

1 **Growth and Elemental Accumulation by Canola on Soil Amended with Coal Fly ash**

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**21** *Abstract***22**

**23** To explore the agronomic potential of an Australian coal fly ash, we conducted two  
**24** glasshouse experiments in which we measured chlorophyll fluorescence, CO<sub>2</sub> assimilation  
**25** (*A*), transpiration, stomatal conductance, biomass accumulation, seed yield and elemental  
**26** uptake for canola (*Brassica napus*) grown on soil amended with an alkaline fly ash. In  
**27** Experiment 1, application of up to 25 Mg/ha of fly ash increased *A* and plant weight early in  
**28** the season, before flowering commenced, and seed yield by up to 21%; but these variables  
**29** including leaf area and chlorophyll concentration were all reduced at ash rate of 625 Mg/ha.  
**30** Increases in early vigour and seed yield were associated with enhanced uptake of phosphorus  
**31** (P) by the plants treated with fly ash. Fly ash application did not influence accumulation of  
**32** B, Cu, Mo or Zn in the stems at any stage of plant growth or in the seed at harvest, except  
**33** Mo concentration that was elevated in the seed. Accumulation of these elements was mostly  
**34** in the leaves, where concentrations of Cu and Mo increased with any amount of ash applied  
**35** while that of B occurred only with ash applied at 625 Mg/ha. In Experiment 2, fly ash  
**36** applied at 500 Mg/ha and mixed into the whole 30 cm soil core was detrimental to growth  
**37** and yield of canola, compared with restricting mixing to 5 or 15 cm depth; whereas at 250  
**38** Mg/ha increasing depth of mixing increased *A* and seed yield. We concluded that fly ash  
**39** applied and mixed into top 15 cm of soil is sufficient to obtain agronomic benefits.

**40**

**41** **Keywords:** boron accumulation, chlorophyll fluorescence, gas exchange, heavy metals, fly  
**42** ash, seed yield

**43****44**

## 45 1.0 Introduction

46

47 There is a renewed interest in Australia for developing opportunities for the beneficial use of  
48 coal fly ash for agricultural soil management. Australia produces more than 13 million  
49 metric tonnes of coal fly ash annually of which less than 40% is effectively utilised, while  
50 the balance is emplaced in landfill at substantial cost (Heidrich, 2003). This absence of fly  
51 ash application is partly due to concerns over risk of elemental toxicity, but this would  
52 appear to be a misplaced fear since Australian coals are noted to have low concentrations of  
53 metals that are of concern to the environment and public health (ACRL, 1996). Much of the  
54 recent and past efforts in Australia have concentrated on using fly ash to improve water  
55 availability to plants on coastal sands. Pathan et al. (2001), for example, obtained a doubling  
56 of root mass in turf (*Cynodon dactylon*) grown on ash-amended soil. Yunusa et al. (2006)  
57 recently argued, however, that fly ash could be used to ameliorate a range of structural and  
58 chemical constraints to crop performance on many of the major agricultural soils in  
59 Australia. These could include management of problems such as soil acidity and soil-water  
60 dynamics.

61

62 Coal fly ash could be a potential source of essential nutrients for plants. For instance,  
63 Kuchanwar and Matte (1997) observed yield increases for peanuts (*Arachis hypogaea*)  
64 treated with ash at up to 10 Mg/ha, which were at par with yields obtained from NPK  
65 (19:38:0 kg/ha) fertiliser. Wallace and Wallace (1989) explained that a 43% increase in the  
66 yield for wheat was due to improvements in soil nutrition and concluded that large quantities  
67 of fly ash could be disposed on farmlands with little or no risk to the environment. Nutrients  
68 commonly associated with yield benefits in crops grown on media amended with fly ash  
69 include S (Elsewi et al., 1978), P (Pathan et al., 2002; Matsi et al., 1999), Zn, Mn, Fe and

70 several trace elements (Khandkar et al., 1996). Other studies, however, have shown that  
71 plants grown on ash-amended media suffer from deficiency of several nutrients including N,  
72 P, K, Cu, Mn and Zn (Adriano et al., 1978), possibly because they are tied up in the ash  
73 (Carlson and Adriano, 1993).

74

75 A major impediment to routine agronomic use of fly ash is potential elemental toxicity to  
76 crops. The element commonly associated with poor plant growth in soils amended with fly  
77 ash is B (Plank et al. 1975; Adriano et al., 1978; Aitken and Bell, 1985; Taylor and  
78 Schuman, 1988), although other elements such as Mo, As, Be, Se and Ba have also been  
79 mentioned (Adriano et al., 2002). Aitken and Bell (1985) reported that B toxicity reduced  
80 growth in beans (*Phaseolus vulgaris*) and Rhodes grass (*Chloris gayana*) grown on sandy  
81 soil amended by weight with between 15 and 100% of fly ash by weight of soil. Seed yields  
82 for both species were reduced by as much 25% with 15% ash, falling further to 48% in both  
83 plants as fly ash was increased to 70%. Also, accumulation of B in the shoot of the beans  
84 increased by 2.5- to 6.0-fold, when grown on ash-treated soil, from 88 mg/kg in the absence  
85 of fly ash; for the grass the increase was 2.5- to 4.5-fold over the 33 mg/kg in the control. A  
86 later study by Aitken and Mccallum (1988) found that once concentration of B rose above  
87 1.9 mg/L in soil solution, sunflower (*Helianthus annuus*) suffered significant growth  
88 reductions. The foregoing demonstrates that species differ in their tolerance to B toxicity and  
89 possibly to other elemental stresses as well.

