Interactions between sulfide and reproductive phenology of an annual aquatic plant, *Zizania palustris* (wild rice)

Sophia LaFond-Hudson[1]
Corresponding author
lafo0062@d.umn.edu

Nathan W. Johnson[1]
nwjohnso@d.umn.edu

John Pastor[2]
jpastor@d.umn.edu

Brad Dewey[2]
bdewey@d.umn.edu

[1]Department of Civil Engineering, University of MN Duluth
221 SCiv, 1405 University Drive Duluth, MN, 55812, USA

[2]Department of Biology, University of MN Duluth
207 SSB, 1035 Kirby Drive Duluth, MN, 55812, USA

Abstract

Aquatic plants live in anoxic sediments that favor formation of hydrogen sulfide, a known phytotoxin. We investigated how the phenology of reproductive life stages of wild rice (*Zizania palustris* Poaceae), an annual aquatic graminoid, is influenced by rooting zone sulfur geochemistry in response to elevated sulfate and sulfide. In addition, we characterized how redox conditions in the rooting zone change throughout reproduction to determine if they are tied to plant life stage. The redox conditions in sediment decreased just prior to flowering, and again just prior to seed production for all plants, allowing sulfide to accumulate at the root surface of sulfate-amended plants. Plants exposed to sulfide initiated seed production later than unamended plants. Sulfide appears to slow plant development in a way that gives the plant less time to allocate nutrients to seeds before senescence. The impact of sulfide in delaying reproductive life stages of wild rice and changing seasonal rooting zone biogeochemistry could extend to other plant species and additional chemical species that change mobility with redox potential, such as phosphate, manganese, mercury, and other metals.

Key words: ontogeny, geochemistry, wild rice, seed production, rhizosphere

1. Introduction

Many aquatic plants grow in sediments with low redox potential that favors formation of toxic reduced compounds like sulfide. To cope with these conditions, some aquatic plants transport oxygen to the roots through hollow aerenchyma tissue, release it into the rhizosphere, and form iron oxide plaques on root surfaces (Stover 1928; Mendelssohn et al. 1995; Colmer 2003; Jorgenson et al. 2012). The released oxygen and iron oxides may protect roots from dissolved sulfide species (Trolldenier 1988; Van der Welle et al. 2007; Schmidt et al. 2011; Soana and Bartoli 2013). Many wetland plants, including wild rice, are vulnerable to dissolved sulfide (Koch and Mendelssohn 1989; Carlson et al. 1994; Lamers et al. 1998; Pastor et al. 2017). Wild rice (*Zizania palustris* Poaceae), an annual aquatic graminoid which forms large monotypic stands in lakes of the Western Lake Superior region, is especially sensitive during the seedling and seed production life stages, suggesting that the ability to withstand sulfide varies throughout their life cycle (Pastor et al. 2017; LaFond-Hudson et al. 2018).

Plants growing in nutrient limited conditions sometimes experience ontogenetic drift, a phenomenon in which morphological development through successive life stages is slowed (McConnaughay and Coleman 1999; Sims et al. 2012). Because the allocation of biomass to different tissues changes throughout a plant's life cycle, delayed development has sometimes been misdiagnosed as morphological plasticity in experiments in which plants are normalized by date or age, rather than size or life stage (Coleman et al. 1994). Nitrogen is the limiting nutrient to wild rice (Sims et al. 2012) and its uptake is tied to specific life stages (Grava and Raisanen 1978). About 30% of nitrogen is taken up during early vegetative growth, 50% is taken up during the growth of the stem until flowering, and 20% is taken up during seed production (Grava and Raisanen 1978). Dissolved sulfide inhibits nutrient uptake (Allam and Hollis 1972; Koch et al.

74 1990; Martin and Maricle 2015). If nitrogen uptake in wild rice is inhibited or slowed by sulfide,
75 it may slow the rate at which the plant progresses through subsequent life stages and limit the
76 quantity of N uptake available for seed production.

