

Relationship between the Al Resistance of Grasses and their Adaptation to an Infertile Habitat

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• **Background and Aims** Original data on Al resistance, relative growth rate and leaf traits of five European grasses as well as literature data on Al resistance, habitat preference and traits of grasses were considered to determine whether (a) Al resistance is correlated to a growth conservative strategy and (b) species occurrence could be useful to assess Al toxicity in meadows on acid soils.

• **Methods** The Al resistance of 15 species was represented by the Al activity in nutrient solution that resulted in a 50 % decrease in root length, $\{Al^{3+}\}_{50}$, or, for published values, in root or plant biomass. The correlations between Al resistance and acidity or nitrogen indices and the correlation between Al resistance and selected traits (relative growth rate, leaf dry matter content, specific leaf area and leaf thickness) were calculated. Principal component analysis was used for the characterization of the relationships between Al resistance and measured traits.

• **Key Results** The $\{Al^{3+}\}_{50}$ values of the resistant species *Molinia caerulea* and *Sieglingia decumbens* were 13 and 26 μM $\{Al^{3+}\}_{50}$, respectively. The known Al resistance of 15 species that were mainly of the intermediate strategy competitor–stress tolerator–ruderal (C-S-R) type and of the S type was correlated with Ellenberg's nitrogen and acidity indices. For the whole set of species, the correlation between Al resistance and traits was not significant.

• **Conclusion** The Al resistance of the C-S-R species was variable and independent of their traits. S-type species, adapted to acid soils and with traits of conservative strategy, displayed Al resistance. The large difference in Al resistance between grasses may help assess Al soil toxicity by using the abundance of grasses.

Key words: Grassland, plant functional traits, acid soils, Al resistance, grasses, *Arrhenatherum elatius*, *Festuca rubra*, *Helictotrichon sulcatum*, *Molinia caerulea*, *Sieglingia decumbens*.

INTRODUCTION

Soil acidity, as defined by a topsoil pH value below 5.5, is observed in about 37 % of the soils in Europe (von Uexküll and Mutert, 1995). It develops under wet climates in northern Europe as well as in the forests and grasslands of the mountain ranges (Falkengren-Grerup and Tyler, 1993; Baize and Roque, 1998; Austrheim *et al.*, 2005). This natural phenomenon limits plant growth, decreases the diversity of grassland species and selects species which are generally lower yielding (Tyler, 1996; Roem and Berendse, 2000; Austrheim *et al.*, 2005). The acidification is promoted by the geological substratum and enhanced by acid rains and ammonium fertilizers.

In organic acid soils, the acidity due to H⁺ ions is, by itself, harmful to plants (Kidd and Proctor, 2001). However, in mineral acid soils, the indirect adverse effects of acidity prevail with Al, Mn and Fe toxicity, and P, Ca, Mg, and K deficiency (von Uexküll and Mutert, 1995). Among these adverse effects, Al toxicity, as measured by its chemical activity $\{Al^{3+}\}$, is often predominant (Hocking, 2001). Al activity is near zero above pH 5.5 and increases markedly from pH 5.0 to pH 4.1 over the range 1–150 μM (Kinraide, 1991, 2003). It is influenced by Ca and Mg concentrations (Kinraide, 2003) and by Al ligand concentrations, particularly phosphates, sulfates and the anionic forms of soluble organic acids (Ritchie,

1995). Al activity in the soil solution and Al toxicity are thus difficult to assess through the determination of the usual parameters measured in soil analysis. In most cases, the Al toxicity hazard is estimated through the measurements of soil pH value, exchangeable Al concentration and Al/Ca ratio in the soil solution (Houdijk *et al.*, 1993; Roem and Berendse, 2000). Al activity in the soil solution is rarely determined or calculated (Kidd and Proctor, 2001; Schöttelndreier *et al.*, 2001; Kinraide, 2003).

Plant resistance to Al toxicity (Kochian *et al.*, 2005) has been measured by the Al activity $\{Al^{3+}\}_{50}$ for which above ground or root biomass (Wheeler *et al.*, 1992; Wheeler, 1995) or root length (Wright *et al.*, 1987; Kinraide, 2003) is decreased by 50 %. The variability in Al resistance between grasses ranges from 1 to 50 μM $\{Al^{3+}\}_{50}$ (Wenzl *et al.*, 2001). However, the $\{Al^{3+}\}_{50}$ value is known only for a small number of meadow grasses of the temperate zone: for the resistant species *Agrostis capillaris* ($\{Al^{3+}\}_{50} > 30 \mu M$), *Festuca rubra* and *Holcus lanatus* ($\{Al^{3+}\}_{50} 10–20 \mu M$), for the moderately sensitive species *Dactylis glomerata*, *Festuca arundinacea*, *Lolium* spp. ($\{Al^{3+}\}_{50}$ approx. 5 μM) and for the sensitive species ($\{Al^{3+}\}_{50} < 2 \mu M$) such as *Bromus* spp., *Cynosurus cristatus*, cultivars of *Lolium perenne*, *Poa pratensis* and *Phleum pratense* (Wheeler *et al.*, 1992; Wheeler, 1995). Besides, an intraspecific variability in the resistance to Al was observed in rye grass and orchard grass (1–5 and 2–10 μM $\{Al^{3+}\}_{50}$, respectively; Wheeler 1992, 1995).

