Supporting Information Methods S1

Phloem transport of arsenic species from flag leaf to grain during grain filling

Leaf feeding of As species

Flag leaf totals for Rb and Sr Mean total Rb and Sr concentrations in flag leaf and grain for leaf fed As species are reported in Fig. S1. Arsenic controls, which were not exposed to As, and As treatments, were all pulsed with 1 mM Rb and Sr as markers for phloem and xylem transport, respectively (Kuppelwieser and Feller, 1991; Carey et al., 2010). Zero exposure controls were not exposed to Rb or Sr. One-way analysis of variance (ANOVA) demonstrated that Rb addition to flag leaves led to significantly higher levels of Rb than the zero exposure controls (P<0.001) and that there were no significant differences in flag leaf Rb between As controls and As treatments, with the exception of the DMA treatment, which pairwise comparisons showed had reduced Rb compared to As control. This can be explained by the fact that total solution uptake from feeding vials was less for the DMA and MMA treatments than that for the control and inorganic treatments. This effect is also evident in flag leaf Sr levels which are also significantly lower for organic treatments. ANOVA showed that zero exposure controls and As controls differed significantly, with Sr addition achieving significantly higher levels of Sr in the flag leaf (P=0.007). Inorganic As treatments led to flag leaf Sr levels that did not differ significantly from those of the As controls, however, both organic As treatments led to significantly lower Sr in the flag leaf than As controls. As observed for Rb concentrations, this is probably due to differences in solution uptake.

Flag leaf exposure to Rb led to significantly higher grain Rb than for the zero exposure controls ((one-way ANOVA, P<0.001), Fig. S1) with pairwise comparisons confirming

that there were no significant differences in grain Rb between As controls and As treatments. These results demonstrate that there is significant phloem transport to the developing grain from the flag leaf, and that this is not affected by the addition of As. ANOVA determined that there were no significant differences in grain Sr between any of the leaf fed treatments, including the zero exposure controls that had not been supplied with Sr, demonstrating that there was no measureable Sr transport from the flag leaf to the grain. The lack of any measurable transport of Sr from the flag leaf to the grain suggests that there was little xylem transport from the flag leaf to the grain although, in view of the large degree of variation between replicates (Fig. S1), it cannot be definitively concluded that there is no xylem transport from the flag leaf to the grain.

Dose Response for DMA and Arsenite

Grain arsenic (As) increased linearly in the developing grain as concentrations of DMA fed to the cut flag leaf were increased from 0 to 333 µM (Fig. S2; R^2=1, $P < 0.001$). Conversely, where flag leaves were exposed to increasing concentrations of arsenite there was no corresponding increase in grain As (Fig. S2; R^2=0.28, P=0.473). The concentration of As in the flag leaf itself did however increase linearly with the increasing level of arsenite exposure (Fig. S2; R^2=0.97, P=0.017), confirming that As was indeed taken up from the feeding solution and accumulating in the leaf in increasing concentrations. Solution uptake from feeding vials is displayed in Fig. S3.

Arsenic speciation in flag leaves

Figs S4 and S5 show chromatograms of arsenic speciation in fresh leaf extracts for flag leaves fed either 333 µM arsenite or arsenate for a period of 7 days, with a fresh vial attached daily, and arsenic speciation in the feeding vials, respectively.

Imaging arsenic unloading

Fluorescence microtomography images showing arsenic distribution in the developing rice grain for grains fed either arsenite, arsenate, DMA or MMA, via the flag leaf or the excised panicle stem, during grain fill, are shown in Fig. S6. It should however be noted that the quality of some of the images is relatively poor due to rapid integration times to eliminate any beam-induced damage to the partially hydrated grains (Fig. S6).