77 Near the end of an annual plant's life cycle when plants allocate resources from leaves
78 into flowers and seeds, photosynthesis declines and radial oxygen loss from roots may also
79 decrease, creating favorable conditions for reduction of iron oxides and sulfate (Schmidt et al.
80 2011). Several mechanisms for maintaining radial oxygen loss from roots have been described,
81 including pressure gradients that actively pump oxygen from new leaves, through roots, to old
82 leaves (Dacey 1980; Armstrong 1980; Armstrong et al. 1992); and production and transport as a
83 byproduct of photosynthesis (Marzocchi et al. 2019). Although the exact mechanism of radial
84 oxygen loss in wild rice is not yet known, the aforementioned mechanisms may be inhibited by
85 the senescence of leaves during reproduction. We previously reported a decline in the redox
86 potential of root surfaces during the seed production life stage (LaFond-Hudson et al. 2018). In
87 plants grown in sediment without sulfur amendment, iron oxides plaques on root surfaces
88 decreased, but in sulfate-amended plants, iron oxide plaques transitioned to iron sulfide, which
89 further accumulated on root surfaces and coincided with production of fewer, smaller seeds with
90 less nitrogen relative to unamended plants. In plants exposed to sulfide, the total uptake of
91 nitrogen ceased during the onset of iron sulfide plaque formation and thickening while
92 unamended plants continued to accumulate nitrogen in seeds (LaFond-Hudson et al. 2018). In
93 this paper, we specifically explore the relationship between sulfur geochemistry and phenology
94 of life stages, as both may control each other through interactions that culminate in the redox
95 potential of root surfaces. We use wild rice (*Zizania palustris,* Poaceae) as our model organism
96 to investigate connections between sulfide and iron geochemistry in the rhizosphere and

97 reproductive phenology and ontogeny. Because wild rice is an annual plant, the ontogeny of
98 development is equivalent to the annual phenology. So in this case, the two words are
99 synonymous, except that ontogeny has the connotation of development whereas phenology has
100 the connotation of seasonality.

101 Wild rice is a culturally, economically, and ecologically important macrophyte that is
102 harvested for its grain (Fond du Lac Band of Lake Superior Chippewa 2018). An advantage of
103 using an annual plant is the relatively simple life cycle; root and shoot growth starts over each
104 year, photosynthesis declines and vegetative structures senesce during the transition from
105 vegetative to reproductive life stages, and seeds are produced at the end of the growing season
106 just prior to death. In addition, standard markers of transitions in life cycle stages for wild rice
107 have been established in prior research in the context of nutrient limitation (Grava and Raisanen
108 1978; Sims et al. 2012).

109 Motivated by acute and population-level impacts of sulfide on aquatic plants, we compare the
110 ontogenetic progression of life stages with the development of iron sulfide plaques throughout
111 the life cycle of wild rice. Sulfide may slow ontogenetic development, but plant life stage may in
112 turn control rhizosphere redox conditions and the amount of sulfur present as reactive sulfide. To
113 investigate these geochemical and phenological interactions, we quantify the timing and length
114 of life stages and seed production along with the concurrent accumulation of iron sulfide
115 plaques.

116

117 **2. Methods**

118 *2.1 Experimental design*

Individual wild rice plants were grown outside in polyethylene buckets, 32 of which were amended with 300 mg L^{-1} sulfate and 32 of which were left unamended. Although many lakes and rivers in central and northern Minnesota have concentrations of sulfate lower than 10 mg L^{-1}, several current and former wild rice lakes and rivers have sulfate concentrations near or above 300 mg L^{-1}. Additionally, 300 mg L^{-1} is close to the EPA secondary standard for drinking water and is a concentration we have used in several prior sulfate-addition experiments with wild rice. Sediment was collected on 01-Jun-2016 from Rice Portage Lake (MN Lake ID 09003700, 46.7038, -92.6829) on the Fond du Lac Band of Lake Superior Chippewa Reservation in Carlton County, Minnesota. This lake is a productive and unpolluted wild rice lake with little or no settlement along its shores and its sediment is organic-rich mud. The sediment was not sieved, but thoroughly homogenized and loaded into 4 L plastic pails that were set inside 12 L buckets (see LaFond-Hudson et. al 2018) on 25-Jun-2016. Water was added from a nearby well (sulfate concentration ranging from 8 to 14 mg L^{-1}) to provide a 12-15 cm water column. Two wild rice seeds obtained from Rice Portage Lake were planted in each bucket on 26-Jun-2016 (Julian day 177). All buckets had at least one seedling by 28-Jun-2016 (day 179), and the less robust plant of the two was removed a week later. Half of the buckets had sodium sulfate added on 28-Jun-2016 and 05-Aug-2016 (days 179, 217) to maintain surface water sulfate concentrations of 300 mg L^{-1}. Plants remained outside for the entire duration of the experiment. Further details on the maintenance of buckets can be found in LaFond-Hudson et al. (2018).