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Intraspecific variabilities, without any mention of the corresponding $\{Al^{3+}\}_{50}$ values, were reported in Yorkshire fog (Kidd and Proctor, 2001) and in fescues (Liu *et al.*, 1996).

The numerous observations on the adaptation of some grass species to soil acidity were synthesized in Ellenberg's acidity indices (Ellenberg *et al.*, 1992). Low values (1–3) of the acidity index correspond, with few exceptions, to species whose pH optimum can be assumed to be below 5.5, i.e. lying in the potential range of Al toxicity (Schaffers and Sykora, 2000). However, a precise correspondence between tolerance to soil acidity (using Ellenberg's indices) and resistance to Al does not appear to have been ascertained.

To our knowledge, since the investigations of Wheeler (1995), neither the Al resistance potential of other grasses of the temperate zone nor the possible links between tolerance to acid soils and resistance to Al have been further investigated. Likewise, the possible link between resistance to Al and adaptation to infertile soils (conservation strategy vs. capture strategy) has not been investigated. The assumption is that Al-resistant grasses are, in the first place, adapted to limited nutrition conditions. The adaptation to infertile soils can be represented by Ellenberg's nitrogen index which is issued from the classification of observed niches and is well correlated to species productivity (Schaffers and Sykora, 2000). Functional traits that are characteristic of the species strategy (Reader, 1998; Wilson *et al.*, 1999; Ansquer *et al.*, 2004), relative growth rate (RGR), specific leaf area (SLA), leaf dry matter content (LDMC) and leaf thickness (LT) were also considered. The aim of the investigations reported here was to analyse the relationships between resistance to Al and these indices and traits so as to ascertain a diagnosis of the extent of Al toxicity from the occurrence of various grass species in acid grasslands.

MATERIALS AND METHODS

Growth conditions and quantification of Al resistance

The grasses to be studied were chosen in relation to their occurrence in moderately acidic soils of grasslands in the Central Pyrenees (1°17'E, 42°51'N, 600–900 m a.s.l.). The abundance of the grass species was determined in three acidic stands with infertile soil and potential Al toxicity (stands 1, 2 and 3) and in a less acidic and slightly more fertile stand with no Al toxicity (stand 4). The disturbance induced by the defoliation pressure increased from stand 1 (only mowed once or twice a year) to stands 2 and 3 (two foliage removals by mowing and grazing) and to stand 4 (three foliage removals: mowed, grazed and re-grazed). To give an index of the fertility differences between the stands, the nitrogen nutrition was measured on the first cut: the hay was dried (80 °C, 48 h), weighed, ground, and analysed for total N content (CHN 2000 analyser, Leco, St Joseph, MI, USA). The nitrogen nutrition index (NNI), expressed as a percentage of the optimum nutrition, was then calculated according to Lemaire and Gastal (1997). The pH (soil water ratio 1:2.5, v/v) and the Al exchangeable content (cobaltihexamine method;

Orsini and Remy, 1976) were measured at 0–5 cm soil depth. The abundance was measured according to Daget and Poissonet (1971) and expressed as the percentage of the abundance of the grasses ($n = 2–4$ replications by stand).

When Al resistance was unknown, the Al resistance of the abundant species was quantified in nutrient solution culture in a growth chamber. One cultivated species, *L. perenne* (Lp) 'Clerpin', and one wild ecotype of *F. rubra* were taken as sensitive and resistant controls, respectively. Seeds of the seven wild grasses were collected in their native habitat: *Arrhenatherum elatius*, *F. rubra*, *Helictotrichon sulcatum* (Gay) Pilger, *C. cristatus*, *D. glomerata*, *Molinia caerulea* and *Sieglingia decumbens*.

Growth conditions

Surface-sterilized and vernalized seeds were germinated for 1 or 2 weeks, depending on the species, on wet filter paper in Petri dishes. After germination, 12–20 uniform seedlings of each species were sorted in four lots. Each lot of 3–5 plants was taken as one experimental unit. When the principal root length was 1 ± 0.5 cm, the seedlings were transferred into the different nutrient solutions, with or without Al, in a growth chamber with the following conditions: day, 14 h, photosynthetic photon flux density at mean plant height $260 \mu\text{mol m}^{-2} \text{s}^{-1}$ of PAR (photosynthetically active radiation), temperature 25 °C, relative humidity approx. 70%; night, 10 h, temperature 18 °C. The species were cultivated in trays floating on four 48 L tanks corresponding to the four Al treatments. The same experimental protocol was time-replicated three times.

