

Photosynthetic Pigment Concentrations, Gas Exchange and Vegetative Growth for Selected Monocots and Dicots Treated with Two Contrasting Coal Fly Ashes

Isa A. M. Yunusa,* Margaret D. Burchett, V. Manoharan, D. Lionel DeSilva, Derek Eamus, and C. Greg Skilbeck
University of Technology, Sydney

There is uncertainty as to the rates of coal fly ash needed for optimum physiological processes and growth. In the current study we tested the hypothesis that photosynthetic pigment concentrations and CO₂ assimilation (*A*) are more sensitive than dry weights in plants grown on media amended with coal fly ash. We applied the Terrestrial Plant Growth Test (Guideline 208) protocols of the Organization for Economic Cooperation and Development (OECD) to monocots [barley (*Hordeum vulgare*) and ryegrass (*Secale cereale*)] and dicots [canola (*Brassica napus*), radish (*Raphanus sativus*), field peas (*Pisum sativum*), and lucerne (*Medicago sativa*)] on media amended with fly ashes derived from semi-bituminous (gray ash) or lignite (red ash) coals at rates of 0, 2.5, 5.0, 10, or 20 Mg ha⁻¹. The red ash had higher elemental concentrations and salinity than the gray ash. Fly ash addition had no significant effect on germination by any of the six species. At moderate rates (≤ 10 Mg ha⁻¹) both ashes increased (*p* < 0.05) growth rates and concentrations of chlorophylls *a* and *b*, but reduced carotenoid concentrations. Addition of either ash increased *A* in radish and transpiration in barley. Growth rates and final dry weights were reduced for all of the six test species when addition rates exceeded 10 Mg ha⁻¹ for gray ash and 5 Mg ha⁻¹ for red ash. We concluded that plant dry weights, rather than pigment concentrations and/or instantaneous rates of photosynthesis, are more consistent for assessing subsequent growth in plants supplied with fly ash.

MANAGEMENT of fly ash is a major environmental and economic concern for the coal-fired power generators all over the world. Substantial amounts of this by-product end up in landfills every year, despite having characteristics that could improve soil conditions for plant growth (Yunusa et al., 2006). This is due in part to conflicting results from earlier studies that evaluated agronomic benefits of fly ash (Adriano et al., 1980; Aitken and Bell, 1985). Consequently there has been a lack of definitive recommendations in terms of application rates needed for achieving optimum plant yield. Inconsistent results from previous studies could be due to differences in plant species and specific fly ashes used. Differences among plant species in their growth response to media amended with fly ash are often associated with the plants' tolerance of trace elements (Adriano et al., 1980; Aitken and Bell, 1985), which may manifest in the responses of their photosynthetic pigment concentrations. Chlorophylls are known to be the most sensitive pigments to heavy metals (Abdel-Basset et al., 1995; Fargašová, 1998), because trace elements such as Cu, Mn, Pb, and Zn readily displace Mg from the chlorophyll molecule (Küpper et al., 1998).

Plant species differ in the way they adapt to elemental stress. Ralph and Burchett (1998) showed that in the presence of heavy metals certain tolerant plant species increase their chlorophyll content both in absolute terms and relative to carotenoid contents, and/or alter chlorophyll *a*/chlorophyll *b* ratios, while the less tolerant species do the reverse. Increases in carotenoid levels are a common response to elemental stress because it is a precursor of abscisic acid (ABA) synthesis and also assists in quenching excess harmful excitation energy absorbed by the chlorophylls (Young, 1991; Botella-Pavía et al., 2004). Mishra et al. (2007) found no significant change in the concentrations of carotenoid and chlorophyll *a* in the leaves of rice when supplied with up to 15 Mg ha⁻¹ of a mildly alkaline fly ash. However, they observed increases in the concentrations of chlorophyll *b* with ash addition. A later glasshouse study by Yunusa et al. (2008) found significant reductions in relative chlorophyll concentrations in canola (*Brassica napus*) only when an alkaline fly ash was added to soil at rates exceeding 125 Mg ha⁻¹. Extreme elemental stress, however, generally results in a complete breakdown of any adaptive mechanisms, including a general decline in all photosynthetic pigments (Ralph and Burchett, 1998; Abdel-Kader and Saleh, 2002).

Properties of the soil (textural, chemical, and physical) may also interact with those of the fly ash to modify the root environment,

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*Corresponding author (isa.yunusa@uts.edu.au).