90

91 Changes in plant growth as a consequence of fly ash treatment could manifest in  
92 physiological processes, such as stomatal conductance, transpiration, photosynthetic  
93 pigments, and CO<sub>2</sub> assimilation (Srivastava et al., 1995; Gupta et al 2002; Siddiqui and  
94 Singh, 2005). Improvements in major nutrient and trace element availability would be

95 expected to enhance CO<sub>2</sub> assimilation or photosynthesis (Enriquez et al., 2004), whereas  
96 elemental toxicity could impair this process through reduced concentration of chlorophyll  
97 directly or by substituting Mg into the pigment, which reduces the efficiency of photosystem  
98 II (Küpper et al., 1998). In this study, we report how physiological processes, elemental  
99 uptake, and seed yield in canola responded to fly ash applied at variable rates and depths into  
100 a loamy and a sandy soil. Our main aim was to explore whether application of fly ash is  
101 beneficial to this crop and if the ash can be routinely used for soil management.

102

## 103 2. Materials and methods

104

105 The study was undertaken in 2004 in a glasshouse equipped to monitor internal temperature  
106 and humidity that were logged twice daily at 0900 and 1500 hrs. Soil cores were collected in  
107 PVC cylinders of 150 mm internal diameter and 300 mm length. The cylinders were  
108 bevelled at one end to ease being pushed into the soil. This study involved two experiments  
109 detailed below.

110

### 111 2.1 Experiment 1

112

113 In this experiment we compared effects of application of different rates and grades of fly ash  
114 on canola grown on loamy or sandy soil types from the teaching farms at University of  
115 Western Sydney, Richmond (37° 33' 35"S, 150° 42' 00"E), Australia. The loamy soil had  
116 7% more clay, but 12% less sand than the sandy soil. The basic textural and chemical  
117 characteristics for these soils are presented in Table 1. The loamy soil had been under  
118 various seasonal vegetable crops in the preceding 10 years, while sandy soil had been under  
119 various *Acacia* spp in the past six years. The soils were collected with PVC tubes of 150 mm

120 ID and 300 mm length. Soil was collected into the PVC tubes either by pushing the tubes  
121 into the soil and carefully digging them up (Intact cores) or by shovelling soil into the PVC  
122 cylinders (Disturbed cores). All cores were sealed at both ends with plastic bags held in  
123 place with elastic rubber bands and were carefully transported to the glasshouse where the  
124 plastic bags were removed. The core were than transferred onto trays and arranged on  
125 benches. An alkaline fly ash was obtained from a power station north of Sydney, Australia.  
126 This ash consisted mostly of particle-size in the sandy category (Table 1). Except for B, Cu,  
127 Cr, S, Se, Mo and Zn that were higher, concentrations of other cations were either similar to  
128 or less, in the ash than in either of the two soils. Extractable B was determined based on hot  
129 water extraction (Aitken et al., 1987).

130

131 Treatments consisted of four factors involving soil type (loamy or sandy), core type (intact  
132 or disturbed), ash grade (fine or unsorted) and rates of ash (0, 5, 25, 125 and 625 Mg/ha),  
133 corresponding to 0, 8.84, 44.2, 222 and 1105 g per core. These made a total of 40 treatments  
134 that were replicated three times making a total of 120 cores. The ash was mixed thoroughly  
135 into top 50 mm of soil cores. The pots were randomly assigned to pre-numbered positions on  
136 the benches and later re-randomised around three times during the course of the experiment.  
137 The cores were watered twice daily at a rate of 40 ml/min for 3 mins for the first four weeks.  
138 After this time, watering regime was maintained to supply enough water to avoid stressing  
139 the plants, while avoiding drainage from beneath the pots. The pre-installed fans were  
140 switched on for extended periods during flowering to facilitate pollination.

141

142 *2.1.1 Plant material*

143

144 Canola variety Surpass 603CL (Pacific seeds, Toowoomba) was used in this study. Ten  
145 seeds were sown per pot at 10–20 mm depth on 26<sup>th</sup> March 2004 and the pots gently  
146 watered. Three weeks later on 14<sup>th</sup> April 2004 each pot was given 200 ml of “Thrive” liquid  
147 fertiliser (8:3:8, NPK) (Yates Garden Supples, Australia), and then once every four weeks  
148 until flowering in late July. The plants were closely monitored for fungal and pest attacks,  
149 and were treated with insecticide (Tau-fluvalinate and myclobutanil) against aphids and with  
150 fungicide (polysulphide sulphur) to control damping off and other fungal infection. Weeds  
151 were manually removed from the cores.

152

### 153 *2.1.2 Plant sampling for growth*

154

155 Emergence of seedlings occurred within 5 days on all cores and number of seedlings was  
156 counted at 20 days after sowing (DAS) to calculate percentage germination. The plants on  
157 each were progressively thinned to 4 at 27 DAS, then to 3 plants at 47 DAS and finally to 2  
158 at 83 DAS when plants were at early flowering stage. At each thinning exercise, the plants  
159 removed were collected in paper bags, dried in oven at 45 °C for 7 days and then weighed to  
160 estimate biomass or dry matter (DM) accumulation.

