

1 **Adaptations to winter-wet ironstone soils: a comparison**
2 **between rare ironstone *Hakea* (Proteaceae) species and their**
3 **common congeners**

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1 **Abstract.** In SW Australia a rare plant community is found on shallow, winter-wet
2 ironstone soils, which occur on coastal plains as isolated islands in a matrix of
3 surrounding deeper sandy soils. To test for local adaptation of species endemic to these
4 communities and potential inhibitory effects of ironstone soils on other species, we
5 compared two rare ironstone *Hakea* species with four of their common congeners. The
6 common congeners were chosen from nearby winter-wet habitats on deeper sandy soils
7 and from non-wetland woodland habitats (i.e. 2 species in each habitat group). Seedlings
8 of all species were grown on ironstone soil and subjected to waterlogging in a glasshouse
9 experiment. Significant habitat related differences only emerged when seedlings were
10 waterlogged. When compared with their controls, shoot and root growth rates of
11 ironstone endemics were less affected by waterlogging than those of their common
12 congeners. This was partly associated with their large accumulation of leaf starch, and
13 their substantial adventitious-root formation. Leaves of ironstone endemics also exhibited
14 consistently higher concentrations of Cu and Zn. In contrast to the effect of waterlogging
15 in the glasshouse experiment, natural waterlogging of seedlings transplanted into
16 ironstone communities led to high mortality, but only in the non-wetland *Hakea* species.
17 Mortality was strongly associated with the intensity of flooding events, with very small
18 differences in inundation level (10-15 mm) strongly influencing seedling survival. Our
19 results suggest that the chemistry of the waterlogged ironstone soil and species
20 adaptations to it, are important to understand these *Hakea* species' distribution patterns.

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23

1 **Introduction**

2 Many of the world's rare plant communities are associated with environments that have a
3 distinctive soil chemistry; local endemics that are associated with unusual substrates like
4 gypsum, serpentine, limestone and soils enriched in heavy metals are well known to field
5 botanists all over the world (e.g. Kruckeberg and Rabinowitz 1985 and references
6 therein). There is little evidence to support the view that these edaphic endemics 'need'
7 the specific chemical features of their native soils. Rather, they can resist their local,
8 sometimes toxic, conditions better than more widespread species. For example, in
9 serpentine soils the unusually high Mg to Ca ratio, the high Ni concentrations and the low
10 availability of many essential nutrients (e.g. N, P, K) are considered to be the most
11 important factors affecting the survival and growth of many non-serpentine species (e.g.
12 Turitzin 1981; Kruckeberg 1984; Huenneke *et al.* 1990). On limestone soils, the low
13 availability of Fe and P, and the inability of many plants to mobilise these nutrients, have
14 been implicated as major factors limiting growth and survival of non-limestone, calcifuge
15 species (Tyler 1992, 1994).

16 In the coastal plains of Mediterranean south-western Australia, a few rare plant
17 communities occur on shallow winter-wet soils, overlaying massive ironstone rock. Many
18 of the perennial woody taxa occurring in these 'ironstone communities' are either
19 restricted to them or have their distribution centred on them (Gibson *et al.* 2000). Since
20 these communities occur as isolated islands in a matrix of deeper, sandy soils, we
21 hypothesised that their high level of endemism is related to the specific chemistry of their
22 soils. This may be especially relevant during winter when ironstone communities
23 experience numerous waterlogging and drainage events. Reduced waterlogged ironstone

1 soils are likely to have high concentrations of reduced Fe and possibly Mn, which are
2 toxic to many plant species (e.g. Marschner 1995; Snowden and Wheeler 1993).

3 To test the ‘home advantage’ of ironstone endemics and the possibly inhibitory effect
4 of these soils on other species, we compared growth, biomass allocation, and leaf nutrient
5 concentrations of two rare ironstone *Hakea* species with those of four of their common
6 congeners. Seedlings of all species were grown on field-collected ironstone soil in a
7 glasshouse and subjected to well watered and waterlogged conditions. To be able to
8 distinguish between characteristics specific for the ironstone endemics and general
9 characteristics of wetland plants, two of the chosen species were from common winter-
10 wetlands on deeper sandy soils, whereas the other two originated from non-wetland
11 habitats on the nearby scarp and plateau. In an earlier experiment with the same set of
12 species (Poot and Lambers 2003b), we showed that the ironstone endemics did not differ
13 from their common ‘winter-wet congeners’ in traits well-known to be adaptive in
14 waterlogged conditions (e.g. adventitious roots, relatively high root porosity). However,
15 these results can only be interpreted in terms of general adaptations to waterlogging,
16 since plants were grown on ‘neutral’ soil (washed river sand). In this paper, we
17 hypothesise that the non-ironstone plants will suffer toxicities or growth reductions when
18 grown on ironstone soil, and that these may be exacerbated by waterlogging.

19

20

21 **Materials and methods**

22 *Ironstone communities*

1 Ironstone communities are winter-wet shrublands that occur on very shallow, reddish,
2 loamy sands (0-20 cm deep) over massive ironstone rock. Only scattered remnants of
3 these unique communities are left on the southern Swan- and Scott River Coastal Plains.
4 They occur as fragmented islands, either on these coastal plains or on the foot slopes of
5 the nearby and geologically much older lateritic scarps and plateaus (Gibson *et al.* 2000).
6 They are believed to have been formed by the precipitation of ferric iron from the
7 groundwater in the zone of water-table fluctuation, and are thought to be still forming
8 today ('bog iron' formation; Johnstone *et al.* 1973; Gibson *et al.* 2000). Although iron-
9 rich impeding layers are common on these coastal plains, they generally occur at much
10 greater depth, and most soils, therefore, are composed of deep layers of Quaternary sand
11 deposits. In contrast, the shallow, sandy, loamy soils of the ironstone communities (0-20
12 cm deep), are mainly derived from the weathering of the underlying rock, and have a
13 distinct red-brown coloration.