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677 S. Segoe Rd., Madison, WI 53711 USA

Plant Functional Biology & Climate Change Cluster, Dep. of Environmental Sciences, Univ. of Technology, Sydney, PO Box 123, Broadway 2007, Australia.

especially in terms of pH and salinity, to influence distribution and availability of nutrients in the ash-treated soils. To minimize artifact effects due to variable environmental conditions in assessing effects of chemicals on plants, the Organization for Economic Cooperation and Development (OECD) developed a set of guidelines (Guideline 208) to standardize the procedure (OECD, 2003). These guidelines provide internationally consistent protocols for the pre-market testing of possible phytotoxicities of soil-applied agricultural chemicals within the first 4 wk of growth. They allow for a quick assessment of plant germination and growth responses to potentially toxic substances at very early stages of growth rather than waiting until maturity. These guidelines have been extensively used for assessing agricultural and non-agricultural native species to elemental stress (Mitchell et al., 1988; Boutin and Rogers, 2000).

To clarify any differences among species in their response to fly ash amendment of growth media, we applied the OECD guidelines to test a selection of six monocotyledonous and dicotyledonous crop and pasture species for their performance on media treated with two contrasting fly ashes under the same set of conditions. We determined photosynthetic pigments, the rate of CO₂ assimilation (*A*), and dry weight to assess tolerance or sensitivity to fly ash. Our aim was to test the hypothesis that responses in photosynthetic pigments could be a more valid test than plant dry weight for assessing agronomic benefits of variable dosages of two types of fly ash.

Materials and Methods

Plant Species and Fly Ashes Used

This study was undertaken in a glasshouse at the University of Technology, Sydney, between April and June 2005. Plants were grown on a loamy soil with a pH of 6.5 and an electrical conductivity (EC) of 0.44 dS m⁻¹, or on a commercial potting mix with a pH of 6.5 and EC of 0.16 dS m⁻¹. The growth media consisted of either soil or potting mix that was treated with either of two chemically contrasting fly ashes (see below). These growth media were used to fill 2-L PVC pots (15 cm internal diameter at the top, 12 cm internal diameter at the base, and 15 cm depth) that had perforated bottoms. Where the soil was used as the growth media, it was dried, ground, and sieved to pass through a 2-mm mesh as given in Guideline 208 (OECD, 2003). The guideline also stipulate a minimum of three plant species, including local ones. The current study used four dicotyledons: canola (*Brassica napus*), radish (*Raphanus sativus*), field peas (*Pisum sativum*), and lucerne (*Medicago sativa*); and two monocotyledons: barley (*Hordeum vulgare*) and ryegrass (*Secale cereale*). These six species represented three families of Brassicaceae (canola and radish), Fabaceae (peas and lucerne), and Poaceae (barley and rye grass). The dicot species were selected on the basis of their being widely grown crops in temperate and subtropical climates, and in Australia they are common in districts where coal-fired power stations are located, such as the Newcastle-Sydney-Wollongong axis of New South Wales, Australia. The monocots were chosen to represent major broad-acre crops and pasture species. Four species were grown on the loam soil, with only ryegrass and radish being grown on a com-

mercial potting mix (50:50 sand/peat mixture) (Richgro Planting Mix, Bunnings Garden Supplies, Australia). The pots were treated with one or other of the two ashes, which were collected fresh from the power stations without prior weathering.

The two fly ashes were sourced from power stations that burnt semi-bituminous (black) coal located just north of Sydney, Australia, or lignite (brown) coal in southeast Victoria, Australia. The ash from the semi-bituminous coal was gray in color, while that from the brown coal was reddish; we therefore refer to them in this paper as *gray ash* and *red ash*, respectively. Both ashes were strongly alkaline and the red ash was highly saline with an EC (1:5 water) of 19.1 dS m⁻¹ compared to 0.51 for the gray ash (Table 1). The high salinity (1:5 water) of the red ash was mainly due to its high soluble salt content, while the red color reflected its high concentration of Fe₂O₃. The concentrations of trace metals and cations, including important plant nutrients Ca and Mg, were generally higher in the red ash than in the gray ash. Other elements, such as Cd, Mo, and Pb were up to three times higher in the red ash than in the gray ash (Table 1).