161

### 162 *2.1.3 Chlorophyll fluorescence*

163

164 We used a Mini PAM (Walz GmbH, Germany) to measure effective quantum yield on 24  
165 May (60 DAS) and 20 July (117 DAS), expressed as the ratio of photon absorbed to photons  
166 emitted through fluorescence  $\Delta F/F'm$ , in which  $\Delta F$  is the difference between instantaneous  
167 fluorescence and the maximum fluorescence in a light-adapted state ( $F'm$ ).  $F'm$  is obtained  
168 by applying a saturating flash of irradiance ( $8000 \text{ mmol m}^{-2} \text{ s}^{-1}$  for 1 s) to the leaf to fully

169 reduce electron receptors of photosystem II. Instantaneous fluorescence (F) is obtained from  
170 application of constant illumination to activate carbon fixation and onset of photochemical  
171 and heat dissipation processes. The  $\Delta F$  is taken as  $F'm - F$  as a measure of efficiency of  
172 photosystem II, and hence the general capacity to fix CO<sub>2</sub> (Küpper et al., 1998) and to  
173 accumulate biomass. Details of the technique and the underlying principles have been  
174 reported (Van Kooten & Snell 1990, Macinnis-Ng and Ralph, 2003). Measurements were  
175 made between 1030 and 1100 hrs AEST, under natural light conditions.

176

#### 177 *2.1.4 Photosynthesis, transpiration & stomatal conductance*

178

179 Photosynthesis was assessed by determining the rate of CO<sub>2</sub> assimilation ( $A$ ) and was  
180 measured along with transpiration and stomatal conductance on tagged youngest fully  
181 expanded leaves on 10 and 26 May, 9 and 30 June and 14 July corresponding to 46, 62, 76,  
182 97 and 111 DAS, respectively. We monitored  $A$  with a portable photosynthesis system (Walz  
183 Portable Photosynthesis System, HCM-1000, Heinz Walz GmbH, Germany) with all  
184 measurements made under constant artificial illumination ( $350 \mu\text{mol m}^{-2} \text{s}^{-1}$  at leaf level)  
185 from a metal halide lamp 400 W (Lowbay Luminaire, Blackwoods, Smithfield, Australia).  
186 The equipment also monitored stomatal conductance ( $g_s$ ) and transpiration simultaneously  
187 with  $A$ .

188

#### 189 *2.1.5 Seed yield and quality*

190

191 At final harvest on 22 October (211 DAS), flower stalks and pods were counted on each  
192 plant. The pods were then removed into bags and then threshed by hand to recover the seeds,  
193 which were dried in an oven at 40 °C for 48 hours before being weighed to determine seed



194 yields. The total number of seeds produced per plant was counted, and 100 seeds were  
195 counted and weighed from each core to estimate mean grain weight (MGW).

196

#### 197 *2.1.6 Chemical analysis of plants*

198

199 This analysis was performed on leaves and stems of plants sampled on 83 DAS (16 July) at  
200 start of flowering, and on stems and seeds taken at final harvest. The samples were ground in  
201 a stainless steel ball mill, acid digested using analytical grade (AR) concentrated HNO<sub>3</sub> and  
202 20% H<sub>2</sub>O<sub>2</sub> and then analysed using Inductively Coupled Plasma-Optical Emission  
203 Spectrometer (ICPOES) or Inductively Coupled Plasma-Mass Spectrometer (ICPMS).

204

#### 205 *2.2 Experiment 2*

206

207 We evaluated how depths of mixing ash into the soil influenced canola physiology, growth  
208 and seed yield in this experiment, and used the same ash and similar size of cores to those  
209 used in Experiment 1. The cores contained intact loamy soil that were taken as was done in  
210 Experiment 1, and the coarse ash applied at three rates equivalent to 0, 250 and 500 Mg ha<sup>-1</sup>.  
211 The ash was then mixed with the soil to depths of 50, 150 or 300 mm. The soil and ash were  
212 mixed as required and 15 seeds were sown in each core on 17 May. The cores were watered  
213 regularly to ensure that neither water-stress nor drainage from the cores occurred throughout  
214 the experiment. Each core received 200 ml of liquid fertiliser Thrive® (12.4 N: 3.0 P: 6.2 K,  
215 and some trace elements) four times during growth, while weeds, diseases and other pests  
216 were controlled as described in Experiment 1.

217

218 We recorded number of days to seedling emergence. Plant numbers were thinned to 4 plants  
219 per core at 45 DAS and finally to 2 plants/core at flowering at 86 DAS. We used portable  
220 photosynthesis system to measure net assimilation rate and quantum yield during flowering  
221 at 90 DAS following the same procedures as used in Experiment 1. The 2 plants left in each  
222 core were harvested at 159 DAS (22 October), and we determined final plant height, seed  
223 yield and seed size.

224

### 225 2.3 Statistical analysis

226

227 All the data were analysed using general linear models (SPSS version 12, SPSS Inc., USA).  
228 We used Turkey *post hoc* tests to determine significant differences between treatment  
229 groups. Log transformation of the data was made before analyses as there were indications  
230 that the variances were not normally distributed. If the transformation failed to normalise the  
231 variance, the ANOVA was undertaken on the form of data that produced the highest *P* for  
232 homogeneity, and the results interpreted cautiously. Treatment means were compared for  
233 statistical differences at 95% level of probability ( $p \leq 0.05$ ).

234

## 235 3. Results

236

### 237 3.1 Conditions inside the glasshouse

238

239 The weather inside the glasshouse was generally warm and humid. Monthly averages for  
240 minimum and maximum temperature were 11 °C and 27 °C, respectively. Temperatures  
241 remained largely stable for much of the study period, showing small falls during the winter  
242 months (June –August), when these averages fell to 10.5 and 24 °C respectively. The relative

243 humidity fluctuated within 80 – 95% range during the study, mostly due to frequent irrigate  
244 of other experiments going on at the time. To facilitate comparison with field based studies,  
245 the time intervals are given in growing degree days (GDD) taking 0 °C as base temperature.

246

### 247 *3.2 Experiment 1*

248

249 Germination was observed within one week in all pots and by 800 GDD within three weeks  
250 after sowing, at least 80% of seeds had germinated and the seedlings well established.