14

15 *Experimental species*

16 We studied six south-western Australian *Hakea* species (Proteaceae) that differed in
17 abundance and originated from three contrasting habitats: endangered ironstone winter-
18 wetlands, "other" winter-wetlands, and non-wetland *Eucalyptus* woodlands. *H. oldfieldii*
19 Benth and *H. tuberculata* R. Br. are both locally common in separate communities on the
20 Swan ('northern ironstone') and Scott ('southern ironstone') Coastal Plain, respectively.
21 Outside these communities they are absent or rare. Due to their restricted distributions,
22 both ironstone species have priority status. *H. ceratophylla* Smith R. Br. and *H. varia* R.
23 Br. are both common species that typically occur in winter-wetlands in south-western

1 Australia. They also occur on very few of the remnant ironstone communities, but are
2 rare in this habitat. *H. lissocarpha* R. Br. and *H. cyclocarpa* Lindl. are both common
3 species that occur on lateritic soils in the undergrowth of eucalypt woodlands on the
4 Darling and Blackwood Plateau. Neither species occurs in winter-wet situations. The
5 phylogenetic status of the different *Hakea* species is still under consideration. However,
6 under the current classification system (Barker *et al.* 1999), the comparison presented in
7 this study is unbiased, since none of the species within a specific habitat are classified in
8 the same taxonomic subgroup.

9

10 *Glasshouse experiment*

11 For each of the *Hakea* species, woody follicles containing the seeds were sampled from a
12 single population in the field during March and April 2000. Follicles of at least 5 plants
13 per species were collected. Within two weeks at room temperature, all woody follicles
14 had dehisced. Forty seeds per population were placed just under the surface of low-
15 nutrient potting mix (Kings Park and Botanic Garden) in germination trays. The potting
16 mix had initially been watered with a diluted fungicide solution (Previcur, group Y
17 fungicide, Bayer-Cropscience Australia) to prevent damping off. All trays were placed in
18 a phytotron during mid-January (20°C/15°C, day/night, light intensities 65% of incident).
19 Germination percentages were high (75-100%), and did not differ between species from
20 the different habitat groups. After germination, the approximately 15-days old seedlings
21 were transferred to individual pots (depth 120 mm, diameter 135 mm) with field-
22 collected ironstone soil. The soil was a dark, reddish brown loam (ferric podzol), which
23 was collected from the ironstone community from which one of the ironstone species, *H.*
24 *oldfieldii*, originated (also see Appendix). It was sampled from the surface (top 10 mm)

1 of a recently (<1 year) made firebreak. None of the other experimental species occurred
2 on this site. Because dieback (*Phytophthora cinnamomi*) is prevalent in this area, we
3 steam-sterilised the soil for 2 hours. Pots were watered regularly with deionised water to
4 keep soil water content above 70% of field capacity.

5 Plants were grown in the glasshouse for 4 months during which a growth analysis was
6 carried out. The first harvest (H1) was conducted the day before the transfer of the
7 approximately 15-days old seedlings to the ironstone soil. The second harvest (H2) was
8 conducted after 55 days of growth in ironstone soil. Thereafter, half of the remaining pots
9 were waterlogged with deionised water, which was maintained at a level approximately 1
10 cm above the soil surface. After 43 days of treatment, both the waterlogged and the well
11 watered control plants were harvested (H3). At each harvest, 6 plants per species were
12 sampled. Roots were rinsed with water to remove all soil. Fresh and oven-dried masses
13 (48 hours at 70°C) of roots (adventitious and main root systems separately), stems and
14 leaves were determined. At the final harvest the fully grown leaves of the six individuals
15 of each species were bulked in two groups for further chemical analyses.

16

17 *Leaf chemical analyses*

18 Waterlogging can cause substantial carbohydrate accumulation in leaves (e.g. starch,
19 soluble sugars; Gravatt and Kirby 1998) which can greatly distort leaf nutrient
20 concentrations. To be able to ‘correct’ for this, we measured leaf soluble sugar and starch
21 concentrations. Fresh leaves from the bulked samples were snap-frozen in liquid N₂ and
22 freeze-dried for 72 hours. Samples were ground to a fine powder in a stainless steel ball
23 mill. Soluble sugars were extracted after boiling the samples (100 mg) for an hour in 10

1 mL of 80% (v/v) ethanol. All other solutes that were extracted by this method, as
2 calculated from weight loss, are referred to as 'rest solutes'. Starch in the residue was
3 hydrolysed after boiling for an additional 3 hours in 10 mL of 3% (v/v) HCl. Both the
4 soluble sugar and starch extracts were measured as glucose equivalents with the anthrone
5 method (Fales 1951). Hydrolysis of standard potato starch was 85%, and glucose spikes
6 gave recovery values above 85%.

7 For the determination of a range of macronutrients (N, P, Mg, Ca) and micronutrients
8 (Fe, Zn, Cu, Mn) as well as Al, Cr and Ni, subsamples (0.5 g) of the bulked leaves were
9 oven-dried for 48 hours. Total N content in dried leaf material was determined by thermal
10 conductivity after combustion using a CHN-1000 elemental analyser (LECO®
11 Corporation, St. Joseph, MI, USA). The remainder of the dried samples was digested in
12 hot concentrated nitric acid. The digests were used for analysing Mn, Mg, Ca and Fe by
13 AAS (PE AAnalyst 300, Perkin Elmer, Norwalk, CT, USA), and the other elements by
14 ICP-MS (PE Elan 6000, Norwalk, CT, USA). P content was determined colourimetrically
15 on the same digests with the malachite green method (Motomizu *et al.* 1983).

16 Potassium content was determined in subsamples of the ethanol extracts obtained for
17 the soluble sugar analysis. After evaporating off the ethanol, potassium was solubilised
18 from the residue by adding 10 mL of deionised water, after which it was measured by
19 flame photometry (Model 410, Sherwood Scientific Ltd, Cambridge, UK).