Low ionic strength nutrient solutions simulated an infertile acid soil solution according to Wenzl *et al.* (2003). The composition of the nutrient solution (pH 4.2) was as follows (in μM): 100 NO_3 , 10 NH_4 , 60 Ca, 30 Mg, 60 K, 1 P, 100 S, 6 B, 5 Fe, 1 Zn, 1 Mn, 0.2 Cu, 5 Si, 5 H_2EDTA , 0.001 Mo, 160 Na. Al was added at concentrations of 0 (control), 40, 80 and 160 μM of acidified AlCl_3 , corresponding to 0, 22, 43 and 83 μM $\{Al^{3+}\}$. These values correspond to the range of $\{Al^{3+}\}$ expected in temperate acid soils (Wright *et al.*, 1987). The solutions were continuously aerated and renewed every 3 d in order to minimize variations in the concentration of the nutrients. The HCl concentration (55.4 μM) was adjusted in order to obtain a pH value of the solution in the range 4.2 ± 0.1 . The activity of Al^{3+} was calculated with GEOCHEM 2.0 (Parker *et al.*, 1987) by using the constants of stability of Nordstrom and May (1989) and the solubility constants of Al (OH)₃ mentioned by Kinraide and Parker (1989).

Quantification of Al resistance

Seedlings were harvested after 14 (species with a high RGR) or 21 d (species with a low RGR) of growth at the different Al concentrations. They were separated into roots and shoots. Roots were stained with a 1% (v/v) dilution of a methyl violet stock solution (1 g 100 mL⁻¹ ethanol) and scanned with a flatbed scanner. The WinRhizo software (Regent Instruments Inc., Quebec,

Canada) was used to obtain the overall length of roots. The data of the three successive replications for each species and treatment were pooled for calculations. Relative root elongation (RRE) of the plants treated with Al and the activity $\{Al^{3+}\}_{50}$ at which RRE was reduced to 50% of the control were adapted from Wenzl *et al.* (2001). Briefly, RRE and $\{Al^{3+}\}_{50}$ were calculated as follows:

$$RRE = 100(RL_{Al} - RL_{\infty}) / (RL_0 - RL_{\infty})$$

where RL_{Al} is the root length in the Al treatment, RL_{∞} is the mean root length at the highest level of Al, and RL_0 is the mean root length in the control solution ($0 \mu M$ Al). RRE values for *C. cristatus* (sensitive to Al), *L. perenne* and *D. glomerata* (moderately sensitive) were taken as control values. The response of RRE to $\{Al\}$ was fitted to a Weibull equation as suggested by Kinraide and Parker (1989):

$$RRE = 100 / \exp[(a\{Al^{3+}\})^b]$$

The activity $\{Al^{3+}\}_{50}$ at which RRE was reduced to 50% of the control was calculated from a and b empirical values according to the formula:

$$\{Al^{3+}\}_{50} = a^{-1} [\ln(2)]^{1/b}$$

As the $\{Al^{3+}\}$ values tested ($22\text{--}83 \mu M$) were high, the calculations were not suitable for sensitive species with low $\{Al^{3+}\}_{50}$ values ($1\text{--}6 \mu M$ $\{Al^{3+}\}_{50}$) such as *C. cristatus*, *D. glomerata* and *L. perenne*.

Leaf traits and RGR measurement

Leaf dry matter content and specific leaf area were measured on the same species. For each species, samples of 5–15 tillers ($n = 5\text{--}12$ samples) developed under sunlight conditions were harvested in the field during May–June, in acidic meadows (pH 4.7–6.6) of Ariège (Central Pyrenees, France). Each tiller was cut at its base and immediately plunged into a test tube filled with water in order to avoid wilting. LDMC and SLA measurements were made following the protocol described by Garnier *et al.* (2001) and Cornelissen *et al.* (2003) evaluating water-saturated blades (excluding sheaths) on the youngest fully expanded leaf of the tiller. Leaf thickness (LT) was calculated from measured SLA and LDMC according to Vile *et al.* (2005).

RGR was measured on the species presenting some Al resistance: *A. elatius*, *F. rubra* (taken as a control), *H. sulcatum*, *M. caerulea* and *S. decumbens*. The young plants were cultivated in nutrient solution in a growth chamber with climatic conditions as previously described. The composition of the nutrient solution was as follows (in μM): 450 NO_3 , 150 NH_4 , 800 Ca, 200 Mg, 350 K, 50 P, 1000 S, 6 B, 5 Fe, 1 Zn, 1 Mn, 0.1 Cu, 0.1 Mo. The solution was renewed every 10 d. Holes in the Styrofoam cover

of the 48 L tanks received bungs with three homogeneous seedlings of each species. RGR was measured using a fresh weight method adapted from Causton (1991). Each bung was removed from the tank, blotted dry, weighed, and then replaced in the solution. By this means, the fresh weight of the same unit of three plants could be monitored throughout the experiments. Let the fresh weight of the sample at date t_i be W_i ; RGR was calculated as follows:

$$RGR = (\ln W_2 - \ln W_1) / (t_2 - t_1)$$

As species differed in growth rate, RGR was calculated over a period when the different species had a similar total dry weight (between 100 and 450 mg dry weight per plant) and yielded high RGRs. Values are the mean of three independent replications of RGR measurement except for *H. sulcatum* (two replications).