251 Germination and establishment were not significantly affected by treatments. The plants  
252 started flowering around 1722 GDD and attained 50% flowering at 2260 GDD on 16 July.

253 Maturity was recorded at 3520 GDD on 22 September (181 DAS).

254

#### 255 *3.2.1 Plant physiology, yield and yield components*

256

257 Treatment with ash did not have a significant effect on quantum yield (QY) in the mid-  
258 season (Table 2), indicating stability in chlorophyll function in canola. At the start of  
259 flowering (1722 GDD), concentration of chlorophyll in the leaf was, however, reduced in  
260 plants treated with 125 Mg/ha or more of ash, but by start of flowering this was reduced only  
261 in plants supplied with 625 Mg/ha. This reduction was as high as 16% during mid-season,  
262 but was only 6% at start of flowering. Treatment with fly ash increased gas exchange and  
263 stomatal conductance early in the season (Fig. 1). At 1330 GDD, *A* was almost 29% larger  
264 for plants supplied with 5 Mg/ha compared to those that did not receive ash. Response in the  
265 rate of  $g_s$  was generally similar to that in *A*, increasing with ash rates early in the season  
266 before onset of flowering. There was no significant influence of fly ash on transpiration,  
267 which was similar for all plants during the study.

268

269 Application of fly ash at 625 Mg/ha reduced leaf area at 1290 GDD (60 DAS) by at least  
270 22% (Table 2), but dry weight for plants measured at the same sampling time was increased  
271 by 52% with 5 Mg/ha of fly ash (Fig. 2). There was a gradual decline in plant weight with  
272 increasing ash rate beyond 25 Mg/ha such that plant weight at 625 Mg/ha of fly ash was only  
273 60% that for control and 40% that for 5 Mg/ha. Plant weight at flowering was also reduced  
274 significantly with fly ash of 625 Mg/ha compared control and either 5 or 25 Mg/ha.

275

276 Fly ash applied at either 5 or 25 Mg/ha increased number of flowers and of pods produced,  
277 while rates of 125 or 625 Mg/ha either produced no benefit or reduced these variables (Table  
278 3). These differences in flowers and pods were not reflected in the amount of seeds  
279 produced. There was considerable shattering of pods at final harvest that resulted in loss of  
280 seeds. However application of fly ash of up to 25 Mg/ha increased seed yield by at least 21%  
281 compared to control, but yield fell by 10% with fly ash at 625 Mg/ha. The weight of  
282 individual seeds was not affected by application of fly ash.

283

### 284 3.2.2 Elemental uptake by canola

285

286 Application of fly ash increased concentrations of B, Cu and Mo in the leaves at flowering,  
287 while accumulation of other elements was not affected by treatment (Fig. 3). There was a  
288 significant concentration of B in leaves only at 625 Mg/ha, while both Cu and Mo both  
289 increased at all rates of ash, compared to control treatment. B in leaves at 625 Mg/ha was  
290 80% more than we found in control. Application of fly ash did not affect concentrations of  
291 any of the elements in the stems, except for Mo that had greater concentration in stems of  
292 plants grown on ash-treated soil compared to those in control. There was a general decline in

293 the concentrations of elements in stems between flowering and maturity, except B that  
294 remained steady while P increased, in all treatments. Seeds accumulated substantial amounts  
295 of Mo, P and Zn, to at least the same concentration as found in the leaves earlier at  
296 flowering. It is noteworthy that application of fly ash promoted accumulation of Mo only in  
297 the grains; this increased at least 3-fold with application of fly ash compared to the control  
298 (Fig. 3e).

299

300 Of all the elements we analysed only P showed any correlation with any of the plant  
301 variables we measured. Plant dry weight at start of flowering (1039 GDD) was correlated  
302 with total (leaf and stem) P content measured at the same time (Fig. 4). This relationship  
303 indicates that P in fly ash enhanced early growth in canola.

304

### 305 3.3 Experiment 2

306

307 Germination was observed within one week in all cores, and by 380 GDD (three weeks after  
308 sowing), at least 80% of seeds had germinated and the seedlings well established.

309 Germination and establishment were not significantly affected by treatments. The plants  
310 attained flowering at 1465 GDD (6 August) and maturity at 2736 GDD (9 Oct), or 82 and  
311 146 DAS respectively. There were significant interactions between rate and depth of  
312 application of fly ash on variables of canola growth that we measured (Table 4). When the  
313 ash was applied at 500 Mg/ha by mixing it into the whole 30 cm depth soil column growth  
314 and yield variables were reduced, compared with restricting the addition to either 5.0 or 15  
315 cm depths. Whereas applying fly ash at 250 Mg/ha mixed into the whole 30 cm core  
316 increased both *A* and seed yield, compared with treating only the shallower depths of 5.0 or  
317 15 cm. On the whole, there was no difference between the mean effects of rates of ash

318 addition, but applying ash to the whole 30 cm profile of the soil decreased *A*, shoot weight at  
319 flowering and plant height at maturity.