The ashes were applied to the pots at rates equivalent to 0, 2.5, 5.0, 10.0, or 25.0 Mg ha⁻¹, which were 0, 6.9, 13.8, 27.6, or 55.2 g pot⁻¹, respectively. Each dosage of fly ash was separately mixed with either the soil or the potting mix before being dispensed into pre-assigned pots to within 2 cm of the tops. The amount of soil dispensed into each pot was 1.7 kg, and 1.0 kg for the potting mix. Therefore the ash constituted 0.0, 0.41, 0.81, 1.62, and 3.25% by weight of soil, respectively, for the five treatment rates; the corresponding percentages with the potting mix were 0.0, 0.69, 1.38, 2.76, and 5.52%. Our aim was to confine treatments to rates that would be within the range of agronomic practice when applying soil amendments. Each of the plant species was therefore subjected to 10 treatments consisting of two types of fly ash at five application rates, with each treatment replicated four times. The pots were treated with a solution of an anti-fungal agent (polysulphide sulfur) before dispensing the soil or potting mix and planting of seeds. The pots were each planted with ten seeds of the assigned plant species on 15 Apr. 2005. They were then arranged randomly on benches, and their positions shuffled every 2 wk. Watering was by automatic spray irrigation lasting 1 min per day at the start, but was later extended to 2 min daily, to account for growth stage. The plants were supplied with 0.2 L of liquid fertilizer (*Thrive*, 8:3:8 NPK; Yates Garden Supplies, Australia) at 20 d after sowing. The first 3 wk of the trial experienced mild conditions when daily temperatures ranged from 15 to 28°C, while relative humidity (RH) averaged 75%. The second half of the trial period was cooler with a daily temperature range of 12 to 26°C and average RH of 90%. The plants therefore predominantly grew in a humid and relatively warm environment.

Measurements

Germination Percentage

The number of emergent seedlings was counted every 1 or 2 d from 3 d after sowing (DAS), and germination was considered to be completed on any given pot once there was no further increase in

Table 1. Selected chemical and physical properties for the two fly ashes and the soil used in the study.

Properties	Grey ash	Red ash	Soil†
pH	10.8	9.0	6.5
Electrical conductivity (dS m ⁻¹)	0.54	19.1	0.8
Specific surface area (m ² g ⁻¹)‡	2.09	2.76	NA
Total nutrient contents (%)			
C	0.70	0.10	NA
Ca	0.26	9.28	0.17
K	0.13	0.18	0.13
Mg	0.05	8.94	0.03
Na	0.02	1.75	0.01
P	0.02	0.02	0.15
Selected metal concentrations (mg kg ⁻¹)			
Al (x10 ³)	14.4	21.1	NA
B	66.0	127.0	11.5
B (hot water extractable)	6.7	6.8	0.46
Ca (soluble)	766	7647	52.5
Cd	0.3	0.1	< 0.1
Co	6.0	42.0	< 0.1
Cu	18.0	24.0	10.3
Fe	7616	343,840	NA
Mo	9.0	5.0	0.5
Mn	453	4713	NA
Pb	18.0	5.0	11.9
Si	794	1124	NA
Zn	56.0	117.0	22.5

† NA, data not available.

‡ From Aitken et al. (1984).

the number of seedlings. For all species germination was completed by 12 DAS, when no new plants emerged. The number of seedlings was expressed as a percentage of the seeds planted to determine germination percentage. The plants were thinned to four plants per pot at 28 DAS (13 May), the other seedlings being carefully removed to recover much of their roots intact. These removed plants were used for determining growth, root length, and pigment concentration, as described below. The remaining four plants in each pot were left for the second phase of the experiment.

Shoot Growth and Root Length

To determine plant dry weight at 28 DAS, two plants were randomly chosen from among those taken out of each pot. The length of the tap root in the dicots and the longest root of the fibrous root system in grasses were measured with a ruler from the root tip to the base of the shoot. The numbers of fully expanded leaves were recorded for each plant, and the roots cut off at the base of the shoot and the latter dried in an oven at 60°C over 48 h before being weighed to obtain dry weight. This procedure was repeated at 76 DAS (29 June) using two of the four plants remaining in each pot. These two plants were carefully removed, dried, and weighed.

Plant growth rate (PGR) and relative growth rate (RGR) were calculated as given by Hunt (1990), but implemented using heat units expressed as growing degree days (°Cd) (Yunusa and Gworgwor, 1991) to facilitate comparison with field trials:

$$PGR = \Delta W_d / \Delta H \quad [1]$$

$$RGR = [\Delta W_d / \Delta H] / W_{di} \quad [2]$$

in which ΔW_d is the change in dry weight per plant, W_{di} is the initial plant dry weight (i.e., at 28 DAS), and H is the temperature index taken as sums of mean daily temperatures between the two dates. This is because base temperature in the southern Australian environment is considered to be 0°C (Karimi and Siddique, 1991). The RGR is often described as the compound interest in growth (Hunt, 1990) and accounts for preexisting weight in assessing subsequent increments. This therefore enabled us take into account any earlier influence of fly ash on plant weight between emergence and the first sampling at 28 DAS.