2.2 Sampling methods

To compare changes in pace of progressions through the life cycle, we examined initiation of life stages from a subset of plants that completed their entire life cycle through seed production. We define initiation as the first date a plant was observed to be in a life stage. Life

142 stages were identified visually and nondestructively according to the descriptions codified by
143 Grava and Raisanen (1978) and further subdivided by Sims et al. (2012).
144 Our observations of the phenology of wild rice began with mid tillering, a life stage in
145 which the main stem, the tiller, grows more than one leaf above the surface of the water (Table
146 1). Prior life stages include emergence of the seedling from sediment (life stage 0), the floating
147 leaf stage (life stage 1), the first aerial leaf (life stage 2), and the formation of the tiller, the main
148 stem that will eventually produce flowers and seeds (life stage 3). We started observations with
149 mid tillering (life stage 4) because it is the last vegetative growth stage before reproductive life
150 stages. After mid tillering, the internodes of the tiller elongate (jointing, life stage 5) and the
151 panicles emerge (boot, life stage 6) in preparation for flowering (life stages 7-9). Flowering is
152 broken into early (7), mid (8), and late (9) flowering by the proportion of flowers emerged and
153 blooming. Once flowers have finished blooming, a seed hull develops and seed production
154 begins (life stage 10). Filled seeds start to drop once they reach maturity (life stage 11), and
155 senescence is reached once all seeds have dropped and leaves have turned completely yellow
156 (life stage 12). Life stages of each plant and date were recorded eight times during the growing
157 season.
158 When at least half of the plants were in a specific life stage, four sulfate-amended plants
159 and four unamended plants in that life stage were destructively harvested to determine root
160 surface geochemistry. When the plants entered the seed production life stage, harvests were
161 made on three separate dates, each approximately a week apart, spanning the duration of the seed
162 production life stage. Sampling at a more frequent temporal resolution during seed production
163 enabled us to make detailed observations of the accumulation of iron sulfide (or lack thereof) on
164 the roots during a potentially critical time for sulfide exposure.

165 *2.3 Biological and chemical analysis*

166 On each sampling date, the same eight plants that were harvested were separated into
167 aboveground vegetative tissue, seed tissue, and root tissue according to LaFond-Hudson et al.
168 (2018). Vegetative tissue and seed tissues were dried for seven days at 65 °C and weighed. Total
169 N concentrations were determined with a Thermo Electron Flash EA 1112 CHNS Analyzer.
170 Fresh roots were analyzed for acid volatile sulfide (AVS) and weak acid extractable iron the
171 same day plants were harvested, taking great care to avoid exposure to oxygen (LaFond-Hudson
172 et al. 2018). Iron and acid volatile sulfur (AVS) were simultaneously extracted from entire roots
173 using 1 M deoxygenated HCl for four hours. AVS was volatilized and trapped in a sulfide
174 antioxidant buffer (SAOB) using a modified diffusion method (Brouwer and Murphy 1994).
175 AVS was quantified using a sulfide selective electrode. Iron was extracted into the 1 M HCl and
176 analyzed for total extractable iron and Fe(II). Fe(III) was estimated from the difference between
177 total iron and Fe(II). Total iron was quantified using a Varian fast sequential flame atomic
178 absorbance spectrometer with an acetylene torch. Fe(II) was quantified on the day of extraction
179 using the phenanthroline method on the spectrophotometer. After extraction, roots were dried at
180 38 °C for 24 hours to determine dry mass.

181 *2.4 Data analysis*

182 Data are publicly available in the Data Repository of University of Minnesota (DRUM)
183 and can be accessed at https://conservancy.umn.edu/handle/11299/208579. We used a two-
184 sample *t*-test to compare differences between sulfate-amended and unamended conditions for
185 seed measurements and root sulfide. Because we sampled destructively to measure root surface
186 sulfide and iron, dates for the initiation of early reproductive life stages contain a larger sample
187 size relative to the seed production life stage. Conclusions about the initiation of life stages

between treatments are based only on the subset of plants that reached seed production in this experiment, and thus are not influenced by changes in sample size. For this subset of plants, we calculate the cumulative distribution of the date on which each life stage was initiated by summing the number of plants that are at or beyond the life stage. We also calculated the duration of seed stage for each plant in this subset using the difference between the first day we observed filled seeds and the first day we observed dropped or missing seeds. In many plants, seed production ended artificially early due to our destructive sampling design. In these cases, we used the harvest date as the end date in our calculation of duration and refer to the resulting value as "experimental duration". We investigated correlations between experimental duration and yield of seed production (seed count, seedhead mass, and seedhead nitrogen mass) to understand progression in seed development within the seed production life stage. We used these linear relationships to infer the seed yield at "true durations", which we define as the probable duration of seed production if plants were not harvested. For true duration, we use the average last date seeds were observed in parallel wild rice experiments. These parallel experiments occurred in the same year, used the same sediment and tested sulfate-addition but did not use non-destructive sampling (Table S1).

Effective redox potential at the root surface was calculated using a modified Nernst equation (Stumm and Morgan 2012).

