Leaf scorching due to foliar application of synthetic acid mine drainage and the effectiveness of an anti-transpirant in protecting leaves

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Abstract

Untreated acid mine drainage (AMD) is being considered for crop irrigation on strategically limed soils, but foliar scorching is of potential concern. If leaf scorching is problematic, it is hypothesized that crops may be protected using anti-transpirants. A field trial and two glasshouse pot trials were undertaken in the 2020/2021 growing seasons. Crops namely *Sorghum bicolor*, *Zea mays*, *Glycine max*, *Vigna unguiculata*, *Triticum aestivum*, *Avena sativa, Medicago sativa* and *Pisum sativum,* were exposed to acid water pH levels of 2.0, 2.5, 3.0, 4.0 and 7.0. The first pot trial and field trial tested the effects of AMD on leaf scorching and the second pot trial focused on effectiveness of the anti-transpirant, Wiltpruf, in protecting crops against leaf scorching. Leaf scorching occurred at pH levels of 2.0, 2.5, and 3, but only to a maximum of 6% leaf area damage in the worst-affected crop species. Contrary to expectations, the anti-transpirant increased the propensity for foliar injury among crops, especially at pH 2.0 and 2.5. It is concluded that scorching with sulphuric acid solutions to emulate AMD, is of limited extent, and crops are likely to recover from this injury. However, it is acknowledged that acid generating metal cations commonly found in AMD should be included in follow-up leaf scorching studies with acidic waters to better emulate typical AMD waters.

It is expected that any crop growth problems possibly encountered when irrigating with AMD are likely to stem from root zone effects.

Keywords; *Acidic waters, AMD, foliar injury, pH levels, Wiltpruf.*

1. Introduction

The mining industry's greatest source of environmental concern is acid mine drainage (AMD) (Kumari et al. 2010). AMD is caused by the exposure of tailings and overburden to air and water. AMD is created from the oxidation of pyrite (FeS_2), which needs to be neutralized through liming before being released into the environment (Jovanovic et al. 2004). Upon exposure to water and oxygen, pyrite reacts to form sulphuric acid (H_2SO_4) and ferric hydroxide [$Fe(OH)_3$] (Sparks, 2003). The use of untreated AMD for irrigation may lead to inhospitable conditions for plant growth, especially for non-acidophilic crops. AMD also causes excessive loading and mobilization of Fe, Al, and Mn. Nonetheless, there are several factors to consider that influence the degree to which such water qualities may negatively influence crop growth. These include crop management, choice of irrigation system, crop tolerance, and the soil's chemical properties.

Most studies have focused on studying the impacts of acid rain on crop plants. Information on the use of untreated AMD for crop irrigation could not be found. AMD is currently neutralized with lime in a high-density sludge (HDS) plant prior to release to the environment or further treatment. A major concern that has not yet been addressed is the high cost of setting up and operating an HDS plant. The possibility of irrigating crops directly with acid mine drainage, on soils that are strategically limed, has been identified as a potentially fruitful endeavor (Madiseng, 2018). One of the questions that arise when considering this unusual practice, is whether or not crop foliage will be scorched by overhead irrigation, thereby reducing photosynthetic leaf area, and reducing production to below economic levels. However, as a first step, special attention was given to the quantification of the degree of leaf scorching due to free proton acidity from AMD. Future trials are planned which will focus on irrigation with waters that more closely emulate actual AMD sources which are enriched with acid generating metal cations.

Leaf scorching resulting from acidic waters begins with the erosion of the epicuticular wax in leaves (Percy and Baker, 1987). Similar to other biological structures, cuticles vary between species, and between individuals, and are influenced by the environments in which the plant grows. According to Sun et al. (2016), when acid rain has a pH below 3.0, it damages plant cuticles, leading to blade chlorosis. Acid rain results in premature defoliation or aging of plants, necrotic patches in their tissues, and other obvious signs when its pH falls below 3.0. Generally, stomatal damage reduces photosynthetic rates, which hinder plant development and productivity. In addition, acid rain can weaken or completely damage chloroplast structures, which will impact some chloroplast activities including photosynthesis. Acid rain-induced impairments in photosynthetic capability lead to lower biomass production in crop species (Sant'Anna-Santos et al. 2006). According to Shi et al. (2021), acid rain can directly reduce the amount of chlorophyll in leaves by 6.7% for every pH unit. Previous studies (Jacobson et al. 1989; Keevar and Jacobson, 1983) revealed that crops are most vulnerable to acid scorching in the early stages of growth. One possible agronomic practice that can potentially contribute to the reduction of foliar injury is the use of anti-transpirants if AMD is the source of irrigation water.