Concentrations of Photosynthetic Pigments in Leaves

These pigments were measured on two plants that were removed from each pot at 28 DAS, and again at 76 DAS using the other two plants remaining at this time. Leaf samples were taken from the plants and then frozen at -80°C until analysis several weeks later. To undertake measurements, the leaf samples were patted dry with blotting paper and then 0.1-g subsamples were placed in 0.015 L amber glass screw cap bottles containing 0.01 L of *N*-dimethylformamide (DMF) and refrigerated at 4°C (Macfarlane and Burchett, 2001) for 7 d. These were used to measure absorbance at 480, 647, and 664 nm using a spectrophotometer with a spectral resolution of 1.0 nm (LKB Ultraspec II UV/VIS, model 4050, England). Scattering due to turbidity of the mixture was corrected by subtracting absorbance at 750 nm from those at lower wavelengths. Concentrations of the three main pigments (chlorophyll *a*, chlorophyll *b*, and carotenoids) were calculated using the extinction coefficient equations (Wellburn, 1994).

Carbon Dioxide Assimilation Rates

The rate of CO₂ assimilation (*A*) was measured along with those of transpiration and stomatal conductance on barley and canola at 65 DAS (18 June) on the uppermost fully expanded leaves using a portable photosynthesis system (Walz Portable Photosynthesis System, HCM-1000, Heinz Walz GmbH, Germany). All the measurements were made under constant artificial illumination (350 μmol m⁻² s⁻¹ at leaf level) from a 400 W metal halide lamp (Lowbay Luminaire, Blackwoods, Smithfield, Australia).

Data Analysis

All data were subjected to two-way ANOVA (General Linear Model) using statistical software (Minitab V13.1, Minitab Inc., Sydney, Australia) to test for the effects of ash type, ash rate, and for their interactions. Means and standard errors (SE) were calculated for each treatment and for each species. Tukey's Post Hoc test was used to identify significant differences between means. Normality of data was tested using the Kolmogorov-Smirnov test, and homogeneity of data was assessed with Bartlett's test (for normally distributed data) and Levene's test (for non-normally distributed data). Differences were considered significant where $p < 0.05$.

Results

Germination of Plants and Number of Leaves Produced

Neither of the fly ashes affected germination rates determined at 12 DAS; an average of 82% emergence was achieved for all species irrespective of treatment. The number of plants

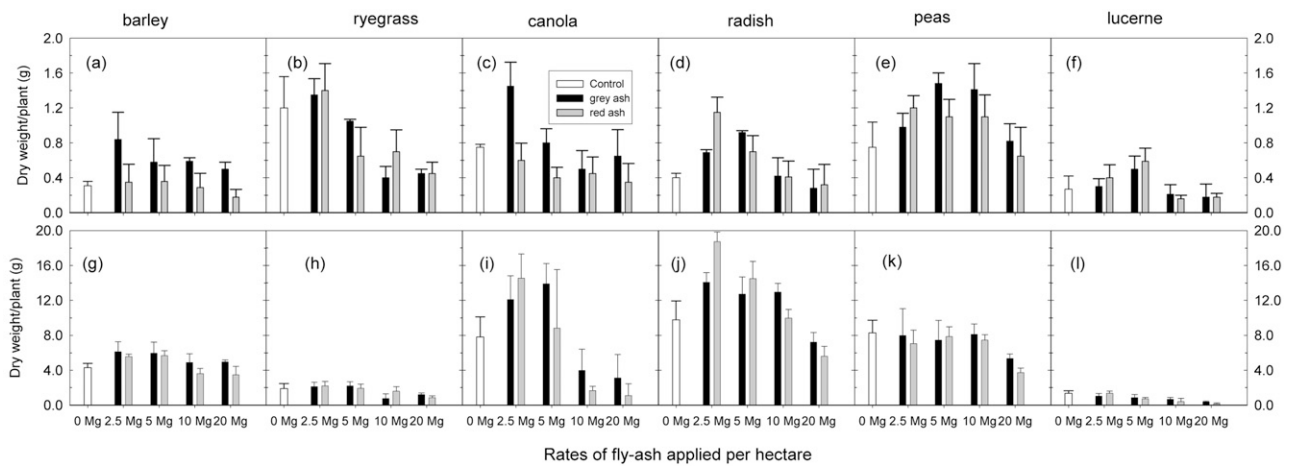


Fig. 1. Means (\pm standard errors) for plant dry weight produced at either 28 (a–f) or 76 (g–l) days after emergence by the various plant species grown on media treated with variable amounts of gray or red fly ash applied to growth media before planting. Open bars are means for the respective plant species on media not supplied with fly ash.

