccumulation, to over double that value. It is notable that soils on site 3 were found to contain a significantly higher mean extractable Mn concentration directly under the trees sampled, in comparison to the corresponding mean of samples collected away from each tree. This result may be indicative of allelopathy (Boyd and Martens 1992). Although within-species variation of hyperaccumulation has been studied, little is known about inter-populational variation of hyperaccumulation by the herbaceous species investigated to date. Macnair (2002) found Zn hyperaccumulation by *Arabidopsis halleri* to be highly polymorphic, both between and within populations.

In contrast to foliar Mn concentrations, we found no evidence of qualitative variation in the spatial pattern of Mn sequestration. Qualitative PIXE/EDAX mapping of Mn in *G. bidwillii* leaves showed that in six trees from two sites, the majority of foliar Mn occurs in their photosynthetic layers. It may therefore be reasonable to hypothesise that this is a species-wide occurrence in *G. bidwillii*. These findings are consistent with previous studies of *G. bidwillii* using quantitative cryo-scanning electron microscopy (SEM)/EDAX and qualitative PIXE/EDAX mapping (Fernando et al. 2006a, b). Both showed primary sequestration of foliar Mn to occur in the photosynthetic tissues, particularly the palisade mesophyll cell layers. The quantitative study found highest mean vacuolar concentrations of around 500 mM Mn in the first-layer palisade cells. The PIXE/EDAX study also showed that in leaf cross-sections of the Mn hyperaccumulator *V. neurophylla* and the Mn-accumulating species, *M. integrifolia* Maiden and Betche (Proteaceae) and *neurophylla* L. A. S. Johnson (Proteaceae), the highest localised Mn concentrations occurred in their photosynthetic tissues.

The major inference drawn from this study is that like Ni and Zn hyperaccumulators in the Brassicaceae, the Mn-hyperaccumulative trait in naturally occurring *G. bidwillii* trees is highly heterogeneous, at both the species and populational levels. This study of hyperaccumulation by a woody species in its native habitat opens up previously unexplored aspects of the phenomenon of hyperaccumulation, and in doing so, increases the framework around which practical applications and theoretical research may be further formulated.

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