Anti-transpirants are categorized into four classes based on their mode of action: (1) reflectants; they reduce leaf temperature and transpiration rate by reducing the absorption of radiant energy from the sun. (2) wax forming materials; these are emulsions of plastic or latex but mostly wax, that form a thin transparent film on the leaf surface preventing the loss of water vapour from leaves. (3) metabolic anti-transpirants; these are chemical compounds that can regulate the opening and closing of stomata by controlling the movement of guard cells, thus reducing the loss of water vapour, and (4) growth suppressants which are chemicals that retard the growth of plants while enhancing their tolerance to extreme weather conditions (Aggag et al. 2015; Guleria and Shweta, 2020). In this study, Wiltpruf was used as it belongs to the wax-forming group of materials and was expected to offer some protection from contact with acidic waters. Wiltpruf is made of natural ingredients like pine oil. When sprayed on crops, it leaves a thin, waxy film over the tiny pores on the surface of the plant's leaves or needles, effectively sealing them off and keeping any moisture inside them from evaporating (Cinque, 1979). Carbon dioxide passes into the leaf through the lower epidermis (Kumari and Chauhan, 2017). Anti-transpirants like Wiltpruf are primarily formulated to help prevent winter scorch, dieback, and foliage browning on most evergreen plants (Windish, 2016). The application of anti-transpirants has been found to prevent and/or reduce transplant shock in young plants. Information on the effect of stress on cuticular waxes and how these protect foliage against the deleterious effects of light, temperature, osmotic stress, physical damage, altitude, and pollution has been documented (Shepherds and Griffiths, 2006).

Rodríguez-Sánchez et al. (2020) worked with simulated acid rain and identified damaged leaf regions by gray or brown flecking and localized chlorosis and necrosis. These authors quantified foliar injury by visually estimating scorched leaf patches as a percentage of the total leaf area. However, in the present study, a more objective, quantitative approach was employed using ImageJ, which requires standard conditions to take photographs of plants without the need for a pure white or black background as is required with other similar image processing software packages. It also allows for manual selection of specific colours for analysis (Schneider et al. 2012). ImageJ is freely available and easy to use.

Evaluated in these trials were four summer crops consisting of two dicots and two monocots. Likewise, four winter crops also consisting of two dicots and two monocots were selected for the anti-transpirant trial. These crops were selected based on their popularity among South African farmers and also to assess if there will be variations in leaf scorching between monocots and dicots.

To examine the potential effects of AMD on foliar damage, three consecutive experiments were carried out with varying AMD pH levels. These trials specifically addressed three questions; 1) Does AMD cause foliar injury in field planted crops, 2) Does AMD cause foliar injury in glasshouse planted seedlings, and 3) whether Wiltpruf, an anti-transpirant, is effective in protecting leaves against foliar scorching from AMD. We hypothesized that increasing acidity will not result in foliar injury until a threshold is exceeded and that the use of Wiltpruf will result in reduced scorching due to enhanced wax coverage on leaf surfaces.

2. Materials and methods

2.1 Location of the study

The study was carried out during the 2020/2021 season at the University of Pretoria, Innovation Africa Farm, South Africa. It involved a field trial and two glasshouse pot trials. Acid mine waters were synthesized in the Soil Science Laboratory of the University of Pretoria. Sulphuric acid (98%) was used as the source of acidity because most acidic mine waters are dominated by sulphates. Sulphuric acid was used without the acid-generating cations commonly found in AMD (Fe, Al, and Mn), as these are expected to be less important from a scorching point of view due to the relatively slow kinetics of their oxidation in water.

2.2 Experimental design

The trials were laid out in a split-plot design with three replicates. Five pH levels were selected as main plots for the pot trials, while crop species formed subplots.Each main plot was represented by four pots while subplots were represented by one pot. Four summer crops selected for the field and first pot trial consisted of two monocotyledonous crops; sorghum (*Sorghum bicolor* L.) and maize (*Zea mays* L.) and two dicotyledonous crops; soybean (*Glycine max* L.) and cowpeas (*Vigna unguiculata* L.). The anti-transpirant pot trial was carried out in winter, hence wheat (*Triticum aestivum* L.), oats (*Avena sativa* L.), lucerne (*Medicago sativa* L.), and field peas (*Pisum sativum* L.) were selected to also represent monocotyledonous and dicotyledonous crops.