20

21 *Field transplant experiment*

22 In May 2002, 320 seeds per species were placed on trays with moistened paper in a
23 temperature-controlled room (15 °C) for germination. After germination, seedlings were

1 planted into trays filled with washed river sand. Approximately 4 to 5 weeks after
2 germination (first and second week of June 2002, i.e. winter) the young seedlings were
3 transplanted into six field sites. Field sites coincided with the 6 locations where seeds of
4 the 6 species had been collected. At each site, four plots were chosen in areas where the
5 ‘home-playing’ species was most numerous. In each plot, 10 seedlings per species were
6 planted randomly in a rectangular area (0.9 by 0.5 m) situated in the middle of a chicken
7 wire enclosure (1.30 by 0.9 m), with seedlings being placed at 100 mm distance from
8 each other. The enclosure was deemed necessary to prevent any interference by wildlife
9 (e.g. kangaroos, rabbits). Before the seedlings were transplanted, the area inside the
10 enclosure and a border area of 0.3 m outside the enclosure were cleared of vegetation by
11 pulling out plants. This was done to create a situation closest to a ‘natural’ recruitment
12 (usually after fire) with enhanced light and nutrient levels. Four weeks after transplanting
13 the seedlings, we removed the larger weeds to facilitate further seedling establishment.
14 Thereafter, no further interference with naturally emerging plants inside the plots took
15 place. To be able to associate seedling death with waterlogging events, one piezometer
16 was installed at each winter-wet site, automatically recording water levels every hour
17 (capacitive water level probe, Dataflow Systems, Christchurch, New Zealand). In this
18 paper we only report seedling survival during the first winter on both ironstone sites (for
19 other results of this work, see Poot and Lambers 2008).

20

21 *Statistics*

22 Data were analysed with the general analysis of variance procedure of the statistical
23 package Genstat (Genstat 4.2 Committee 2000). We used a model with habitat (3),

1 species nested within habitat (2), and treatment (2) as main fixed factors. Possible
2 differences in relative growth rate between the species from the different habitat groups
3 were tested by analysing the interaction between their ln transformed dry weights and
4 harvest time. Other, mainly one-way ANOVAs or t-tests were used in specific cases as
5 indicated in the text. All mass-based data were log-transformed before testing, to ensure
6 homogeneity of variances and normality. For the same reason, all percentage-based data
7 that were either in the lower or upper end of the scale (i.e. 0-20 or 80-100%) were arcsin
8 transformed before testing.

9

10 **Results**

11 *Glasshouse experiment: growth and biomass allocation*

12 At the initial harvest, before transfer to the ironstone soil, seedlings of the ironstone
13 endemics tended to be smaller than most of those from the other habitats (Fig. 1;
14 significant differences with *H. varia* and *H. cyclocarpa*; $P < 0.05$, Tukey after one-way
15 ANOVA). This was partly related to their 35% (compared with *H. varia*) to 87%
16 (compared with *H. cyclocarpa*) smaller seed mass ($r^2 = 0.64$, for relationship between seed
17 mass and plant mass at first harvest). The young seedlings of the ironstone species had
18 also invested considerably more of their biomass into roots (7-10% point higher root
19 mass ratio, $P_{\text{habitat}} < 0.001$, one-way ANOVA). This habitat-related difference in biomass
20 allocation pattern remained throughout the experiment (results not shown).

21 When grown on well drained ironstone soil, the common species did not show any
22 growth suppression (harvests 2 and 3C, Fig. 1): plants of all six species attained similar
23 shoot and root relative growth rates ($P_{\text{habitat} \times \text{time}}$ was 0.425 and 0.524 for ln transformed

1 shoot and root dry masses, respectively). However, once waterlogged, growth of the
2 common species was affected more, when compared with their controls, than that of the
3 ironstone endemics. Waterlogging reduced root growth of the common species to a
4 greater extent ($P_{\text{habitat} \times \text{treatment}} = 0.028$), and ‘stimulated’ shoot growth to a lesser extent
5 ($P_{\text{habitat} \times \text{treatment}} = 0.004$), when compared with the ironstone species (Fig. 1, harvests 3C
6 and 3WL).

7 The apparent increase in shoot mass in the wetland species (Fig. 1a, compare harvest
8 3C with 3WL) was not due to additional leaf or stem growth: leaf number and stem
9 length were similar for control and waterlogged treatments (results not shown). Rather, it
10 was due to a substantial increase in leaf as well as stem dry mass percentage (DM%,
11 $P_{\text{treatment}} < 0.001$). Compared with control plants, waterlogged ironstone endemics tended
12 to have a larger increase in shoot DM% (from 28.2 to 40.8%), than species from the other
13 habitat groups (from 31% to 39%, and from 34.2 to 41.4%, for the winter-wet and non-
14 wetland species, respectively; $P_{\text{habitat} \times \text{treat}} = 0.058$). The increase in leaf DM% was strongly
15 related to the accumulation of starch ($r^2 = 0.82$; $P < 0.001$). In the common non-wetland and
16 to a lesser extent the common wetland species, this accumulation almost completely
17 accounted for the increases in leaf biomass of the waterlogged plants (Fig. 2). However,
18 in the rare ironstone species their relatively large increase in starch content was not
19 enough to explain the difference between the treatments, and changes in structural
20 biomass and solutes must have occurred also.

21 All species responded to waterlogging by producing adventitious, superficial roots,
22 whereas a large part of the main root system turned blackish and seemed dead and
23 decaying. Although there were clear differences amongst the six species, the statistical

1 analyses did not show any habitat-related differences in adventitious root formation and
2 characteristics ($P>0.05$). This was mainly due to large differences between the two
3 common winter-wet species. The ironstone species and one of the common winter-
4 wetland species (*H. ceratophylla*) formed 8 to 50 times more adventitious root mass than
5 the other species, and generally produced more and longer adventitious roots (Table 1).

6

7 *Glasshouse experiment: leaf nutrient concentrations*

8 Leaf nutrient concentrations were expressed on the basis of dry mass without starch, to
9 ‘correct’ for the large increase in starch concentrations upon waterlogging. On average,
10 waterlogging reduced ‘corrected’ leaf nutrient concentrations by 25 to 60%, except for
11 Fe, which tended to increase in some species, but overall did not differ significantly
12 between the treatments (Table 2). The reduction in leaf P concentrations upon
13 waterlogging was strongly correlated with the increase in leaf mass upon waterlogging
14 ($r^2=0.93$), with the ironstone species showing a stronger decrease than species from the
15 other habitat groups ($P_{\text{habitat} \times \text{treat}} < 0.001$). The ironstone endemics also had higher overall
16 leaf Zn and Cu concentrations (Table 2, $P_{\text{habitat}} = 0.005$ and 0.004 , for Zn and Cu,
17 respectively). No habitat-related differences in Al, Cr and Ni concentrations were
18 observed. A comparison of the chemistry of ironstone soils, including the one we used,
19 with soils from the other habitats our species originated from revealed that, despite
20 significant variation amongst soils, the ironstone soils were only distinguishable from the
21 other soil types by their intermediate gravel content and their substantially higher iron
22 concentrations (see Appendix).