that germinated and survived to 28 DAS, and the number of leaves counted per plant at this time, was only reduced in canola supplied with red ash; these plants had 70% survival and 4.5 leaves per plant compared with 78% survival and 5.3 leaves per plant for plants in the control treatment (data not presented). Application of the red fly ash increased the length of the main roots measured at 28 DAS in barley from 7.9 cm for the control plants to 13.2 cm for ash-treated plants. A similar response was observed for peas in which ash application increased root length by 37% compared with the control plants (5.5 cm). All the responses in survival, leaf number, and root length to fly ash were achieved at the lowest ash application rate of 2.5 Mg ha⁻¹; higher ash rates produced no further effect.

Dry Matter Accumulation

Plant weight produced at 28 DAS increased for all the species except ryegrass, when ash was applied at up to 5 Mg ha⁻¹ (Fig. 1 a–f). Plant weight for all species was either increased or unaffected by ash type applied at up to 5 Mg ha⁻¹ of fly ash addition. Above this rate there were significant reductions in weight for most species. Ryegrass showed no positive response to fly ash at any rate. At final harvest (76 DAS) plant weights showed clearer responses to the ash treatments (Fig. 1 g–l). Barley, canola, and radish showed significant increases in dry weight with 2.5 and 5.0 Mg ha⁻¹ treatments. At ash doses of 10 and 20 Mg ha⁻¹, barley was unaffected by gray ash but inhibited by the red ash, while ryegrass and canola were significantly inhibited by either ash. In radish, inhibition of growth was greatest in the highest rates of ash additions. However, dry matter accumulation in peas was inhibited only at 20 Mg ha⁻¹. Lucerne, in contrast, showed a linear decline in plant dry matter with increasing rates of either type of fly ash.

Growth Rate Estimates

Growth rates based on changes in shoot dry weight between 28 and 76 DAS were generally low. Detailed data on comparative growth rates are presented in Fig. 2 for barley, radish, and lucerne, as representatives of their respective families. Applica-

tion of either ash at up to 5 Mg ha⁻¹ increased PGR for barley and radish, but PGR was significantly reduced with either ash applied at 20 Mg ha⁻¹. In lucerne, PGR was largely insensitive to application of either ash. Application of fly ash had no significant effect on RGR for radish, but RGR was reduced for barley and lucerne.

Concentrations of Photosynthetic Pigments

To rationalize data presentation, detailed pigment concentration in leaf tissue are given here for the three representative species of the three families and only for the second measurement made at 76 DAS (Fig. 3), since these responses were mostly similar to those observed at 28 DAS.

The fly ashes generally increased concentrations of the two chlorophylls in all the three species; this response was more dramatic in radish where the pigments were increased by as much as 50%, particularly with the red ash. The red ash applied at up to 5 Mg ha⁻¹ increased the chlorophylls by up to 40% in lucerne; it also produced a similar response in the barley but to a smaller extent. Although carotenoid concentration was not significantly affected by application of fly ash in either barley or lucerne, it was reduced in the radish with application of either fly ash. Data in Fig. 3 show that the chlorophyll *a/b* ratios for plants grown with fly ash were within 5% of those for plants grown without fly ash. However, carotenoid/total pigment ratios for radish grown with ash were half the values for those plants grown without ash. Similar patterns were observed in the pigment data collected at 28 DAS when the chlorophyll *a/b* ratio changed by <5% among plants irrespective of fly ash treatment, but carotenoid/total pigments ratios radish were reduced to 86% when grown with gray fly ash, and to 25% with red ash, compared with radish grown without fly ash (data not presented). At this earlier measurement, carotenoid/total pigment concentration for lucerne was 82% for plants grown with gray ash and 25% for those grown with red ash, relative to those grown without ash. Ratio of carotenoid/total pigment concentration was largely unaffected in barley irrespective of fly ash application.