320

#### 321 4.0 Discussion

322

323 Application of coal fly ash at moderate rates enhanced early vigour increasing plant weight  
324 by as much as 20% in canola treated with 5 Mg/ha of ash. This increase in early growth was  
325 associated with P accumulation in the shoot (stems and leaves) with which dry weight was  
326 correlated at flowering (Fig. 4). An earlier study by Pathan et al. (2001) found that  
327 unweathered fly ash contained as much as 410 mg/kg of extractable P, and when applied to a  
328 sandy soil increased extractable P by at least a factor of two, from 19 mg/kg to more than 43  
329 mg/kg. They however found no difference in the concentration of P the tissue of turf  
330 (*Cynodon dactylon*) due to sufficient supply of this nutrient from the applied fertiliser.  
331 Several other studies have associated improvements in growth and/or yield with enhanced P  
332 uptake from media amended with fly ash for species such as corn (Sims et al., 1995) and  
333 ryegrass (Matsi et al., 1999). Although our soils had high total P (Table 1), most of it must  
334 have been in non-extractable forms, but addition of fly ash would have promoted  
335 solubilisation. An increase of just 20% in solubilisation of total P in the ash-soil mixture  
336 would have provided additional 35 mg of P for the plants supplied with 5 Mg/ha.

337

338 Fly ash became particularly detrimental to canola growth only when rate of application  
339 exceeded 25 Mg/ha (Fig. 2). The initial poor growth for plants supplied with 25 and 125  
340 Mg/ha was overcome with the plants producing dry weights similar to those in the control  
341 and in the 5 Mg/ha treatment at maturity, when only plants treated with 625 Mg/ha of ash  
342 suffered reductions in weight. This suggests that any detrimental effect on canola from

343 application of moderate rates of fly ash was transitional so that from mid-season onwards  
344 there was similarity in the physiological processes for the plants irrespective of amount of  
345 ash additions (Fig. 1). Declines in these physiological processes in all treatments from mid-  
346 season onwards could not be due to environmental stress such as high vapour pressure deficit  
347 or limited water supply (Nissanka et al., 1997; Yunusa et al., 2005), but most likely to age  
348 and ontogeny. Lewis et al. (2002) found that narrowing the gap between capacities of source  
349 and sink, as a determinate plant grows older, often leads to declines in photosynthesis.

350

351 Phytotoxicity caused by B is widely associated with reduced plant growth on media  
352 amended with fly ash (Plank et al., 1975; Aitken and Bell, 1985; Matsi and Keramidis,  
353 2001). Results presented show that either ash additions of up to 125 Mg/ha was not high  
354 enough to cause phytotoxicity, or that canola was tolerant of moderate levels of B, or both.  
355 These results were contrary to those reported by Aitken and Bell (1985) who applied fly ash  
356 at between 15 and 100% by weight of soil, which was equivalent to between 180 and 1200  
357 Mg/ha if applied to the top 10 cm of a field soil having a bulk density of 1.2; these rates were  
358 well beyond what would be appropriate for routine agronomic use. Furthermore, they grew  
359 the plants in only 2 kg of soil thereby confining the root systems to a substrate that was  
360 highly fortified with B (and salt) throughout the growing period. Excessively large amounts  
361 of fly ash (216 or 288 Mg/ha) used by Plank et al. (1975) could also be the major cause of  
362 phytotoxicity suffered by corn (*Zea mays*). Another feature of our current study is the use of  
363 fine textured soil, which had high capacity to adsorb B and so limits its availability to the  
364 plant, unlike the coarse soil used by Aitken and Bell (1985) that generally have low capacity  
365 for elemental adsorption (Matsi and Keramidis, 2001). This phenomenon was demonstrated  
366 by Plank et al (1975) who reported that B concentration in leaf increased by between 9 and  
367 21% on a silt loam soil, by up to 78% on loamy sand, compared with control plants that had

368 6.8 mg/kg on the silty soil and just 4.7 mg/kg on sandy soil. The study of Aitken and Bell  
369 (1985) also showed differences amongst species in their accumulation of B, which was 33  
370 mg/kg in the grass compared with 88 mg/kg in the beans, due to possible differences  
371 between the species in their rate and partitioning of this element (Wimmer et al., 2005).  
372

373 Reductions in plant biomass at high rates of fly ash were consistent with declines in *A* early  
374 in the season (Fig. 1). These reductions did not seem to have resulted from impaired  
375 efficiency of the photosynthetic mechanism since QY was unaffected. Küpper et al. (1998)  
376 discussed how photosynthetic efficiency could be impaired by substitution of Mg in  
377 chloroplast with any other elements such as Cu, Hg, Ni, Pb and Zn when they accumulate in  
378 large amounts in the leaf. Of all the metals we analysed, only Cu accumulation was elevated  
379 in the leaf of plants treated with fly ash (Fig. 3), but we found no significant correlation  
380 between any of the growth variables with accumulation of any of the metals. Reductions in  
381 biomass and *A* at high rates of fly ash could in part be associated with declines in the  
382 concentrations of chlorophyll (Table 2). Furthermore, improvements in early growth and in  
383 number of flowers produced in Experiment 1 (Table 3) were not significantly correlated with  
384 seed yield, most probably because of pod shattering mentioned earlier.  
385

386 It is also possible that poor growth and yield of canola at high rates of fly ash were simply  
387 due to salinity resulting from relatively large amounts of soluble salts in the ash (data not  
388 presented). Although canola is often regarded as a tolerant of high salinities, varieties differ  
389 widely in this regard (Francois, 1994). An assessment of canola on saline scotched land  
390 found a linear decline yield when salinity exceeded 0.5 dS/m (McCallum et al., 2001). We  
391 found no significant correlation ( $p \leq 0.05$ ) between any growth variable and accumulation of



392 any of the metals analysed, but the combined effects of salinity and metal accumulation  
393 could have restrained growth and yield of canola when fly ash was applied at 625 Mg/ha.