(1) $p\varepsilon = p\varepsilon^{\circ} + \frac{1}{n} log \frac{\{ox\}}{\{red\}}$

(2) $p\varepsilon = 16 - 3pH + log \frac{\{Fe(III)\}}{\{Fe(II)\}}$

(3) $E_h{}^* = \frac{2.3RTp\varepsilon}{F}$

While not strictly representative of the activity in solution, we use root surface Fe(III) and Fe(II) as a proxy for the activity of oxidized and reduced Fe in the rooting zone. Because the system is

dynamic, root surface (solid-phase) quantities likely mirror the activity of iron in solution enough to draw general conclusions about the direction of the flow of electrons.

3. Results

3.1 Sulfide effects on phenology

When life stage observations began (Julian day 210), unamended plants were ahead by nearly a full life stage (mean life stage of 4.5 ± 0.5 unamended compared with mean life stage of 3.8 ± 0.6 amended, $p < 0.01$, two-sample t test), indicating that vegetative growth life stages were delayed by sulfate amendment. Most amended plants initiated jointing later than unamended plants (mean Julian day 217 ± 9 unamended, mean Julian day 226 ± 9 amended, $p = 0.005$, $n = 17$), but both treatments initiated the boot stage at similar times (mean Julian day 237 ± 3 for both, Fig 1a, 1b). Because the boot stage occurs quickly, our temporal resolution may not have captured any differences in timing if they existed. From days 220-235, about half of the unamended plants initiated mid flowering, compared to only a quarter of amended plants (Fig 1c). During the same time frame, one third of unamended plants initiated seed production, compared to no amended plants (Fig. 1d). Eight days later, day 243, a comparable number of amended plants entered seed production. Amended plants entered seed production during a narrower range of time, with ~75% of plants reaching this life stage between days 240-250 (mean Julian day 247 ± 5), while the initiation of seed production was spread over a 2 week window for unamended plants (244 ± 7 days). Due to the destructive sampling required by our experimental design, we were unable to quantify the end date of seed production in this experiment, but we estimated the end date of seed production from parallel, non-destructive experiments involving sulfate-addition to wild rice mesocosms. The final date of seed collection

was consistently close to day 260 for several years and experiments (Table S1). Using day 260 as the final date of seed production, we estimated a 20% decrease in the true duration of seed production in amended plants compared to the unamended plants.

3.2 Seed production and vegetative biomass

Sulfate amended plants produced 33% fewer seeds ($p = 0.03$), 50% less total seedhead mass ($p = 0.01$), and 40% total seedhead nitrogen ($p = 0.02$) compared to unamended plants (Table 2). Individual seeds were smaller by 33% ($p = 0.02$), but individual seed N mass did not differ significantly between treatments. Sulfate amended plants had lower vegetative biomass (leaves and stems) during late flowering ($p < 0.01$, $n = 4$), but not prior life stages (Fig. S1). The experimental duration of seed production, calculated from the difference between first day seeds were observed and the day the plant was destructively sampled, was positively correlated with more filled seeds ($p = 0.027$), greater seed mass ($p = 0.042$), and more seed nitrogen ($p = 0.012$, Fig. 2).

3.3 Root geochemistry

Concentrations of AVS on amended root surfaces were one to two orders of magnitude higher than on unamended root surfaces during jointing, boot, mid-flowering, and seed production (Table 2, Fig. S2). Porewater sulfate decreased from mid-flowering until senescence, indicating that sulfate-amended plants were likely exposed to sulfide as a consequence of sulfate reduction (Fig. S3). On amended roots, AVS increased from about 10 µmol g^{-1} to about 65 µmol g^{-1} between jointing and boot. Concentrations of root surface sulfide then remained around 65 µmol g^{-1} until seed production. The AVS concentration doubled during seed production (life stages 10-11). On unamended roots, the concentration of sulfide steadily increased from 0.5 to 5 µmol g^{-1}, with the highest concentrations occurring during seed production. However, roots

were not visibly black on unamended plants. Decreases in effective redox potential (E_h*), calculated from the ratio of Fe(III) to Fe(II) at root surfaces (Fig. 3, Fig. S4), occurred near both amended and unamended root surfaces between boot and jointing (life stage 5 to 6) and at the end of flowering (life stage 8 to 9). During seed production, the effective redox potential decreased more steeply at amended root surfaces.

4. Discussion

The phenology of seed production was delayed in sulfate-amended plants, suggesting ontogenetic drift induced by sulfide. Across both amended and unamended conditions, seed mass, seed number, and N-mass correlated with length in the seed production life stage. In the presence of sulfate, delayed seed production and lower seed N-uptake both co-occurred with a precipitous drop in redox potential and rapid accumulation of sulfide on roots.