23

1 *Field transplant experiment*

2 Seedlings were transplanted in a relatively dry year with annual rainfall in large parts of
3 SW Australia some 20 to 50% below average (Bureau of Meteorology). Piesometer data
4 indicated that the colder and wetter southern site experienced waterlogging for two
5 months longer than the northern site, and waterlogging levels were generally higher (Fig.
6 3). Note that the exact level of waterlogging as indicated in Fig. 3 is dependent on the
7 location of the piesometer, as micro-topographic differences of 200-300 mm were
8 common at both sites.

9 Waterlogging only caused mortality in the species from the non-wetland communities
10 and differed greatly between the two sites. On the drier northern ironstone site only *H.*
11 *cyclocarpa* experienced some mortality (33%), and this was mainly associated with the
12 wettest plot at this site. In contrast, on the wetter, southern site both non-wetland species
13 suffered high mortality (90 and 95% for *H. lissocarpha* and *H. cyclocarpa* respectively).
14 Seedling health and likelihood of mortality at this site were strongly related to micro-
15 topography within the plots: individuals that died or were visibly unhealthy were
16 positioned significantly lower in the landscape (4 to 15 mm) than still healthy ones
17 ($P < 0.05$ for both species, Fig. 4)). However, there was no association between health
18 category and seedling height. Throughout the winter, seedlings from the species
19 originating from the common winter-wet habitats (i.e. *H. ceratophylla* and *H. varia*) were
20 significantly taller than those from the ironstone and non-wetland habitats ($P < 0.05$,
21 results not shown).

22

23 **Discussion**

1 The common *Hakea* species did not show any growth suppression when grown on well
2 drained ironstone soil. This suggests that the chemistry of the ironstone soils would not
3 preclude the establishment of the common *Hakea* species. However, when the ironstone
4 soil was waterlogged, shoot and root growth of the common species was more affected
5 than that of the ironstone endemics. Since these habitat-related differences were not
6 observed on sand (Poot and Lambers 2003b), our results suggest that the chemistry of
7 waterlogged ironstone soil and adaptations to it play a role in these species' distribution
8 patterns.

9

10 *Effects of waterlogging on shoot growth*

11 Surprisingly, constant waterlogging to a level of 1 cm above the soil surface did not
12 negatively affect shoot growth in any of the six *Hakea* species, with the four wetland
13 species even showing apparent growth stimulation when compared with their controls.
14 This confirms results of an earlier study on sand for the winter-wetland species (Poot and
15 Lambers 2003b), and would rank them among the most waterlogging-tolerant species.
16 However, in contrast to this earlier study, the rare ironstone species showed a stronger
17 growth stimulation than the common wetland species, and were the only species that
18 showed a significant increase in shoot mass compared with their controls. The apparent
19 growth stimulation was partly the result of an accumulation of starch. Waterlogging-
20 induced starch accumulation has been found in many other woody species (e.g. *Pinus*
21 *serotina*, Topa and Cheeseman 1992; four hardwood tree species, Gravatt and Kirby
22 1998), and is generally considered a consequence of the strongly decreased demand for
23 carbohydrates by the anoxic or hypoxic roots. The stronger shoot growth stimulation and

1 starch accumulation, and the smaller reduction in root growth of the ironstone endemics
2 suggests that they maintained a more positive carbon balance during waterlogging. As
3 accumulated carbohydrates can aid in the rapid re-growth of roots during the recovery
4 phases after waterlogging, this may be relevant for field performance, especially in the
5 drought-prone ironstone habitat where root access to water, through crevices in the
6 underlying rock, is crucial for first summer survival (Poot and Lambers 2003a, 2008).

7

8 *Effects of waterlogging on root growth*

9 Many wetland and non-wetland species produce superficial adventitious roots in response
10 to waterlogging (Justin and Armstrong 1987; Kozłowski 1997). Apart from their
11 placement in soil horizons that most likely contain some oxygen, they also often have
12 higher porosities than the ‘old’ root system (Justin and Armstrong 1987; Poot and
13 Lambers 2003b for the species in this study), resulting in a better oxygen and thus better
14 metabolic energy supply. In a previous study we demonstrated that, when grown on sand,
15 ‘wetland’ *Hakea* species produced similar amounts of adventitious roots, whereas the
16 non-wetland species produced half the amount of the wetland ones (Poot and Lambers
17 2003b). Compared with these previous results, waterlogging on ironstone soil greatly
18 suppressed the initiation of adventitious roots in all but one of the non-ironstone species
19 (*H. ceratophylla*). Interestingly, this is the winter-wet species that occurs most regularly
20 in at least some of the southern ironstone communities which suggests it is of adaptive
21 significance. The importance of adventitious roots for plant survival during waterlogging,
22 through sustaining nutrient and water uptake as well as maintaining hormone supply is
23 well established (Armstrong *et al.* 1994; Vartapetian and Jackson 1997). Although none

1 of the seedlings in our study died, the strong suppression of adventitious-root formation
2 in three of the four common *Hakea* species likely has fitness consequences under much
3 harsher field conditions, and may partly explain their absence from ironstone habitats.
4 Further work is needed to identify the exact mechanisms causing the suppression of
5 adventitious roots in non-ironstone species.

6

7 *Fe toxicity*

8 In contrast to most leaf nutrients, which decreased substantially upon waterlogging, Fe
9 concentrations tended to increase in most of the species. However, there was no clear
10 pattern between the increase in leaf Fe and habitat of origin. Most likely the increase in
11 leaf Fe was due to a large increase in the availability of soluble ferrous iron (Fe^{2+}) in the
12 strongly reduced ironstone soil (cf. Ponnampertuma 1972). Higher leaf Fe concentrations
13 under flooding conditions have been observed in many species, and the possible toxic
14 effects of Fe have been implicated as a determinant of waterlogging sensitivity and
15 species distribution (e.g. Jones and Etherington 1970; Talbot *et al.* 1987; Snowden and
16 Wheeler 1993, 1995). However, leaf iron concentrations were well below those being
17 reported as toxic for other species (e.g. $300 \mu\text{g g}^{-1}$ for rice, Howeler 1973; $700\text{-}800 \mu\text{g g}^{-1}$
18 for a variety of species, Snowden and Wheeler 1995). The high iron content of clay
19 particles attached to the root systems in our plants, made any quantitative analyses of Fe
20 in root biomass impossible. However, we observed that young adventitious roots of the
21 two ironstone species and *H. ceratophylla* developed a distinctive ochreous (red-brown)
22 discolouration over time. Similarly, during the harvests, all waterlogged plants showed an
23 ochreous discolouration of the stelar tissue at the root-shoot junction. The observed

1 ochreous discolourations are most likely precipitates of iron oxide (cf. Snowden and
2 Wheeler 1995; Smolders and Roelofs 1996), and suggest that external or internal
3 precipitation of Fe plays an important role in maintaining Fe homeostasis in these
4 species. Clearly, more work is needed to elucidate the role that Fe may play in
5 suppressing adventitious root formation and the importance of internal and external Fe
6 precipitation for species survival on waterlogged Fe rich soil.