394

395 Responses in physiological and other growth and yield variables in Experiment 2 could be  
396 associated with benefits of P and detrimental effects of salinity resulting from treatment with  
397 fly ash. At relatively low rate of 250 Mg/ha, mixing fly ash into the whole of the soil profile  
398 ensured access to P by all the roots, while diluting salinity level, throughout the soil profile  
399 resulting in increased yield (Table 4). Applying fly ash at 500 Mg/ha to shallow layers,  
400 however, meant large portions of the soil remained relatively favourable for root growth and  
401 function. The deep roots that experienced minimal impediment are able to function close to  
402 normality, while the shallow roots in ash-treated layer were able to access nutrients,  
403 especially P, from the ash. Whereas applying large amounts of ash to most or whole of the  
404 soil profile meant that majority or all of the roots were exposed to soil of high salinity and  
405 elevated metal concentrations.

406

#### 407 **Concluding remarks**

408

409 Results presented in this study show that fly ash at low to moderate rates of up to 25 Mg/ha  
410 enhanced growth and yield variables in canola. At these low rates, ash can be applied to soil  
411 at depths similar to those used for soil amendments such as lime and gypsum, because  
412 toxicity due to salinity will be negligible at these low rates of fly ash. At these low rates,  
413 there was no evidence of excessive bioaccumulation of any element that we assessed; even  
414 the elevated accumulation of Mo in the seed was well below regulatory limit to be of any  
415 concern. It was only on soil supplied with 625 Mg/ha that plant physiological processes were  
416 adversely affected with subsequent penalty on growth and yield, even though canola

417 established successfully. The adverse effects of high rates of fly ash was most probably due  
418 to salinity and/or B toxicity, none of which was manifested at 125 Mg/ha or less of fly ash.  
419 We concluded that that this fly ash applied at rates of not more than 25 Mg/ha and mixed  
420 into top 15 cm of soil is sufficient to provide some yield benefits to the crop. Future studies  
421 with Australian fly ashes should involve field evaluation of interactions between different  
422 ashes and a range of soils and crop types.

423

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425

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437

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439

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441 toxic trace elements in comparison to internationally traded thermal coals from other

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**517** agroforestry system under conditions of soil-water deficit in a temperate environment.

**518** *Plant Soil*, 275: 195–206.

**519**

**520 Table 1. Selected chemical properties for fly ash and soils used in the study.****521**

Property	Fly ash	Loamy soil	Sandy soil
Proportions (%) of particles in the various size-classes ( $\mu\text{m}$ )			
$\leq 2$ (clay)	0	10.9	3.3
2–20 (silt)	0	17.4	13.1
20–200 (sand)	100	71.7	83.6
pH	10.2	6.57	6.38
EC (dS/m)	0.66	0.41	0.35
CEC (cmol/kg)	0.9	4.90	5.95
<i>Elemental concentrations (mg/kg)</i>			
B	66	18.0	5.0
B (extractable)	6.7	0.48	0.44
Na	210	85	20
Ca	2100	2000	1400
Mg	500	430	280
P	200	1000	700
K	1300	2500	800
Cu	18.0	13.0	7.6
Cr	42.0	10.0	3.5
Pb	9.4	15.0	8.7
S	200	74	73
Se	3.7	0.2	0.1
Mo	6.1	0.5	<0.3
Zn	56	29.0	16.0

**522****523**

524 **Table 2. Growth characteristics for canola measured at two growing degree days**  
 525 **(GDD) on ash-amended soil during Experiment 1: mean leaf area per plant, quantum**  
 526 **yield (QY) and chlorophyll content of leaves.**

527

Ash rate (Mg/ha)	Leaf area (cm <sup>2</sup> ) at 1290 GDD	QY		Chlorophyll conc. (µg/g)	
		1290 GDD	1722 GDD	1290 GDD	1722 GDD
0	334a	0.77	0.74	49.0a	49.8a
5	316a	0.74	0.74	50.4a	52.6a
25	322a	0.77	0.75	48.0a	51.2a
125	314a	0.75	0.76	45.6b	49.4a
625	244b	0.75	0.76	41.4c	46.8b
<i>SED</i>	20.0	0.034	0.058	3.10	4.32

528 GDD of 1290 and 1722 correspond with 60 and 83 days after sowing, the latter was early

529 flowering phase. Means within each variable followed by the same letter(s) are similar at  $p \leq 0.05$

530

531

532



**533 Table 3. Yield characteristics for canola grown on ash-amended soil during**  
**534 Experiment 1.**

**535**

Ash rate (Mg/ha)	Number of flowers/plant	Number of pods/plant	Seed yield (g/plant)	Mean seed weight (mg)
0	145b	59c	2.17b	3.95a
5	174a	75a	2.64a	3.97a
25	156ab	65b	2.65a	4.02a
125	148b	60bc	2.42ab	3.53b
625	125c	54c	1.96b	3.49b
<i>SED</i>	21.3	5.39	0.364	0.215

**536** Flower numbers were determined from flower stalks soon after flowering. Means within each  
**537** variable followed by the same letter(s) are similar at  $p \leq 0.05$

**538**

**539 Table 4. Selected plant variables for canola grown with different rates of fly ash that**  
**540 was mixed into soil at different depths during Experiment 2.**