Al-traits relationships

In order to gain an insight on a greater number of species (15 species), the Al-traits relationships were studied using a collection of values published by other authors in addition to measured values on five species from this study. Published values of $\{Al^{3+}\}_{50}$ are scarce. Data issued from long-term measurement of $\{Al^{3+}\}_{50}$ on shoot biomass, which had been suggested to be in accordance with those on root biomass (Wheeler *et al.*, 1992), were found in Wheeler *et al.* (1992) and Wheeler (1995). When a range of resistance was known for a species, the value used was the median value of the resistance range. For the other species, the estimated $\{Al^{3+}\}_{50}$ value was used. The $\{Al^{3+}\}_{50}$ value of *D. flexuosa* was estimated from growth data and nutrient concentrations of Rorison (1985), using GEOCHEM 2.0.

Published values of traits were collected in Poorter and Remkes (1990), Hunt and Cornelissen (1997), Meziane and Shipley (1999), Poorter and de Jong (1999), Ryser and Urbas (2000), Ryser and Wahl (2001), Taub (2002), Al Haj Khaled *et al.* (2005) and Sugiyama (2005). Ellenberg indices were obtained from Ellenberg *et al.* (1992). Details are given in Table 3.

Statistics

One-way (species) analysis of variance of RRE was performed with the StatBox 2.0 statistical package (Grimmersoft, Paris, France) on log-transformed data at each level of Al. Significant differences between means refer to the probability level of 0.05 by Newman and Keuls's bilateral test. For $\{Al^{3+}\}_{50}$ estimation, the Weibull function was fitted using the non-linear square procedure in R software 1.9.0 (Ihaka and Gentleman, 1996). The standard error s.e. $\{Al^{3+}\}_{50}$ was calculated according to Wenzl *et al.* (2001). Significance of the $\{Al^{3+}\}_{50}$ values refers to the unilateral comparison to the 0 value (t -test; $n = 12$; *A. elatius*, $n = 11$).

Relationships between $\{Al^{3+}\}_{50}$ and traits were tested with the linear correlation coefficient r . For the overview of $\{Al^{3+}\}_{50}$ and traits relationships, principal component analysis (PCA) was performed on the correlation matrix of the selected variables using R 1.9.0. Groups of species in the first plane of the PCA were distinguished using a distance analysis based on Ward's criteria.

RESULTS

Ranking of Al resistance

In the acidic stands 1, 2 and 3, with Al toxicity hazard, *A. capillaris*, *F. rubra* and *H. sulcatum* were always more abundant than in the control stand 4 (Table 1). *Molinia caerulea*, *S. decumbens* and *B. pinnatum* were observed only in the acidic stand 1. Conversely, the species that were abundant in the control plot with no Al toxicity hazard (*D. glomerata*, *H. lanatus* and *L. perenne*) were scarce or absent in the acidic stands. The number of grass species in the acidic stands was rather restricted in comparison with the control plot. Thus, the abundances of the grasses may be related to the Al toxicity hazard of the stands.

In nutrient solution culture, the $\{Al^{3+}\}$ 22 μM level (Fig. 1A) was highly toxic to the controls *C. cristatus* and *D. glomerata* (RRE 2 and 5 %, respectively). In contrast, *S. decumbens* and *M. caerulea* displayed a markedly higher relative growth (59 and 38 %, respectively). The growth of *L. perenne* 'Clerpin' (11 %), *A. elatius* (20 %), *F. rubra* (25 %) and *H. sulcatum* (35 %) was intermediate. The $\{Al^{3+}\}$ 43 μM level (Fig. 1B) allowed two groups of species to be distinguished: sensitive (RRE below 3 %: *C. cristatus*, *D. glomerata* and *L. perenne* cv. Clerpin) and resistant species (RRE ranging from 11 to 38 %: *F. rubra*, *S. decumbens*, *M. caerulea* and *H. sulcatum*). *Arrhenatherum elatius* (11 %) was intermediate.

When the response of RRE to $\{Al^{3+}\}$ was fitted to a Weibull function, the resistance of *M. caerulea* and *S. decumbens* was corroborated with calculated activities $\{Al^{3+}\}_{50}$ of 13 and 26 μM (Table 2). Higher data variability and/or a lower $\{Al^{3+}\}_{50}$ value resulted in non-significant $\{Al^{3+}\}_{50}$ values for *A. elatius*, *F. rubra* and *H. sulcatum*. Most of the resistant species (*H. sulcatum*, *S. decumbens* and *M. caerulea* and the red fescue ecotype selected as a control) displayed traits that are characteristic of a conservation strategy (low SLA, high LDMC, low RGR; Table 2). *Arrhenatherum elatius*, with a high SLA, a median LDMC value and a relatively low RGR, presented traits of an intermediate strategy between capture and conservation.