3.0 Experiment 1. Response of field planted crops to foliar wetting with AMD

3.1 Methodology

Land preparation was undertaken through a tractor-drawn plough, which was followed by disc-harrowing as the last step to prepare a fine seedbed. The soil has a sandy loam texture and is classified as a deep Hutton loamy, kaolinitic, mesic, typic, highly weathered soil (Soil Classification Working Group, 1991). The pH (CaCl) of this soil was 5.49, and no lime was added. The trial evaluated four pH levels of foliage wetting: 2.0, 3.0, 4.0, and 7.0 (control) as main plot treatments with three replicates, giving a total of 12 plots, each measuring 20 m by 3.4 m. These were further divided into four sub-plots each measuring 4 m by 2.4 m with borders between them. The four crops were assigned randomly to subplots. Maize and sorghum were planted in 0.8 m rows with 0.25 m between maize plants and 0.2 m for sorghum. Sorghum was planted with three seeds per planting position. Both cowpeas and soybean were planted in rows of 0.6 m by 0.15 m. Due to the non-availability of an irrigation system at the time of planting, fertilizer was applied after crop emergence to avoid fertilizer burn. A week after emergence, fertilizer was applied using the dollop method (Edje and Ossom, 2009). Maize and sorghum received the recommended amounts of nitrogen (100 kg/ha), phosphorus (40 kg/ha) and potassium (40kg/ha). Cowpeas and soybean also received similar amounts, but nitrogen was halved, as these are leguminous crops.

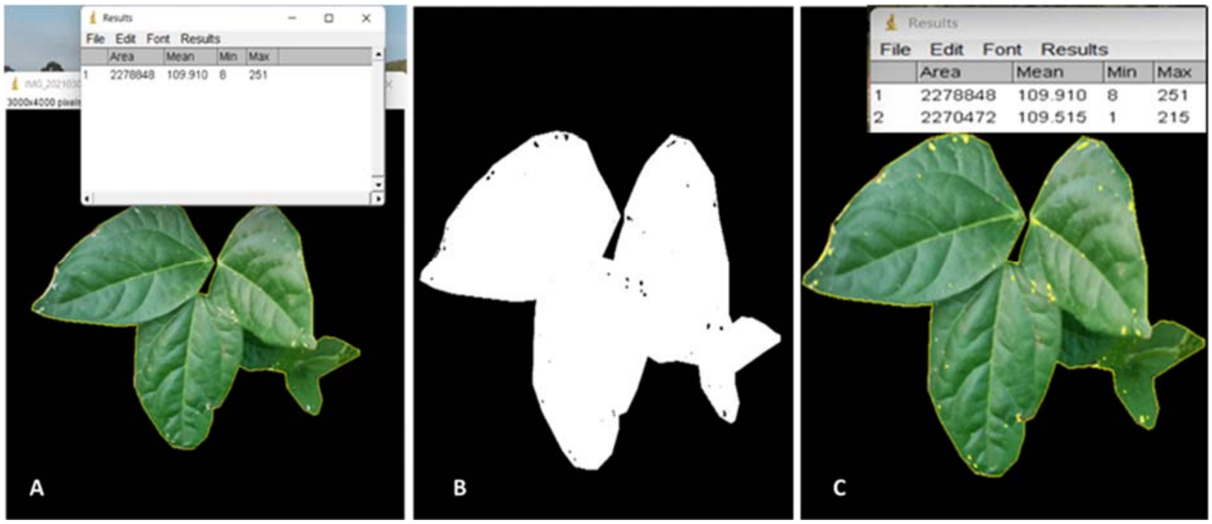
Soybean failed to germinate due to a dry spell at the emergence stage. Replanting was done after the irrigation system was set up. However, the crop did not emerge again following failure of the water pump at the farm which coincided with another dry spell. With soybean, soil surface wetting is critical for germination. Soybeans were, therefore, eliminated from the trial. The other crops were able to germinate since they are not as badly hindered by a hard soil crust during emergence. Supplemental irrigation commenced after the pump was repaired at three weeks after emergence. Foliar spraying with simulated AMD was done on alternate days beginning 14 days after crop emergence. A hand-held sprayer was used to ensure that all leaves were adequately wetted to just below the point of run-off. All crops were irrigated 10 mm with borehole water every third day in the absence of rainfall. Weeds were controlled through hand-hoeing. The trial was terminated ten weeks after planting.

3.2 Data collected

To collect plant data, five plants were selected per plot on which crop growth and canopy-related data (number of scorched leaves per plant, and fractional areal foliar damage) were measured on a weekly basis. Chlorophyll content was indirectly determined using a SPAD-502 Plus chlorophyll meter (Konica Minolta, 2017) at the end of the experiment. Dry mass was also determined at the end of the experiment with five plants cut off at ground level and oven-dried at 65 °C until constant mass.