Ge phloem and xylem transport – Rb and Sr concentrations in tissues

Mean concentrations of the phloem marker, rubidium (Rb), and the xylem marker, strontium (Sr), in the grain, husk and flag leaves for the stem-girdling experiment are reported in Fig. S7. Germanic acid controls, which were not exposed to germanic acid and germanic acid treatments were all pulsed with 1 mM Rb and Sr as markers for phloem and xylem transport, respectively. Zero exposure controls were not exposed to either germanic acid, Rb or Sr. Mean flag leaf values of Rb and Sr for stem girdled panicles were about half those for non-stem girdled panicles, and two-way (ANOVA) revealed highly significant reductions in flag leaf Rb and Sr where panicles were stem girdled, however this corresponds with the 50% reduction in solution uptake for these panicles (Fig. S7). Levels of Rb in the flag leaf were 3-fold higher than those of the husk, and 16-fold greater than those of the grain. For germanic acid control panicles, stem girdling had no significant effect (P=0.1781) while for germanic acid treated panicles there was a significant difference between stem girdled and non-stem girdled counterparts (P=0.0129). However, mean flag leaf Rb was not significantly different for germanic acid control and germanic acid treated panicles (P=0.6534). Stem girdling had no significant affect on mean flag leaf Rb for zero exposure control panicles which were significantly lower than all Rb pulsed panicles (P<0.001).

Two-way ANOVA showed that treatment with Sr yielded significant increases in flag leaf Sr (P<0.001) and that stem girdling had a significant effect (P<0.001) in all cases, and there was no interaction between the stem girdling and germanic acid treatments (P=0.173). However, as stated earlier, the decrease in flag leaf Sr corresponds to the differences in solution uptake (Fig. S7). Mean Sr levels in the flag leaves of germanic acid controls and germanic acid treated panicles were about 10-fold greater than those of the corresponding husk and over 1000-fold greater than those of the grain. There were no significant differences in flag leaf Sr between the germanic acid controls and germanic acid treated panicles (P=0.9218).

Two-way (ANOVA) revealed that stem-girdling had a highly significant effect on grain concentrations of the phloem marker, Rb (P<0.001), with stem girdling leading to a 90% reduction in grain Rb for Rb treated panicles (88% for germanic acid control panicles, 91% for germanic acid pulsed panicles). Pairwise comparisons showed that zero exposure controls demonstrated no significant differences between their stem girdled and non-stem girdled counterparts (P=0.9979) and were significantly lower in grain Rb than those panicles pulsed with Rb (P<0.001 in all cases). There were no significant differences between grain Rb for germanic acid controls and germanic acid treated panicles (P=0.403) demonstrating that germanic acid addition did not significantly affect Rb (and, thus, phloem) transport.

Two-way ANOVA determined that treating excised panicles with 1mM Sr led to significant increases in grain Sr compared with the Sr controls (P<0.001) while the stem girdling treatment had no significant effect on grain Sr, P=0.151 (and there was no significant interaction between the treatments, P=0.618), indicating that xylem transport remained intact. Pairwise comparisons confirmed that there were no significant

differences in mean grain Sr between germanic acid controls and germanic acid treated panicles (P=0.9384) demonstrating that germanic acid addition did not significantly affect Sr (and, thus, xylem) transport to the rice grain.

These results confirm that the stem-girdling treatment was effective in limiting phloem transport without damaging xylem transport.

Two-way analysis of variance (ANOVA) revealed that stem-girdling had a highly significant effect on husk concentrations of the phloem marker, Rb, (P<0.001), with stem-girdled plants having significantly less Rb in the grain than non-stem girdled plants. Husk values of Rb in non stem girdled panicles were more than 5 fold greater than those of the grain (Fig. S7). However, as with the rice grain, phloem interruption via stem girdling reduced husk Rb levels by 90% (86% in germanic acid control, and 88% in germanic acid treated, panicles). Zero exposure controls demonstrated no significant differences between their stem girdled and non-stem girdled counterparts (P=0.9950) and were significantly lower in husk Rb than those panicles pulsed with Rb (P<0.001 in all cases). Pairwise comparisons showed that there were no significant differences in mean grain Rb between Germanic acid controls and Germanic acid treated panicles, demonstrating that Germanic acid addition did not significantly affect Rb (and, thus, phloem) transport to the husk (P=0.4038).

Two-way ANOVA demonstrated that Sr addition led to significantly increased husk Sr, with Rb/Sr controls significantly lower in husk Sr than the germanic acid control and germanic acid treated panicles (P<0.001 in all cases), and confirmed that stem girdling did not significantly affect husk concentrations of Sr (P=0.055). Husk levels of Sr were more than a hundred fold those of grain (Fig. S7). There was no significant difference in

husk Sr between germanic acid control panicles and germanic acid treated panicles (P=0.7939), demonstrating that germanic acid addition does not affect Sr husk levels, i.e. germanic acid addition did not affect xylem transport into the husk.

As with the grain, these results demonstrate that the stem-girdling treatment was effective in interrupting phloem transport without damaging xylem transport.