Relationships with plant functional traits

For all the species whose acidity index was expressed numerically, the resistance to Al was correlated negatively to the acidity index (H index; Table 3). The grasses that are most resistant to Al were referenced as resistant to acidity (H index 2–6) or indifferent to acidity (H index x). Conversely, the species with an H index of 7–8 are weakly resistant to Al. However, large discrepancies are observed for the species listed as indifferent to acidity (H index x) which include species that are sensitive to Al (*C. cristatus* and *P. pratensis*), as well as species that are moderately sensitive (*D. glomerata* and *P. pratense*) or resistant (*H. lanatus* and *M. caerulea*). The negative correlation between N index and Al resistance reflects the low N index (2–3) of the resistant species (*D. flexuosa*, *M. caerulea* and *S. decumbens*) and the high N index (6–7) of some sensitive grasses (*D. glomerata*, *L. perenne*, *P. pratense* and *P. pratensis*). However, the relationship is rather loose: for example, the Al-susceptible species *C. cristatus* and *B. inermis* had the same moderate N index (4–5) as the resistant species *H. lanatus* and *A. capillaris*.

TABLE 1. Comparison of the abundance of grass species in three acidic stands (1, 2 and 3) with differing exchangeable Al rates and in a control plot with no Al toxicity (stand 4)

	Stand 1 (\pm s.e.; n = 3)	Stand 2 (\pm s.e.; n = 2)	Stand 3 (\pm s.e.; n = 2)	Stand 4 (\pm s.e.; n = 4)
Soil characteristics				
pH	5.0 (0.2)	5.2 (0.1)	5.2 (0.1)	6.0 (0.1)
Al [cmol (+) kg ⁻¹ soil]	4.5* (0.2)	0.7 (0.5)	0.2 (0.2)	0.1* (0.1)
Grass nutrition				
Nitrogen index (%)	39.4 (0.3)	58.0 (4.0)	58.0 (1.4)	64.1 [†] (1.6)
Species abundance (%)				
<i>Festuca rubra</i>	19.8 (11.8)	30.8 (9.2)	33.7 (6.9)	3.0 (3.0)
<i>Agrostis capillaris</i>	5.8 (2.9)	31.0 (0.6)	45.8 (15.9)	6.9 (1.5)
<i>Helictotrichon sulcatum</i>	17.9 (4.5)	16.3 (15.8)	10.5 (1.7)	0.0 –
<i>Molinia caerulea</i>	15.1 (2.9)	0.0 –	0.0 –	0.0 –
<i>Sieglingia decumbens</i>	17.2 (1.3)	0.0 –	0.0 –	0.0 –
<i>Brachypodium pinnatum</i>	24.3 (13.1)	0.0 –	0.0 –	0.0 –
<i>Anthoxanthum odoratum</i>	0.0 –	9.6 (2.4)	0.0 –	4.7 (1.8)
<i>Arrhenatherum elatius</i>	0.0 –	0.0 –	0.0 –	7.1 (2.4)
<i>Holcus lanatus</i>	0.0 –	12.3 (8.3)	8.5 (5.9)	19.9 (10.2)
<i>Lolium perenne</i>	0.0 –	0.0 –	0.0 –	21.4 (3.0)
<i>Dactylis glomerata</i>	0.0 –	0.0 –	1.4 (1.4)	37.0 (5.9)

*n = 2.

[†]n = 3.

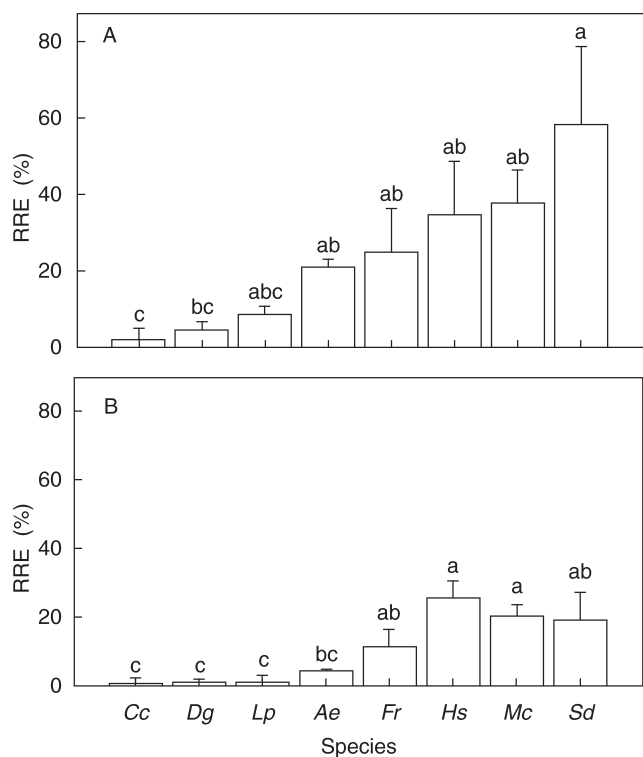


FIG. 1. Relative root elongation at 22 µM {Al³⁺} (A) and 43 µM {Al³⁺} (B). Relative root elongations of species followed by the same letter are not significantly different at $P = 0.05$. For species' abbreviations, see Table 3 footnote. Bars indicate the s.e.