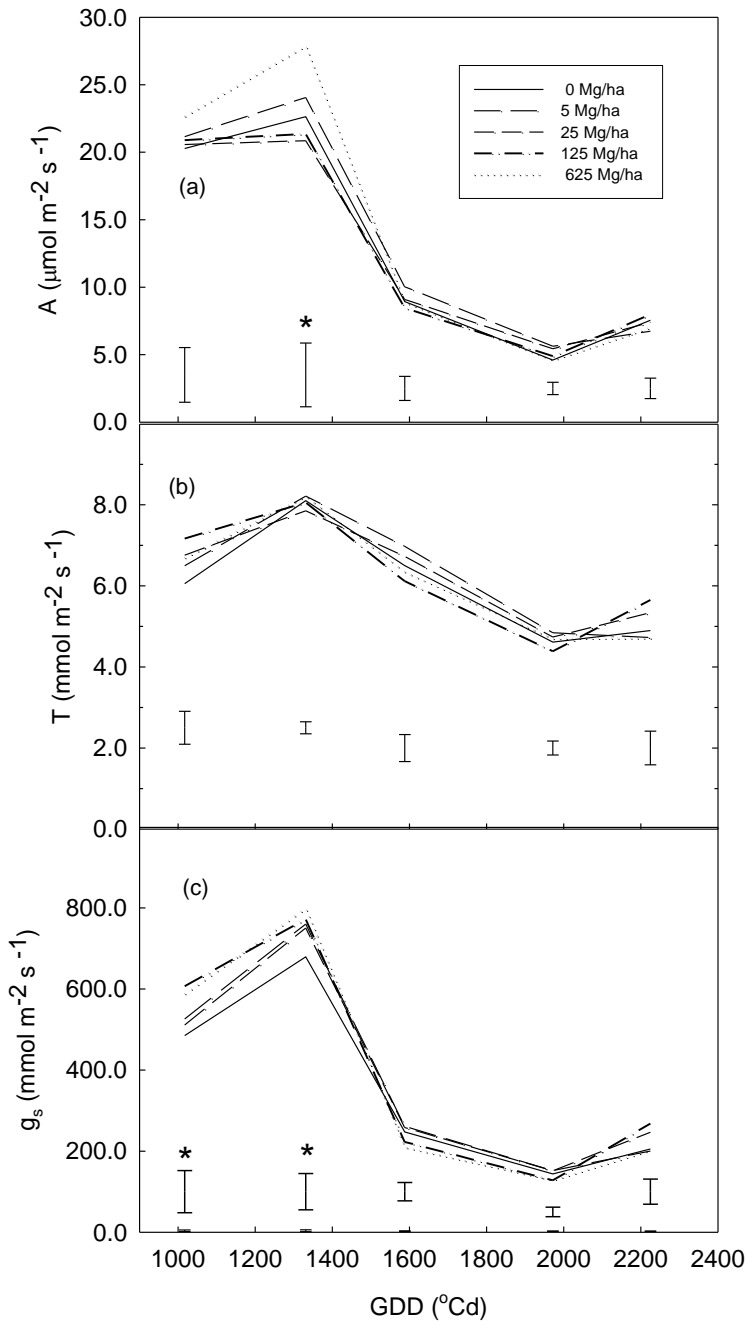
**541**

Depth of application (cm)	Ash rate (Mg/ha)		Mean
	250	500	
<i>Leaf area per plant (cm<sup>2</sup>) at flowering</i>			
5	292b	434a	363A
15	286b	369ab	327A
30	357ab	98c	227A
Mean	311A	300A	
<i>A (μmol m<sup>-2</sup> s<sup>-1</sup>) at flowering</i>			
5	8.13a	6.56ab	7.34A
15	8.68a	8.68a	8.68A
30	5.80b	5.43b	5.61B
Mean	7.53A	6.88A	
<i>Shoot dry weight (g/plant) at flowering</i>			
5	8.3a	11.7a	10.0A
15	8.9a	11.3a	10.1A
30	10.8a	2.6b	5.6B
Mean	9.3A	8.5A	
<i>Plant height at maturity (cm)</i>			
5	115b	142a	129A
15	105b	126ab	115A
30	115b	61c	88B
Mean	112A	110A	
<i>Seed yield (g/plant)</i>			
5	3.03b	4.93a	3.98A
15	3.70b	3.83b	3.77A
30	4.60a	1.94c	3.27A
Mean	3.77A	3.57A	
<i>Mean seed weight (mg/seed)</i>			
5	3.44a	3.36a	3.40A
15	3.17a	3.85a	3.51A
30	3.35a	3.16a	3.25A
Mean	3.32A	3.46A	

**542** Within each variable, means followed by the same letter(s) are similar at  $p \leq 0.05$ ; small letters compare depth x

**543** rate interactions, and capital letters main effects of either depth (column) or ash rate (row)

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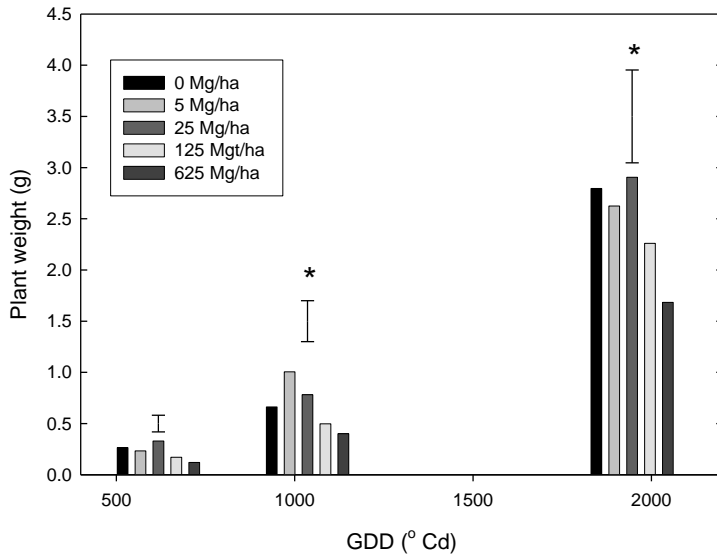
547 **Fig. 1. Response in physiological variables of canola to soil amended with fly ash during**  
 548 **experiment 1: (a) rate of gas exchange, (b) transpiration and (c) stomatal conductance.**