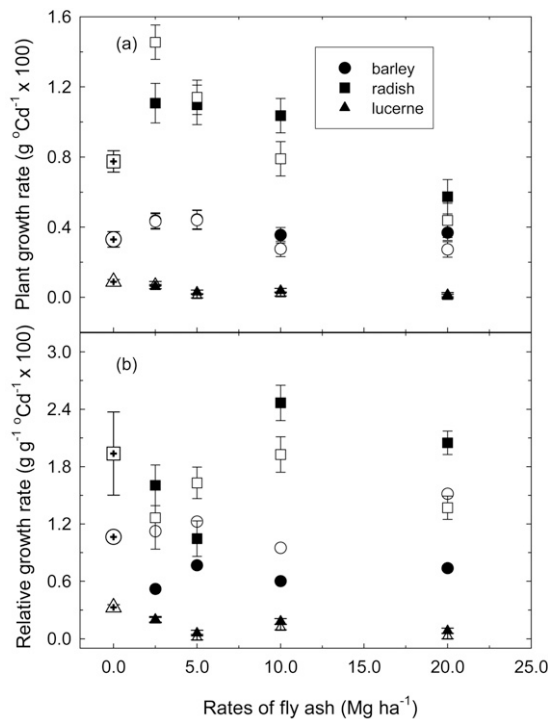


Fig. 2. Means (\pm standard errors) for the growth variables in barley (circles), radish (squares), and lucerne (triangle) in response to variable amounts of gray fly ash (closed symbols) or red fly ash (open symbols) applied to growth media before planting: plant growth rate (a) and relative growth rate (b). Open symbols with crosshair are means for the respective plant species on media not supplied with fly ash.

The magnitudes of the pigment concentrations changed between the two dates in some species and these are summarized

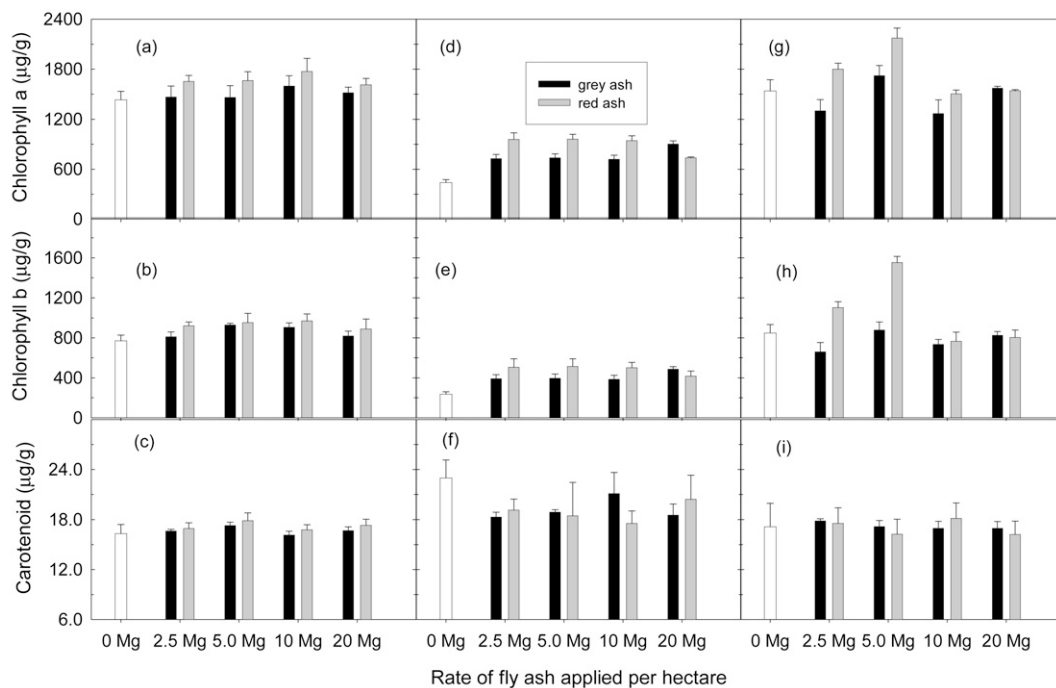


Fig. 3. Means (\pm standard errors) for concentrations of photosynthetic pigments in the leaves of barley (a–c), radish (d–f), and lucerne (g–i) measured at 76 d after sowing in response to amounts of either gray or red fly ash applied to growth media before planting: chlorophyll a (a, d, and g), chlorophyll b (b, e, and h), and carotenoids (c, f, and i). Open bars are means for the respective plant species on media not supplied with fly ash.

in Table 2 for selected representatives of the three families. Concentrations of chlorophyll *a* declined in barley, remained largely unchanged in radish, but increased in lucerne, between the two sampling dates. The reductions in chlorophyll *a* were less pronounced in plants supplied with red ash. The response in chlorophyll *b* was similar to that in chlorophyll *a*, except that with red ash the change was less pronounced. Carotenoid concentrations were stable in barley and lucerne between the two dates, but were reduced at 76 DAS compared with 28 DAS in radish supplied with the red ash.