As the traits are correlated between themselves (SLA – LDMC, $r = -0.77$, $P = 0.01$; SLA – LT, $r = -0.83$, $P = 0.001$; LDMC – LT = 0.33, non-significant) and the nitrogen index is correlated to SLA (0.63, $P = 0.05$) and LDMC (-0.74 , $P = 0.01$), a PCA was carried out on a selection of less redundant variables: {Al³⁺}₅₀, LDMC, LT and RGR (Fig. 2). Axis 1 separates capture strategy species (right) and conservation strategy species (left). Axis 2 opposes Al-susceptible (bottom) to Al-resistant (top) species. This distribution pattern underlines the independence between

TABLE 2. Resistance to Al and traits values of the species presenting some Al resistance in Experiment 1 (s.e. in brackets)

Species	{Al ³⁺ } ₅₀ (µM)	RGR (mg · g ⁻¹ d ⁻¹)	SLA (m ² kg ⁻¹)	LDMC (g kg ⁻¹)
<i>F. rubra</i>	8.9 (5.7) ns	203 (3)	11 (1)	354 (10)
<i>A. elatius</i>	9.4 (5.9) ns	203 (4)	30 (2) [†]	246 (6) [†]
<i>M. caerulea</i>	13.0 (4.3)**	140 (11)	19 (1)	310 (4)
<i>H. sulcatum</i>	13.9 (8.7) ns	211 (7)	18 (1)	302 (6)
<i>S. decumbens</i>	25.5 (6.0)***	196 (7)	15 (1)	351 (8)

{Al³⁺}₅₀ and RGR were measured in a culture chamber, and foliar traits were measured in the field. {Al³⁺}₅₀ values significantly superior to zero at * $P = 0.05$, ** $P = 0.01$, *** $P = 0.001$; ns: not significant.

[†] P. Ansqer, INRA, Toulouse, France, pers. commun.

Al resistance (represented on axis 2) and functional traits (represented on axis 1).

The datum points corresponding to the grass species constitute five main groups. Species with low RGR and high LDMC (group 1) are on the whole more resistant than species with high RGR and low LDMC (group 4). However, *A. capillaris* with capture strategy traits is resistant to Al (group 2), whereas *B. inermis*, *C. cristatus*, *P. pratensis* and *F. arundinacea* with traits of intermediate strategy between capture and conservation (group 5) are sensitive to Al. *Helictotrichon sulcatum* and *A. elatius* (group 3) are intermediate between group 1 and group 4.

DISCUSSION

Ranking of Al resistance

The slow growth of *C. cristatus*, *L. perenne* and *D. glomerata* in the presence of 22 µM Al is in agreement with their high sensitivity ({Al³⁺}₅₀ 0–6 µM; Wheeler et al., 1992). The resistance of *M. caerulea* is consistent with its occurrence on acid soils with high Al/Ca ratios (Houdijk et al., 1993). The Al resistance of *S. decumbens* appears to be in agreement with observations of Antuna et al. (1980) in acid soils and with the resistance of the related species *Danthonia linkii* (Crawford and Wilkens, 1997). To our knowledge, there are no data on the Al resistance of *A. elatius*, *H. sulcatum* and the {Al³⁺}₅₀ value of these species in the literature. The value obtained for the *F. rubra* ecotype (9 µM) used as a control is lower though compatible with the range (10–20 µM {Al³⁺}₅₀) previously defined by Wheeler et al. (1992).

Stand 1 displayed an exchangeable Al rate (4.5 cmol (+) kg⁻¹; Table 1) which was intermediate between the values associated with low (2.8) and high (7.2) Al toxicity by Curtin and Smillie (1986). In spite of the acidity (pH 5.2), the much lower exchangeable Al values (0.7 and 0.2, respectively) of stands 2 and 3 suggest a very low level or the absence of Al toxicity in these stands. The absence of the species *B. pinnatum*, *M. caerulea* and *S. decumbens* in stands 2, 3 and 4 may be related to their low ability to withstand increased defoliation (disturbance indices of *M. caerulea* and *B. pinnatum* 0.10, *S. decumbens* 0.24; Grime et al., 1988). The increase in fertility from stand 1 (NNI 39%, Table 1) to stands 2 and 3 (NNI 58%) and to stand 4 (NNI 64%) may also give a competitive advantage to the species with the higher relative growth rate, as shown for *H. lanatus* and *L. perenne* by Mountford et al. (1993). Thus, increasing disturbance and competition may contribute to the exclusion of the relatively slower growing species *B. pinnatum*, *M. caerulea* and *S. decumbens* in stands 2, 3 and 4, and to the reduced abundances of *A. capillaris*, *F. rubra* and *H. sulcatum* in stand 4.

Taking into account the Al resistance values of *H. sulcatum*, *M. caerulea* and *S. decumbens* (Table 2) and those of *A. capillaris*, *F. rubra* and *H. lanatus* (Wheeler et al., 1992), the resistant grass species in the stands with Al toxicity hazard and in the control, represented up to 90 and 30% of the observed grasses, respectively (Table 1).