Leaf scorching data

Every second day after treatment commenced, crops were inspected for the inception of scorching. Crops were visually assessed for symptoms of foliar injury. If there were noticeable symptoms of the scorching, control crops were checked for similar symptoms. If the control crops did not display similar symptoms, the symptom was attributed to the water quality sprinkled on leaves. Damaged/scorched leaves were photographed, and images were imported into ImageJ to determine fractional leaf area damage (Figure 1) (Alheeti et al. 2021).



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   Area     Mean     Min  Max
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2  2270472  109.515  1    215
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Figure 1. Calculating injured leaf area using ImageJ, A) measuring total leaf area, B) deselecting injured leaf area and C) measuring remaining leaf area.

3.3 Data analysis

Data collected were subjected to statistical analysis using Microsoft Excel and R Statistical software, version 2.15.3 (R Core Team, 2013). Separation of means of significant variables was assessed with the DNMRT Least Significant Difference (LSD) test at a 95% probability level ($P < 0.05$).

3.4 Results

Chlorophyll content and dry mass

There were no significant differences in dry mass (Table 1) and chlorophyll content (Table 2) across the different pH levels in the field trial. The failure of acid waters to negatively affect dry mass production may be due to crops having developed somewhat hydrophobic leaf surfaces and possibly quite high epicuticular wax concentrations. No foliar injury was evident either. This is an indication that yield would most likely not have been reduced in these crops had they been grown to maturity.

Table 1. Above-ground dry mass (kg/ha) of crops sprayed with acid mine waters of different pH levels

pH	Crop		
	Maize	Sorghum	Cowpeas
pH 2	4431	5562	3126
pH 3	5050	5833	2737
pH 4	5533	7577	2637
pH 7	4683	6071	2658
Significance	ns	ns	ns

ns – Non-significant at P = 0.05

Table 2. Chlorophyll content (SPAD units) of crops sprayed with synthetic acid mine waters of different pH levels

pH	Crop		
	Maize	Sorghum	Cowpeas
pH 2	51.4	45.8	50.9
pH 3	53.2	46.7	51.5
pH 4	54.8	51.9	49.6
pH 7	56.0	50.5	48.5
Significance	ns	ns	ns

ns – Non-significant at P = 0.05

4. Experiment 2. Glasshouse leaf scorching trial

It was noteworthy that no leaf scorching was apparent in the field trial, possibly because crops were already quite well established before foliar treatments were initiated. For this reason, a pot trial was established to ascertain whether young seedlings would be more susceptible to foliar scorching.

4.1 Methodology

Tapered flowerpots (6 L by volume) with a top diameter of 22 cm, a height of 22 cm, and a base diameter of 18 cm were filled with soil and coir in the ratio of 5:1. Coir assisted with free drainage. Soils and fertilizers used are described in section 3.1. Main plots comprised foliage wetting with water at five different pH levels [2, 2.5, 3, 4 and 7 (control)]. Each pot had one of the crops under investigation. These were replicated three times, and five seeds were planted per pot.

Domestic tap water was used for irrigation for the duration of the trial. Crops were irrigated to field capacity once a week after emergence. Irrigation frequency was gradually increased as crops' water demand increased with growth. Before each irrigation, pots were weighed to determine the amount of water depleted. Irrigation water was applied to the soil surface using a beaker to avoid wetting the foliage. All treatments were allowed to drain freely.

Beginning seven days after crop emergence, simulated acid mine drainage was sprayed daily onto leaves with a spray bottle. This wetting frequency was selected to increase the chances of scorching foliage. The leaves were sprayed to just below the point of runoff. To eliminate acid drip into the growth media when spraying foliage, the soil was covered with plastic discs. The plastic was sliced to allow plants to grow through them and was not removed during irrigation as the water was able to seep past the sides. Leaf wetting with simulated acid waters continued until the trial was terminated five weeks after crop emergence.

4.2 Data collection and analysis

Data on fractional foliar damage were collected beginning 14 days after planting. Chlorophyll content was measured at the end of the trial using a SPAD-502 Plus chlorophyll meter (Konica Minolta, 2017). Destructive sampling was done at the end of the experiment for dry mass determination. Three plants were cut off at ground level and oven-dried at 65 ºC until a constant mass was achieved. Leaf scorching data was collected as outlined in the field trial section. Data were analysed as stated for the field trial.