Carbon Dioxide Assimilation (A) and Transpiration

These measurements were made on barley and radish only, as representative of monocots and dicots, respectively. Detailed data on *A* and transpiration (Fig. 4) showed that gray ash applied at up to 10 Mg ha⁻¹ increased *A* for the two species. Assimilation was increased by red ash at all rates in radish. Both ashes applied at rates ≤ 5 Mg ha⁻¹ increased transpiration for barley. In radish, transpiration was reduced by the gray ash, but was enhanced by the red ash applied at ≥ 5 Mg ha⁻¹. The red fly ash generally reduced rates of *A* relative to the gray ash, but the trend was reversed in radish. Except for transpiration that was larger in radish with red ash than with gray ash, this variable and the ratio of internal concentrations of CO₂ in the leaf tissue to that in the external environment were similar with application of either ash type to the two species.

Discussion

The benign response in germination to addition of fly ash was consistent with an earlier finding in canola (Wong and Wong, 1989) and faba bean (*Vicia faba*) (Singh et al., 1997).

Table 2. Mean concentrations (\pm standard errors) of photosynthetic pigments for representative species of the three families of plants used in this study.

Plant	Ash treatment	Chlorophyll <i>a</i> ($\mu\text{g g}^{-1}$)		Chlorophyll <i>b</i> ($\mu\text{g g}^{-1}$)		Carotenoid ($\mu\text{g g}^{-1}$)	
		28 DAS	76 DAS	28 DAS	76 DAS	28 DAS	76 DAS
Barley	Control	1779 \pm 103	1433 \pm 93	957 \pm 112	771 \pm 61	17.3 \pm 4.8	16.3 \pm 1.22
	Grey ash	1727 \pm 67	1510 \pm 55	971 \pm 78	866 \pm 52	16.1 \pm 2.2	16.7 \pm 0.40
	Red ash	1757 \pm 78	1675 \pm 59	986 \pm 56	993 \pm 31	16.0 \pm 5.4	17.2 \pm 0.82
Radish	Control	475 \pm 25	438 \pm 76	247 \pm 29	236 \pm 42	27.3 \pm 0.4	23.0 \pm 1.22
	Grey ash	466 \pm 31	770 \pm 95	254 \pm 57	415 \pm 39	28.1 \pm 3.5	19.2 \pm 1.06
	Red ash	971 \pm 88	898 \pm 160	511 \pm 45	484 \pm 83	19.9 \pm 1.2	18.9 \pm 1.61
Lucerne	Control	1264 \pm 82	1540 \pm 222	594 \pm 35	849 \pm 235	17.3 \pm 0.67	17.1 \pm 0.47
	Grey ash	1540 \pm 155	1455 \pm 25	764 \pm 120	764 \pm 120	17.4 \pm 0.54	17.3 \pm 0.58
	Red ash	1217 \pm 136	1514 \pm 237	621 \pm 88	856 \pm 190	18.0 \pm 1.87	16.9 \pm 1.00

Unlike germination and initial seedling establishment that mostly depend on seed storage reserves, later growth depends on photosynthesis and anabolism. Subsequent growth is therefore expected to be affected by any changes in the concentrations of photosynthetic pigments induced by fly ash. The fly ashes used in the current study, however, had mostly benign, and in some cases beneficial, effects on the concentration of the two chlorophyll pigments (Fig. 3). We earlier found that relative chlorophyll concentration was reduced only when canola was supplied with more than 125 Mg ha⁻¹ of the gray ash (Yunusa et al., 2008). The mostly benign response of carotenoid to fly ash treatments, and also the relative stability of its concentrations between the two measurements (Table 2), in all the test species further suggested an absence of significant metal stress. Carotenoids protect chlorophyll from photooxidative degradation (Middleton and Teramura, 1993), and are also a precursor for the synthesis of ABA, when plants are exposed to significant elemental stress (Young, 1991; Botella-Pavía et al., 2004). Earlier studies of plant response to media treated with large amounts (equivalent of 180–1200 Mg ha⁻¹) of several Australian fly ashes, including the two used in the current study, identified B as the only phytotoxic element (Aitken and Bell, 1985). The rates used in that study were definitely excessive and the consequent plant stress was not entirely unexpected.