7

8 *Leaf P*

9 Of all measured leaf nutrients, only for P the ironstone endemics responded differently to
10 waterlogging than the other species. The ironstone species showed a much stronger
11 reduction of leaf P concentrations upon waterlogging. Half of this difference can be
12 calculated as being an effect of dilution, resulting from the relatively stronger shoot
13 growth of the ironstone endemics under waterlogging. The reason this dilution effect is
14 only observed for P suggests that P was the most limiting nutrient on these ironstone
15 soils, which is not surprising as many SW Australian soils are characterised by an
16 extremely low P availability (Foulds 1993). However, the ironstone soil was not different
17 in total P or available P compared with the soils the non-ironstone species originated
18 from (see Appendix). The low leaf P concentrations of the waterlogged ironstone species
19 may also partially result from their presumably higher dependence on soil P as they had
20 the smallest seeds and thus most likely the lowest P storage. The latter may be relevant as
21 Lamont and Groom (2002) showed that in 3-month old seedlings of two SW Australian
22 *Hakea* species, an average of 76% of total plant P originated from the seeds.

23

1 *Higher Cu and Zn concentrations in leaves of ironstone endemics*

2 Of all measured leaf nutrients only Cu and Zn concentrations were consistently higher
3 in the ironstone endemics. As similar results were obtained when species were grown on
4 washed river sand (Poot, unpublished results), ironstone species may have a higher
5 demand for these micronutrients. This might be related to their role as cofactors in
6 protective enzymes such as superoxide dismutases (SODs), as ‘ZnCu-SODs’ are the most
7 abundant and active SODs in higher plant cells (Alscher *et al.* 1997), and Zn deficiency
8 has been implicated in increases in oxidative damage (Cakmak 2000). Compared with the
9 habitat of common congeners, the ironstone habitat is likely harsher and more exposed to
10 environmental conditions resulting in oxidative damage such as: (1) multiple oxygen re-
11 entry events (i.e. a high frequency of waterlogging and drying events in winter; see Fig.
12 3; Hendry and Brocklebank 1985; Blokhina *et al.* 1999), (2) extreme drought (i.e.
13 photoinhibition as a result of stomatal closure in response to the fast drying of their very
14 shallow soils in spring and/or early summer), and (3) potentially high levels of iron in
15 plant tissues (Becana *et al.* 1998; Cakmak 2000; Hell and Stephan 2003).

16

17 *Seedling survival in the field*

18 Unlike in the glasshouse experiment, natural waterlogging in the southern ironstone
19 community quickly led to seedling mortality, but in the non-wetland species only. In
20 contrast, no clear differences in mortality were observed in the drier northern community.
21 The major difference between the communities was the much higher level and much
22 longer period of waterlogging on the southern ironstone site which included periods of
23 several days of complete seedling inundation (deduced from Figs 3B and 6B). The

1 importance of the latter is further substantiated by the observation that seedling health
2 and micro-topographic position were strongly correlated in this community, with
3 individual plants placed just 4 to 15 mm higher in the plot surviving for much longer. It is
4 well accepted that energy deficit, caused by O₂ shortage is one of the most severe
5 problems encountered by plants when subjected to complete inundation (e.g. Voesenek *et*
6 *al.* 2006). Our results strongly suggest that oxygen supply via the shoots is crucial for
7 these non-wetland species, as waterlogging at a level just above the soil surface did not
8 cause any mortality in glasshouse-grown plants (even after 4 months of waterlogging,
9 personal observation P. Poot), and only caused some mortality in the shortest species (*H.*
10 *cyclocarpa*) in the wettest plot of the northern ironstone community.

11

12 *Conclusions*

13 The results presented in this study show that, when waterlogged, ironstone soil affects the
14 growth of common *Hakea* species more than that of rare *Hakea* species originating from
15 these soils. This strongly suggests that local adaptation to the specific conditions imposed
16 by waterlogging on these soils (e.g. high availability of iron) plays a role in these species
17 distribution patterns. The suppression of adventitious root formation in three of the non-
18 ironstone species, likely has consequences for survival in the field, either during the
19 waterlogging period itself (as was shown for the non-wetland species) or during the
20 recovery when a fast resumption of root growth is likely essential to survive the summer
21 drought (see Poot and Lambers 2008). Future research will have to further elucidate the
22 exact mechanisms causing these habitat-related differences.