549 **Bars are standard errors of difference (SED) and \* indicate that means are**

550 **significantly different at  $p \leq 0.05$ .**

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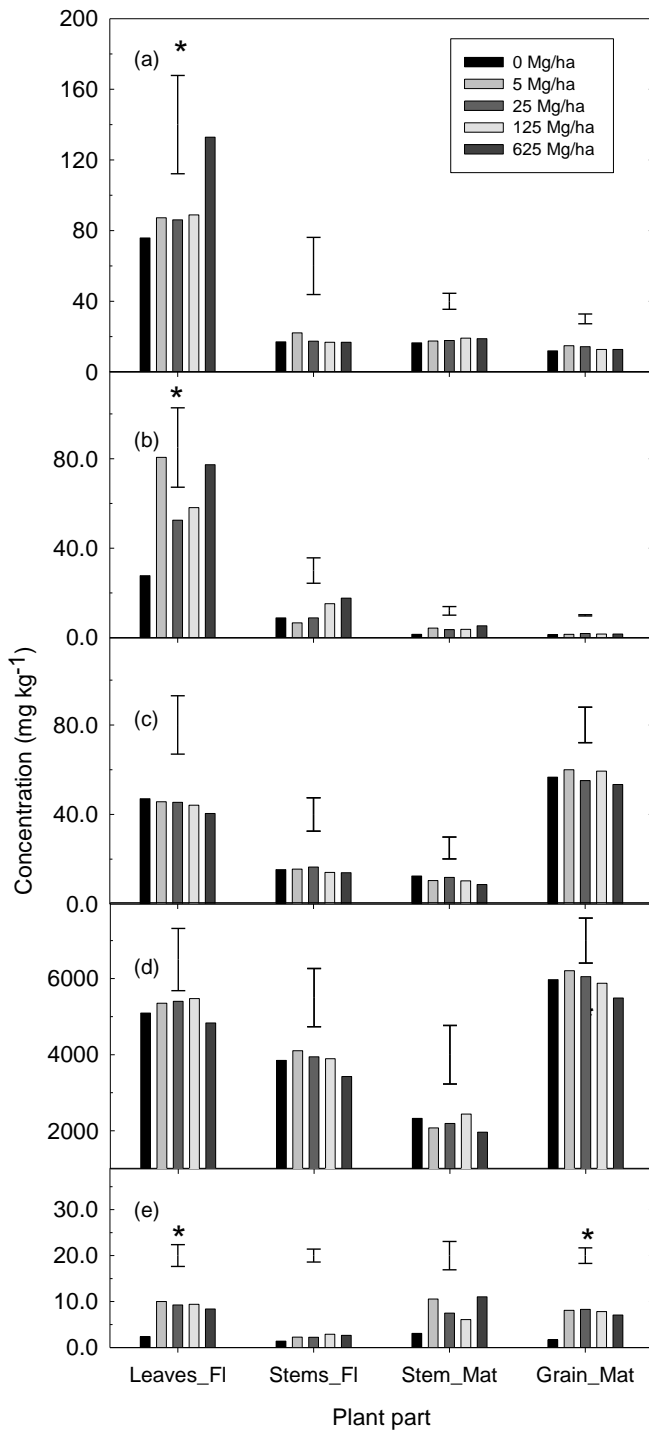
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557 **Fig. 2. Mean dry weight per plant for canola grown on ash-amended soil at 618, 1036**  
 558 **and 1946 growing-degree days (GDD) during experiments 1. The three periods**  
 559 **coincided with 27, 47 and 96 days after sowing (DAS); the plants were in early**  
 560 **flowering at 96 DAS. Bars are standard errors of difference (SED) and \* indicate that**  
 561 **means are significantly different at  $p \leq 0.05$ .**

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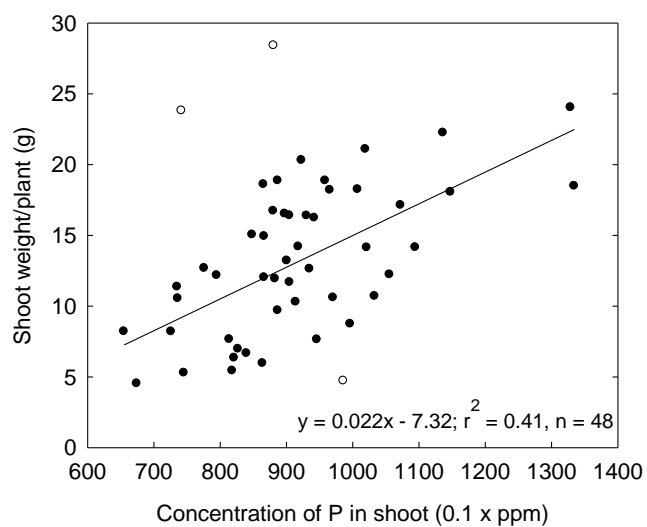
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566 **Fig. 3. Elemental concentrations in canola partitioned into the leaves, stems or grain at**

567 **either early flowering (FI) or maturity (Mat): (a) B, (b) Cu, (c) Zn, (d) P, and (e) Mo.**

568 **Bars are standard errors of difference (SED) and \* indicate that means are**

569 **significantly different at  $p \leq 0.05$ .**



570

571

572 **Fig. 4. Relationship observed between shoot dry matter and concentration of**  
573 **phosphorus in shoot of canola early flowering (1946 GDD or 96 DAS). The open**  
574 **symbols are outliers that were excluded from regression.**

575