Our results differ in some important ways from those found with rice in a study by Mishra et al. (2007). Although they reported increases in the concentrations of all the three pigments in plants supplied with fly ash, as found in the current study, they also reported increases in the ratios of chlorophyll *a/b* and of carotenoid/total pigment concentrations by up to a factor of 3.0, in plants supplied with fly ash compared with untreated plants. In our current study, the chlorophyll *a/b* ratios were relatively stable (0.97–1.01) for the six species irrespective of the ash treatment. This was possibly because our ashes had large amounts of soluble Ca and Mg and low concentration of heavy metals (Table 1). Cations, especially Ca, complex with heavy metals thereby reducing their availability to plants or they may block the heavy metal binding sites on the thylakoid membrane (Issa et al., 1995).

It appears that the differential effects of the two ashes on plant growth were largely attributable to their salinity. The salinity reduced *A* (Fig. 4) and plant dry weight, especially in plants grown with the highly saline red ash. Reductions in growth were, therefore, lower in the grasses that are relatively tolerant of salinity. This is because grasses have superior ability to exclude salt uptake and

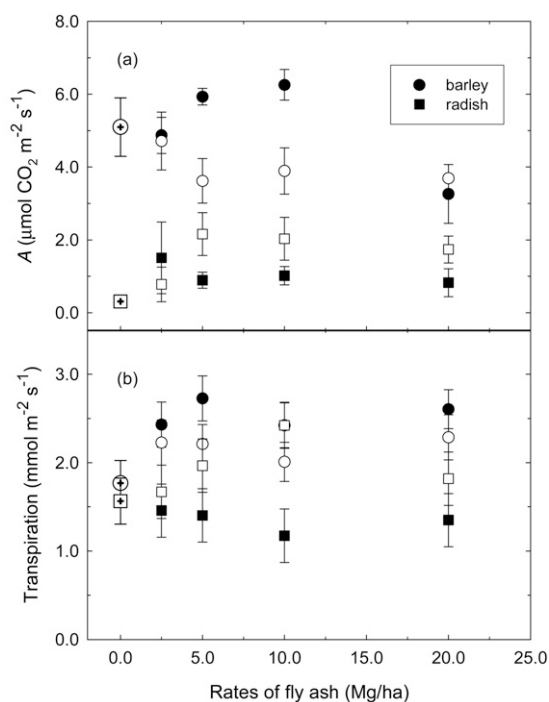


Fig. 4. Means (\pm standard errors) for the photosynthetic process for barley (circles) and radish (squares) measured at 65 d after sowing in response to amounts of gray flash ash (closed symbols) or red fly ash (open symbols) applied to growth media before planting: (a) rate of carbon dioxide assimilation (*A*) and (b) rate of transpiration. Open symbols with crosshair are means for the respective plant species on media not supplied with fly ash.

accumulation, compared with dicotyledonous species (Aitken and Bell, 1985; Steppuhn et al., 2001; Loureiro et al., 2006). The six test plants could be ranked for their tolerance of saline media as barley > ryegrass > canola > lucerne > radish > peas (Steppuhn et al., 2001). We could not, however, find a strong correlation between plant growth responses (plant weight and *A*) and concentrations of any or all of the pigments or their ratios. Also, the lower rate of *A* for radish relative to that of barley was not reflected in their dry weight, suggesting that the former could be more efficient in exporting its photosynthates to the sink, i.e., organs of final storage or utilization, better than barley. Grodzinski et al. (1998) found C3 dicotyledonous species exported more assimilates to sinks by up to a factor of two compared with monocotyledonous species. Thus differences in the instantaneous rates of *A* and transpiration are not always reflected in plant dry weight

due to differences in maintenance respiration and water use efficiency (Yunusa et al., 2005), in addition to those in export rates. Also, increases in *A* with concomitant reductions in transpiration in radish due to addition of gray ash would suggest improvements in water-use efficiency. The general reductions in RGR with increasing rates of ash application (Fig. 2), however, suggest that the plants were not able to make up for initially poor growth rates (Fig. 1). This does not, however, preclude a possible late surge in growth by plants supplied with high rates of ash as reported earlier (Yunusa et al., 2008).

Conclusions

Implementation of the OECD protocol that simply evaluated early growth on the basis of dry matter accumulation, while providing only limited physiological understanding, produced definitive information on plant response to addition of coal fly ash. The use of photosynthetic pigments as a predictive tool for plant responses to fly ash addition was therefore not an improvement over the OECD protocol. The two fly ashes used in this study enhanced vegetative growth when applied at low rates ($\leq 10 \text{ Mg ha}^{-1}$), but the high salinity of the red ash would limit its potential for routine use.

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