TABLE 3. Values for the resistance to Al ($\{Al^{3+}\}_{50}$, μM), specific leaf area (SLA, $m^2 kg^{-1}$), leaf dry matter content (LDMC, $g kg^{-1}$), leaf thickness (LT, $10^{-6} m$), relative growth rate (RGR, $g kg^{-1} d^{-1}$) and Ellenberg's acidity (H index) and nitrogen (N index) indices for 15 grass species

Species*	$\{Al^{3+}\}_{50}$	SLA	LDMC	LT	RGR	H index	N index
Cc	0.5 ¹	17 ⁴	264 ⁴	223	176 ¹⁰	x	4
Po	0.5 ¹	25 ⁶	220 ⁶	182	179 ¹¹	x	6
Bi	1.4 ²	15 ⁶	310 ⁶	215	231 ⁹	8	5
Pp	2.3 ²	31 ⁵	245 ⁵	132	227 ⁹	x	7
Lp	3.0 ¹	28 ⁵	196 ⁵	182	245 ⁸	7	7
Fa	3.5 ¹	17 ⁴	210 ⁴	280	198 ¹²	7	5
Dg	6.0 ¹	26 ⁵	225 ⁵	171	255 ⁹	x	6
Ae	9.4	30	246	136	203	7	7
Mc	13.0	19	310	170	140	x	2
Hs	13.9	18	302	184	211	–	–
Fr	15.0 ¹	11	354	257	203	6	x
Hl	15.0 ¹	33 ⁵	198 ⁵	153	278 ⁸	x	5
Df	20.4 ³	13 ⁷	316 ⁷	243	173 ⁸	2	3
Sd	25.5	15	351	190	196	3	2
Ac	30.0 ¹	32 ⁵	242 ⁵	129	244 ⁸	4	4
n	15	15	15	15	15	8	13
r $\{Al\}_{50}$	1.00	–0.05	0.42	–0.17	0.03	–0.86	–0.61
Significance	–	ns	ns	ns	ns	P = 0.01	P = 0.05

* Species codes: Ac, *Agrostis capillaris*; Ae, *Arrhenatherum elatius*; Bi, *Bromus inermis*; Cc, *Cynosurus cristatus*; Dg, *Dactylis glomerata*; Df, *Deschampsia flexuosa*; Fa, *Festuca arundinacea*; Fr, *Festuca rubra*; Hl, *Holcus lanatus*; Hs, *Helictotrichon sulcatum*; Lp, *Lolium perenne*; Mc, *Molinia caerulea*; Po, *Poa pratensis*; Pp, *Phleum pratense*; Sd, *Sieglingia decumbens*.

Superscripts refer to the following publications: ¹Wheeler *et al.* (1992); ²Wheeler (1995); ³recalculated from Rorison (1985); ⁴R. Al Haj Khaled, INRA, Toulouse, France, pers. commun.; ⁵Al Haj Khaled *et al.* (2005); ⁶Meziane and Shipley (1999); ⁷S. Gaucherand, CEMAGREF, Grenoble, France, pers. commun.; ⁸Hunt and Cornelissen (1997); ⁹Taub (2002); ¹⁰Poorter and Remkes (1990); ¹¹Ryser and Wahl (2001); ¹²Sugiyama (2005). Acidity and nitrogen indices: x, species classified as indifferent; –, species not classified; Ellenberg *et al.* (1992).

Al resistance variability at the ecotype level limits the possibility of diagnosis of Al toxicity which would be based upon the presence of a particular species (e.g. *H. lanatus*; Kidd and Proctor, 2001). However, an analysis based upon the presence or absence of several species might be more reliable. The assessment of the Al toxicity hazard through the abundance of the Al-resistant grasses had the advantage of taking into account simultaneously 5–6 grass species that represented the majority of the grass species in acidic stands (Table 1). The knowledge of the Al resistance of the two other grasses investigated (*Anthoxanthum odoratum*, estimated optimum pH 4.0–6.0; *Brachypodium pinnatum*, estimated optimum pH 5.5–6.5; Antuna *et al.*, 1980; Grime *et al.*, 1988) might improve this approach.

Relationships with plant functional traits

For the species with known RGRs, the trait values obtained here are in good agreement with those reported previously (*A. elatius* 214, *F. rubra* 196; Hunt and Cornelissen, 1997). Likewise, the proposed SLA values are close to those expected for observations on infertile soils (*A. elatius* 35–37, *F. rubra* 13–15, *M. caerulea* 21–24; Poorter and de Jong, 1999). The LDMC value of *A. elatius* is in general agreement with that reported previously (218; Al Haj Khaled *et al.*, 2005).