In a natural setting, plants with a delayed start to seed production would have to compensate by either increasing N uptake rate or delaying senescence until a later calendar date. In our experiment, sulfate-amended plants contained less seedhead nitrogen than unamended plants, so the N uptake rate likely did not increase much, if at all. Our experimental design, requiring destructive sampling during seed production, was unable to test the completion of the seed production life stage. To address these limitations, we examined average end dates of seed production in parallel wild rice experiments. The date of last seed collection happened at similar dates or even earlier dates for sulfate-amended plants in these other experiments (Table S1). Thus, it seems likely that sulfate-amended plants do not extend the seed production life stage to compensate for a delay in the initiation of seed production and have a shorter true duration of seed production. Because the seed production yield (number of filled seeds, seedhead mass, seedhead nitrogen) is positively and linearly correlated with experimental duration of seed

281 production (Fig. 2), we suggest that the implications of delayed initiation without delayed
282 completion of seed production are lower reproductive outputs by plants.

283 The curious timing of iron sulfide precipitation on root surfaces coincident with the
284 beginning of seed production suggests that plants influence the geochemistry of the sediments
285 and that this influence changes during the plant's life cycle. The redox potential at the root
286 surface, calculated from the ratio of Fe(III):Fe(II), decreased from jointing to boot (life stage 5-
287 6), and again at the end of flowering (life stage 8-9). AVS concentrations increased on amended
288 roots at the same life stages that redox declined. During seed production, the redox potential of
289 amended and unamended plants diverged as the redox potential declined precipitously in
290 amended plants. These decreases in redox potential reflect a net flow of electrons toward the
291 plant root surfaces, suggesting a loss in the oxidizing capacity of the root surface. Transitions
292 into new reproductive life stages are plausible times for plants to reallocate resources from
293 photosynthetic tissues to reproductive tissues (Grava and Raisanen 1978; McConnaughay and
294 Coleman 1999; Sims et al. 2012). Experiments with white rice (*Oryza sativa*), a closely related
295 plant, have shown changes from iron oxide to iron sulfide in rhizosphere sediment as the plant
296 entered flowering (Schmidt et al. 2011). We suggest that the change in redox conditions of the
297 root surface at reproductive life stage transitions could be explained by a decrease in radial
298 oxygen loss tied to the life stage of the plant, creating conditions conducive to iron sulfide
299 formation in environments with elevated sulfur.

300 Plants concomitantly control and are controlled by sulfide. During vegetative growth life
301 stages, plants maintain low sulfide in the rooting zone by releasing O_2 and accumulating Fe(III).
302 However, at key reproductive life stage transitions, excess sulfide appears to overwhelm the
303 plant's ability to oxidize the rhizosphere. The geochemical consequences of both life stage

304 transition and excess sulfide is a precipitous drop in Fe(III):Fe(II) ratio and an accumulation of
305 solid-phase sulfur on roots. The ecological consequences of life stage transitions in the presence
306 of excess sulfide is a delay in reproductive phenology and a decrease in N uptake to seeds.
307 Slower development rates in the presence of sulfide may delay life stage transitions and the
308 geochemical consequences of these life stage transitions for redox potential. Our experimental
309 design was not able to directly determine if redox potential decreased at a later date due to
310 delayed phenology in amended plants. However, our observations do provide evidence that the
311 net effect of sulfide-induced ontogenetic drift is shortened and decreased seed production. This
312 finding hints at a phenological mechanism underlying sulfide-induced inhibition of nitrogen
313 uptake observed in prior work (LaFond-Hudson et al. 2018). Considering that seedlings also
314 experience high mortality when exposed to sulfide, 50% less total seed mass in the presence of
315 elevated sulfide may lead to rapid population declines, as has been previously observed in a
316 mesocosm experiment (Pastor et al. 2017). Additionally, decreased density of plants in
317 subsequent generations may lead to lower oxygen fluxes into sediment and exacerbate redox
318 conditions that favor production of sulfide.
319 Sulfide limitation of nutrient uptake has been demonstrated in other plants (Koch et al.
320 1990; Martin and Maricle 2015), as has ontogenetic drift ((McConnaughay and Coleman 1999;
321 Sims et al. 2012), so other freshwater annual aquatic plant populations may face similar
322 reproductive challenges if exposed to sulfide. Additionally, sulfide and iron interact with
323 nutrients besides nitrogen. Iron plaques can adsorb phosphorus and metals, controlling their
324 availability for uptake (St-Cyr and Campbell 1996; Christensen and Sand-Jensen 1998).
325 Reduction of iron plaques in the presence of sulfide may affect uptake of both macro- and
326 micronutrients. Some studies have investigated changes in radial oxygen loss over the growing

327 season in perennial aquatic plants (Soana and Bartoli 2013, 2014). However, because perennial
328 plants may have different life-cycle patterns of radial oxygen loss, the ways sulfide might
329 interact with phenology or reproduction of perennial aquatic plants remains unknown. Clarifying
330 how sulfide interacts with nutrients in rhizospheres of both annual and perennial plants may be
331 important for understanding how wetlands or vegetated littoral zones respond to elevated sulfide
332 conditions on an ecosystem level.