23

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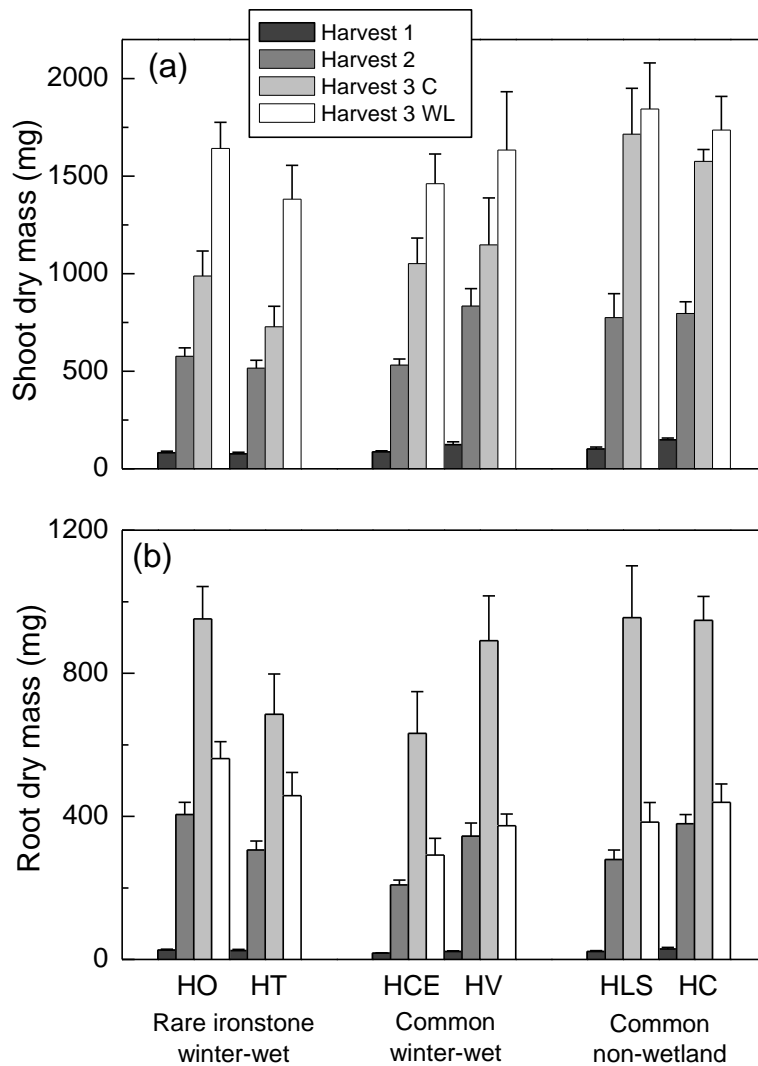
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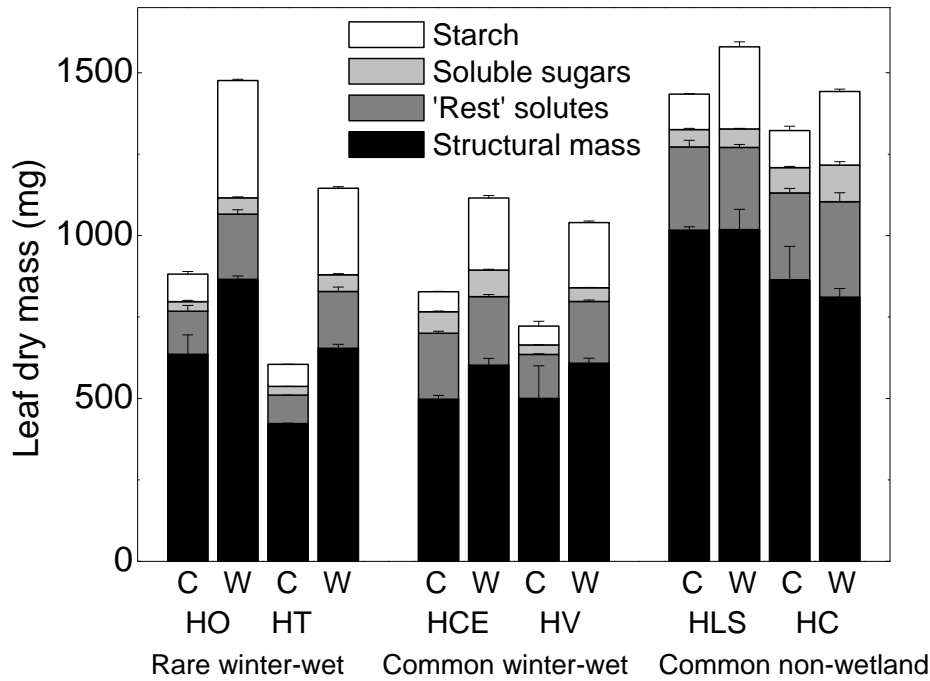
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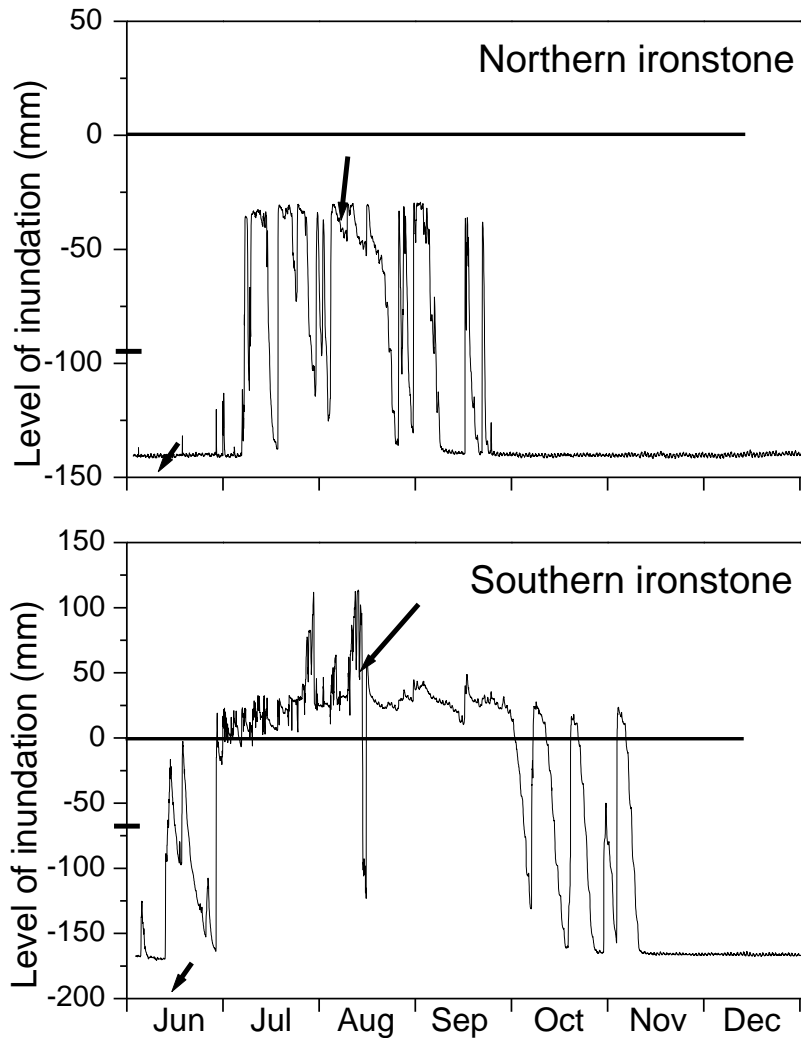