As the species listed as indifferent to acidity (H index x) by Ellenberg were not included in the calculation of the correlation, the agreement between Al resistance and the acidity index is overestimated. In fact, the species

C. cristatus, *D. glomerata*, *P. pratense* and *P. pratensis*, listed as indifferent to acidity, appear actually to be susceptible to Al. The discrepancy may be accounted for in terms of occurrence of these species in slightly acidic grasslands with low Al activity, as a result of soil parent rock and/or organic matter content. From these discrepancies between

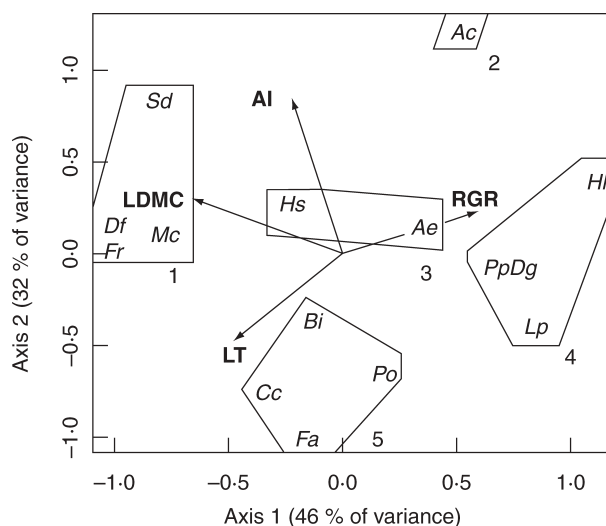


FIG. 2. First plane of the principal component analysis of traits (RGR, LDMC and LT) and Al resistance measured by the $\{Al^{3+}\}_{50}$ value for 15 grasses. Units on the axes refer to the correlation between the variables and the axes. Arbitrary units are used for the species points. For species' abbreviations, see Table 3 footnote.

H index and Al resistance and the loose negative correlation between resistance to Al and Ellenberg's nitrogen index, it can be concluded that Ellenberg's indices are only moderately useful to evaluate Al resistance of grasses.

The range of variation of traits in plants is much wider than that observed on these C₃ grasses (SLA 2–50, LDMC 100–600, LT 200–1200, Shipley *et al.*, 2005; RGR 100–380, Poorter and Van der Werf, 1998). As a result, the independence between resistance to Al and traits may be due to the limited range of trait variation taken into account in considering C₃ grasses only. The consideration of other plant groups such as C₄ grasses, herbs, shrubs and trees might improve the relationships.

The absence of relationships between Al resistance and traits can also be accounted for in terms of data classification into several groups of species with differing Al resistance and traits (Fig. 2). Group 1 species include S-type species (*D. flexuosa*, *M. caerulea* and *S. decumbens*) and the polymorphic species *F. rubra* (C-S-R, S, C or S-C) which all exhibit a resistance to Al. Although the subject is little documented in the literature, a physiological mechanism allowing these S-type species simultaneously to adapt to infertile soils and to become resistant to Al can be considered. As a matter of fact, in *D. flexuosa*, the exudation of dicarboxylic anions such as malate is involved in a higher efficiency of P uptake in infertile soils (Tyler and Ström, 1995) and in a higher resistance to Al toxicity in acid soils (Schöttelndreier *et al.*, 2001). The distribution of *F. rubra* and *M. caerulea* in acid soils and in calcareous soils (Grime *et al.*, 1988) also suggests the involvement of an exudation capacity for di- or tri-carboxylic anions (Tyler and Ström, 1995). However, as the exudates of group 4 grasses and S-type grasses remain insufficiently documented, the assumption cannot be generalized.

Species groups 2, 4 and 5 correspond to C-S-R type species with regard to their ecological strategies (*A. capillaris*, *C. cristatus*, *D. glomerata*, *H. lanatus*, *L. perenne*, *P. pratense* and *P. pratensis*; Grime *et al.*, 1988). Their Al resistance is different between groups and in some cases within the same group (*H. lanatus* vs. *L. perenne*, group 4). The comparison of the traits of the Al-resistant species of groups 2 and 4 (*A. capillaris* and *H. lanatus*) with those of the Al-resistant species (*D. flexuosa*, *F. rubra*, *M. caerulea* and *S. decumbens*) of group 1 suggests that low RGR, low SLA and high LDMC are not causally related to Al resistance. It may be in accordance with the implication of a limited number of genes in Al resistance, as suggested by Delhaize *et al.* (2004), with little or no relationship to global traits.

The usually low fertility (N, P) of acid soils may account for conservation strategy traits in Al-resistant species of group 1 which exhibit the highest LDMC and the lowest RGR values. These species occur in reduced abundance in more fertile and more disturbed stands. Conversely, the Al-resistant species *H. lanatus* exhibits capture strategy traits (with the highest SLA and RGR values) and presents a limited abundance in the infertile soils. The limited abundances of *A. capillaris* on stands 1 and 4 may deserve further attention. In conclusion, Al resistance alone

cannot explain the relative abundance of Al-resistant grasses in infertile or fertile acid soils. As Al-resistant species have to compete with other grasses, their abundance depends, in the first place, on their adaptation to the fertility level and to the disturbance of the habitat and, only in the second place, on their Al resistance.

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