333 Redox conditions at root surfaces are closely tied to wild rice phenology. Sulfide, through
334 delaying phenology, has the potential to control the timing of changes in redox conditions. By
335 changing the timing and duration of reproductive life stages, sulfide's effects on phenology
336 likely plays a role in decreased survival of wild rice populations.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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441 Tables:

442 **Table 1**. Descriptions of the life stages of wild rice and the range of dates for each life stage in
443 which at least one *Zizania palustris* plant was observed (initially $n = 64$, followed by
444 incrementally smaller sample sizes due to destructive sampling). Ranges are described for plants
445 grown in water amended with 300 mg L^{-1} sodium sulfate or grown in water unamended, with
446 background sulfate concentrations of ~8-14 mg L^{-1}. Life stages 1 through 3 pertain to seedling
447 and early emergent stages that did not develop iron sulfide plaques on roots. Designation and
448 description of life stages from Grava and Raisanen (1978) and Sims et al. (2012)

LIFESTAGE NAME	LIFESTAGE NUMBER	CHARACTERISTICS	DATES OBSERVED	
			Amended	Unamended
MID TILLERING	4	Tiller (main stem) grows more than one leaf	210-235	210-222
JOINTING	5	Internodes elongate	210-240	210-240
BOOT	6	Panicles emerge from stems	235-249	235-245
EARLY FLOWERING	7	A few flowers bloom, some not yet emerged	235-245	235-245
MID FLOWERING	8	Most flowers bloom	235-249	235-245
LATE FLOWERING	9	Most panicles empty, few flowers still bloom	235-249	235-249
SEED PRODUCTION	10	Seed hull develops, seed filling occurs	240-263	235-263
SEED MATURITY	11	Filled, ripe seeds present, a few dropped	255-263	255-263
SENESCENCE	12	All seeds dropped, green tissues disappear	280	280

449

450

Table 2. Comparisons of acid volatile sulfide (AVS) concentration on root surfaces (μg g^{-1}) and of seed data in sulfate-amended (300 mg L^{-1}) and unamended conditions using a two-sample *t* test. AVS concentrations are compared during four reproductive life stages. The average for each treatment is reported with the standard deviation in parentheses (*n* = 4 for AVS during jointing, boot, and flowering; *n* = 12 for AVS during seed production, *n* = 10-12 for seed data; not all replicate plants had seeds)

Reproductive life stage	**Sulfate-amended**	**Unamended**	**P value**
Jointing	9.7 *(± 3.7)*	0.6 *(± 0.3)*	***P* < 0.01**
Boot	64.9 *(± 39.7)*	1.4 *(± 0.2)*	***P* = 0.05**
Flowering (mid)	68.9 *(± 42.9)*	2.6 *(± 0.5)*	***P* = 0.03**
Seed production	144.8 *(± 61.6)*	3.3 *(± 0.8)*	***P* < 0.01**
Seed Measurements			
Filled seed count (# per plant)	10.5 *(± 7.3)*	16 *(± 7.1)*	***P* = 0.03**
Total seedhead mass (g)	0.14 *(± 0.07)*	0.28 *(± 0.16)*	***P* = 0.01**
Total seedhead N mass (mg)	3.05 *(± 1.36)*	4.93 *(± 2.28)*	***P* = 0.02**
Individual seed mass (mg)	11.1 *(± 3.27)*	15.26 *(± 4.75)*	***P* = 0.02**
Individual seed N mass (mg)	0.26 *(± 0.15)*	0.28 *(± 0.08)*	*P* = 0.38
Seed N%	2.28 *(± 0.63)*	1.89 *(± 0.48)*	*P* = 0.06