1
 2 **Fig. 1.** Shoot (a) and root (b) dry mass of seedlings of 6 *Hakea* species differing in their
 3 habitat of origin (winter-wet versus non-wetland) and their degree of rareness (rare versus
 4 common), at three harvests (15, 70 and 113 days after germination, respectively). All
 5 species were grown on well-watered ironstone soil. After harvest 2, half of the seedlings
 6 were subjected to a waterlogging treatment (WL), with the other half remaining as well-
 7 watered controls (C). Bars indicate means \pm SE (n=6). HO: *H. oldfieldii*, HT: *H.*
 8 *tuberculata*, HCE: *H. ceratophylla*, HV: *H. varia*, HLS: *H. lissocarpha*, HC: *H.*
 9 *cyclocarpa*.

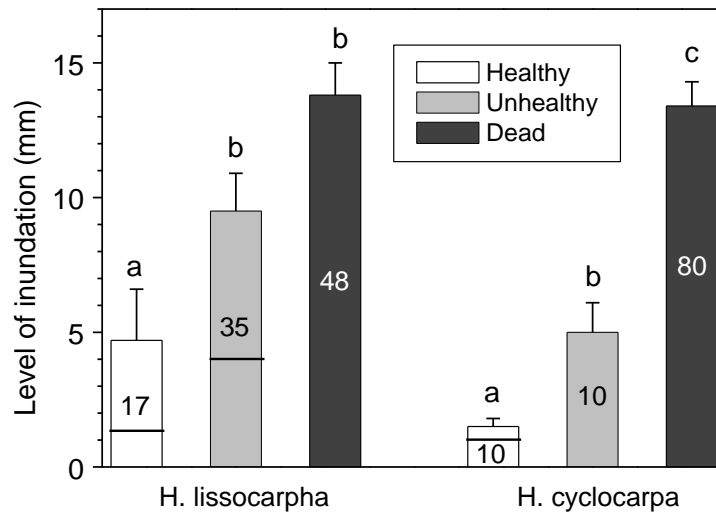


1
 2 **Fig. 2.** Total leaf dry mass and its major components (means \pm SE, n=2 bulked samples)
 3 at the final harvest for control (C) and waterlogged (W) seedlings of 6 *Hakea* species
 4 differing in their habitat of origin (winter-wet versus non-wetland) and their degree of
 5 rareness (rare versus common). For abbreviations of species names, see legend to Fig. 1.

6



1
 2 **Fig. 3.** Levels of inundation (mm above soil surface) in two ironstone communities
 3 during the winter of 2002 as obtained from piesometers. The arrows indicate the date of
 4 transplantation of seedlings (June 2002) and the level of inundation at the position of the
 5 piesometer during a field visit in August. The depth to ironstone rock in both
 6 communities is indicated by the horizontal line on the y-axis. Changing water levels
 7 below this depth indicate changes in water level in the holes that were drilled into the
 8 rock for stabilizing the piesometers.



1

2 **Fig. 4.** Association between level of inundation and seedling health for the two non-
3 wetland species at the southern ironstone site. Inundation levels (mm above ground
4 surface) were determined during a site visit 58 days after transplantation of the
5 glasshouse-grown seedlings into the field (August 2002). Values indicate the percentage
6 of individual seedlings within each category. Bars represent means \pm SE. Different letters
7 denote significant differences within a species ($P < 0.05$, Tukey after one-way ANOVA).
8 Horizontal lines inside a bar indicate the average level of inundation of the few plants that
9 survived until October.

10

1

2 **Table 1. Adventitious root characteristics for seedlings of six 6 *Hakea* species**
 3 **differing in their habitat of origin, and their degree of rareness, after 43 days of**
 4 **waterlogging on an ironstone soil. Values are means of six individuals. Letters**
 5 **denote significant differences among values within rows (Tukey after one-way**
 6 **ANOVA; P<0.05). For abbreviations of species names see legend to Fig. 1.**

7

	Rare ironstone		Common		Common	
	winter-wet		winter-wet		non-wetland	
	HO	HT	HCE	HV	HLS	HC
12 Total dry mass (mg)	29.0 ^a	45.2 ^a	47.5 ^a	3.7 ^b	1.7 ^b	0.92 ^b
13 % of root dry mass	5.0 ^b	10.2 ^a	16.1 ^a	0.91 ^c	0.37 ^c	0.15 ^c
14 % of total plant dry mass	1.36 ^b	2.59 ^a	2.62 ^a	0.19 ^c	0.07 ^c	0.04 ^c
15 Number	6.0 ^a	7.2 ^a	6.3 ^a	1.3 ^b	1.8 ^b	0.83 ^b
16 Length* (mm)	52.7 ^a	57.7 ^a	71.7 ^a	47.0 ^{ab}	21.0 ^{bc}	15.0 ^c

17 * zero values for plants without adventitious roots are not included in mean value.

Table 2. Leaf nutrient concentrations of seedlings of 6 *Hakea* species differing in their habitat of origin and their degree of rareness, under control (C) and waterlogged conditions (WL). The statistical model had ‘habitats’ (Hab) and ‘treatment’ (Treat) as fixed factors and ‘species within habitat’ as nested factor in the model. Model components including the nested factor are not shown. Data were log-transformed to ensure normality and homogeneity of variances. For abbreviations of species names, see legend to Fig. 1. Data were based on two bulked samples (N=2) of leaves of three individual plants, and were not corrected for incomplete starch hydrolysis (i.e. 85%, see Materials and Methods). NS: non-significant, *: P<0.001, **: P<0.01, *: P<0.05.**