459 **Figure captions**
460
461 **Fig 1.** Cumulative frequency of sulfate-amended (300 mg L^{-1}, filled circles) and unamended
462 plants (open squares) that have initiated a) jointing, life stage 5, when internodes elongate just
463 prior to reproduction, b) boot, life stage 6, when panicles emerge, c) mid flowering, life stage 8,
464 when most flowers bloom, and d) seed production, life stage 10, when seed filling occurs
465
466 **Fig 2.** Relationship between the experimental duration of seed production (days) and a) filled
467 seed count, b) seed mass (g), and c) seed nitrogen (mg). Filled circles indicate sulfate-amended
468 plants (300 mg L^{-1}) and open squares indicate unamended plants. End dates of duration for each
469 plant were determined either by the date they entered seed maturity or by harvest date if they
470 were harvested before reaching seed maturity.
471
472 **Fig 3.** Effective redox potential calculated from Fe(III) and Fe(II) concentration on roots
473 amended with sulfate (300 mg L^{-1}, filled circles) or left unamended (open squares). Error bars
474 show one standard deviation (n = 4). Life stages were assigned as 10, 10.5, and 11 for Julian
475 dates 252, 257, and 264 respectively to show chronological progression in E_h* during seed
476 production and maturity.
477
478

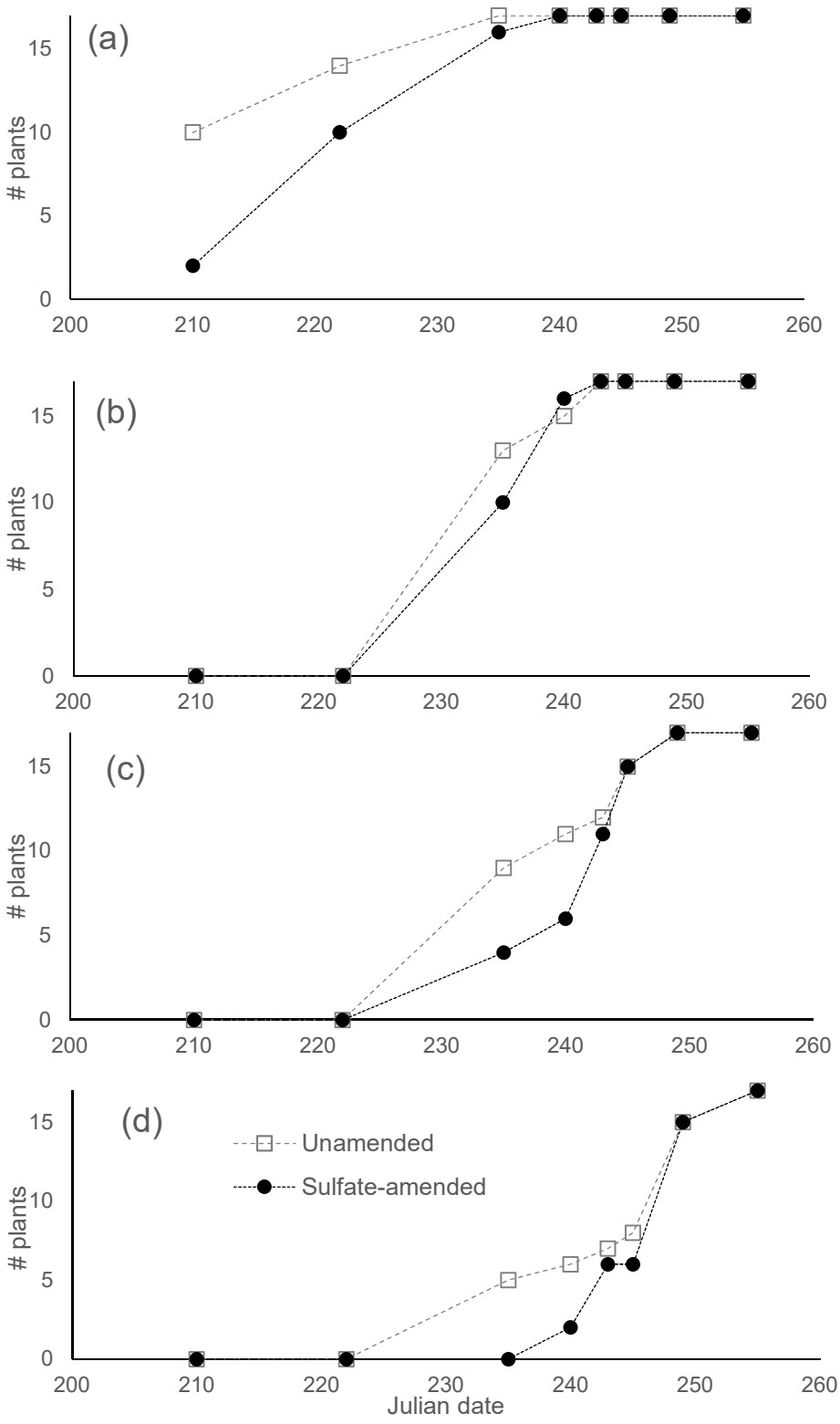
(a)
(b)
(c)
(d)
plants
Julian date
Unamended
Sulfate-amended
0
5
10
15
200
210
220
230
240
250
260

Fig 1

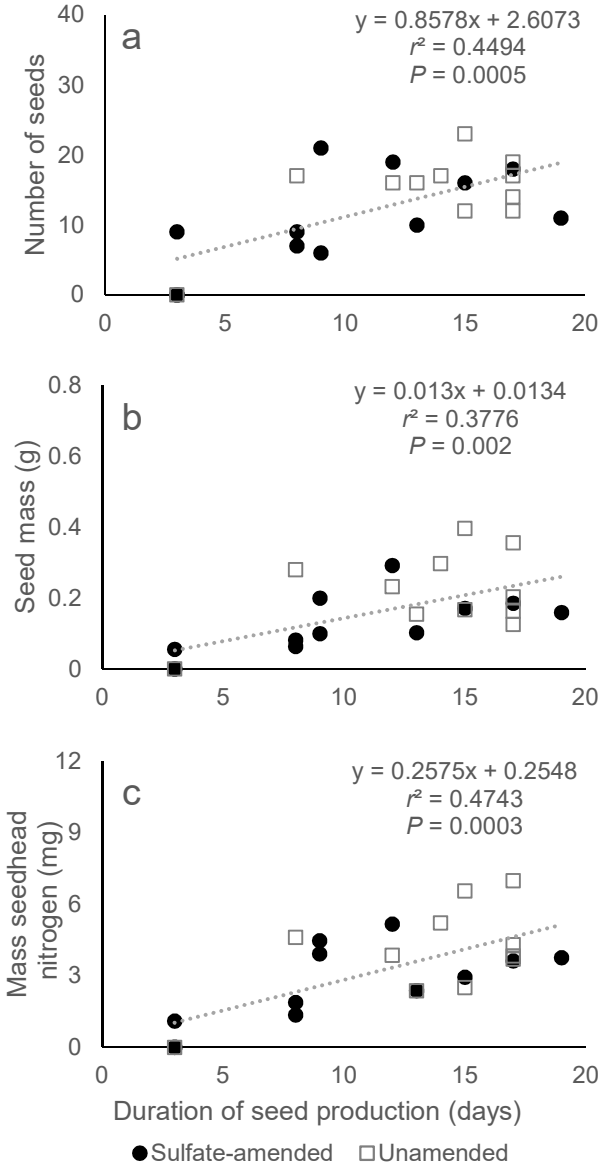
a
y = 0.8578x + 2.6073
r² = 0.4494
P = 0.0005
Number of seeds
b
y = 0.013x + 0.0134
r² = 0.3776
P = 0.002
Seed mass (g)
c
y = 0.2575x + 0.2548
r² = 0.4743
P = 0.0003
Mass seedhead nitrogen (mg)
Duration of seed production (days)
Sulfate-amended
Unamended

Fig 2.

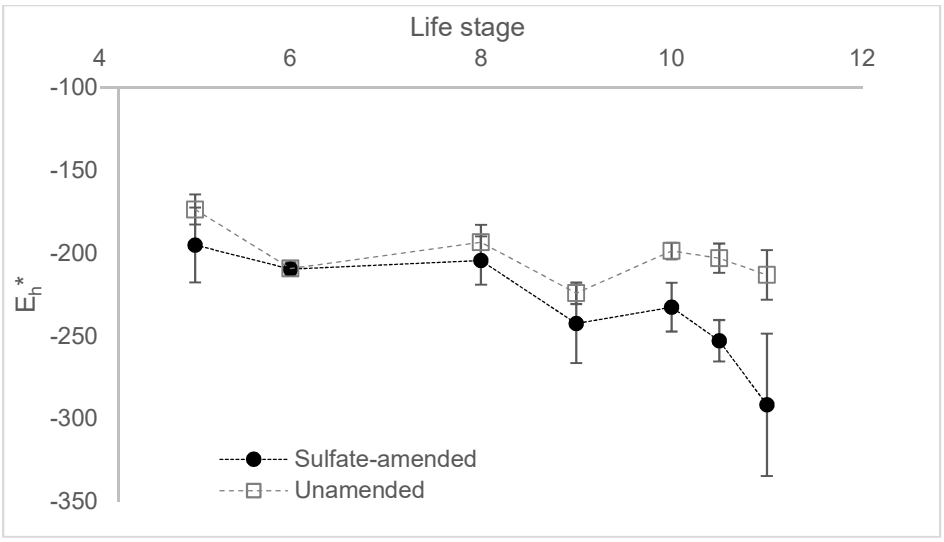
Life stage
4
6
8
10
12
-100
-150
-200
-250
-300
-350
E_{fn}^*
Sulfate-amended
Unamended

Fig 3.