Nutrient	Treat- ment	Rare		Common		Common		Statistical analyses		
		winter-wet HO	HT	winter-wet HCE	HV	non-wet HLS	HC	Hab	Treat	Hab.Treat
<i>Macronutrients (mg g⁻¹)</i>										
N	C	17.8	19.6	17.1	19.1	14.8	14.6	NS	**	NS
	WL	9.2	14.9	11.6	12.5	9.0	8.8			
P	C	0.39	0.42	0.35	0.51	0.39	0.40	NS	*	***
	WL	0.19	0.20	0.24	0.38	0.33	0.38			
K	C	3.8	3.8	5.4	6.0	2.9	2.3	*	*	NS
	WL	2.0	3.4	3.2	3.4	1.8	1.7			
Ca	C	8.0	11.1	7.6	10.8	5.9	5.7	NS	**	NS
	WL	4.6	6.4	5.4	6.2	2.6	1.4			
Mg	C	1.9	2.2	2.5	2.3	1.4	1.7	NS	**	NS
	WL	1.3	1.7	1.8	1.2	1.0	1.1			
<i>Micronutrients (µg g⁻¹)</i>										
Fe	C	71.1	69.7	43.7	74.6	52.7	47.4	*	NS	NS
	WL	69.3	81.1	117	89.1	56.1	72.4			
Mn	C	155	56.5	160	46.8	71.3	108	NS	**	NS
	WL	63.0	26.6	104	19.3	36.9	30.4			
Zn	C	15.3	17.7	7.6	11.1	6.1	7.8	**	**	NS
	WL	6.7	5.7	5.1	4.6	4.2	4.1			
Cu	C	6.5	7.4	4.2	4.0	4.4	3.8	**	**	NS
	WL	4.0	3.7	2.9	2.7	2.1	2.2			

1 *Appendix 1: Soil chemical analyses*

2 **Table 1. Soil characteristics of the three habitats from which the 6 *Hakea* species**
 3 **originate (2 sites per habitat). ‘Ironstone 1’ soil was used in the glasshouse study.**
 4 **Values are averages (N=4 per site). Means with different letters are significantly**
 5 **different (Student Newman Keuls pairwise test after one-way ANOVA). All**
 6 **variables were log-transformed before testing. NS: non-significant, ***: P<0.001, **: P<0.01, *: P<0.05. For methods refer to text.**

	Ironstone		Winter-wet		Non-wetland	
	1	2	1	2	1	2
pH (CaCl ₂)	4.6 ^{ab}	4.4 ^a	4.4 ^{ab}	4.5 ^{ab}	4.7 ^b	4.9 ^c
EC (mS m ⁻¹)	58	61	54	31	39	28
Gravel (%)	10.6 ^b	8.1 ^b	0 ^a	0 ^a	32.5 ^c	25 ^c
CEC (meq 100 g ⁻¹)	2.0 ^{ab}	1.1 ^a	2.5 ^{ab}	1.5 ^{ab}	8.7 ^c	3.2 ^b
Organic carbon (%)	2.5 ^{bc}	1.9 ^{bc}	1.4 ^{ab}	1.0 ^a	5.5 ^c	2.5 ^c
<i>Macronutrients</i>						
P (total, mg kg ⁻¹)	250 ^b	60 ^a	74 ^a	48 ^a	139 ^b	54 ^a
P (mg kg ⁻¹)	5.8 ^{ab}	4.5 ^{ab}	4.0 ^{ab}	3.8 ^a	6.8 ^b	5.0 ^{ab}
K (meq 100 g ⁻¹)	0.08 ^{bc}	0.03 ^a	0.08 ^{bc}	0.06 ^{ab}	0.20 ^c	0.10 ^{bc}
Ca (meq 100 g ⁻¹)	0.82 ^b	0.35 ^a	1.18 ^{bc}	0.65 ^b	5.7 ^d	2.0 ^c
Mg (meq 100 g ⁻¹)	0.77 ^b	0.24 ^a	0.75 ^b	0.40 ^{ab}	2.0 ^c	0.70 ^b
<i>Micronutrients</i>						
Fe (reactive, mg kg ⁻¹)	3120 ^c	6780 ^c	230 ^a	300 ^a	1260 ^b	900 ^b
Fe (mg kg ⁻¹)	190 ^b	203 ^b	83.7 ^a	95.1 ^a	79.7 ^a	64.2 ^a
Mn (mg kg ⁻¹)	22.6 ^a	0.5 ^b	0.6 ^b	2.5 ^b	10.1 ^a	6.2 ^a
Zn (mg kg ⁻¹)	0.39	0.37	0.32	0.76	0.71	0.45
Cu (mg kg ⁻¹)	0.28 ^{ab}	0.25 ^{ab}	0.17 ^a	0.26 ^{ab}	0.27 ^{ab}	0.35 ^{bc}
<i>Others</i>						
Al (mg kg ⁻¹)	0.75 ^a	1.8 ^b	2.2 ^b	1.7 ^b	2.3 ^b	0.65 ^a
Na (m 100 g ⁻¹)	0.24 ^{ab}	0.28 ^a	0.22 ^{ab}	0.15 ^{ab}	0.24 ^{ab}	0.10 ^b

1 To obtain an estimate of the variation in soil characteristics among the three habitat types
2 our species originated from, we randomly sampled topsoil (top 100 mm) from 2 sites for
3 each habitat type (n=4 per site). The sites coincided with the populations seeds were
4 collected from. Soil analyses were performed by CSBP Futurefarm analytical laboratories
5 (Bibra Lake, Australia). Soil samples were air-dried and passed through a 2-mm sieve.
6 For total P, soils were digested by a Kjeldahl procedure (sulfuric acid-potassium sulfate-
7 copper sulfate). Bicarbonate-extractable P was determined after extraction with 0.5 M
8 sodium bicarbonate at pH 8.5 (Colwell 1963). P in the extracts/digests was determined
9 colourimetrically using the phosphomolybdenum blue method (Murphy and Riley 1962).
10 Trace elements (Cu, Zn, Mn and Fe) were extracted with EDTA solution at a ratio of 1:5
11 for 1 hour, and their concentrations were determined by ICP-AES. Reactive Fe and Al
12 were extracted by acid oxalate (Parfitt and Childs 1988). Fe was then determined by AAS
13 and Al by ICP-MS. Exchangeable cations (Ca, Mg, Na, K) were extracted with 0.1 M
14 BaCl₂/0.1 M NH₄Cl, and determined using ICP-MS. For determining soil pH (water) and
15 electrical conductivity (EC), soils were stirred in deionised water for 1 hour at 25°C using
16 a soil:solution ratio of 1:5. After measurements of pH (water) and EC, pH (CaCl₂) was
17 measured by adding CaCl₂ to a concentration of 0.01 M.

18

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