4.3 Results

Fractional leaf area damage

There was a significant ($P<0.05$) difference in areal foliar injury assessed among the different crops (Figure 2). Cowpeas initially exhibited significantly ($P<0.05$) higher injury, while sorghum had significantly greater injury than the other crops from 18-24 days after planting. It can be noted for sorghum and cowpeas that damage declined after a few weeks, partly due to cessation of lesion development in older leaves and the initial scorched area became a small fraction of total leaf area as new leaves emerged. Limited chlorosis and necrosis was observed on injured leaves, and this was accompanied by some leaf curling in soybean (Figure 3). It was noteworthy that both maize and cowpeas had their peak areal damage at pH 2.5. However, this was still very low at only 2 % and 4 % damage, respectively.

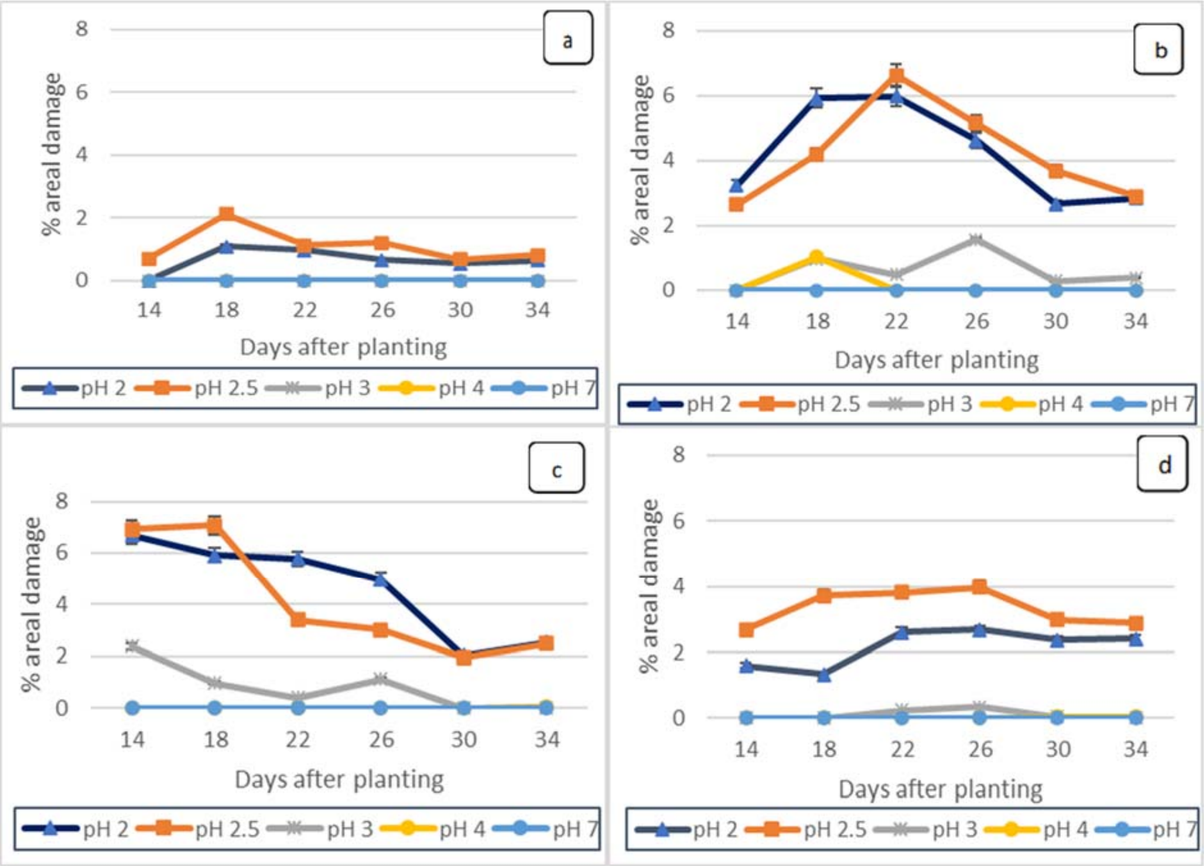

Figure 2. Fractional leaf area damage (%) calculated using ImageJ for a. maize b. sorghum c. cowpeas and d. soybeans sprayed with synthetic acid mine water of pH 2, 2.5, 3, 4, and 7.

Chlorophyll content and dry mass

As was the case with the field trial, no significant differences were noted in chlorophyll content in the glasshouse trial (Figure 4). Dry mass within each crop also shows that pH levels did not significantly affect dry mass per plant. Though not significant, maize dry mass showed a decreasing trend from pH 7.0 down to 2.0 (Figure 5).

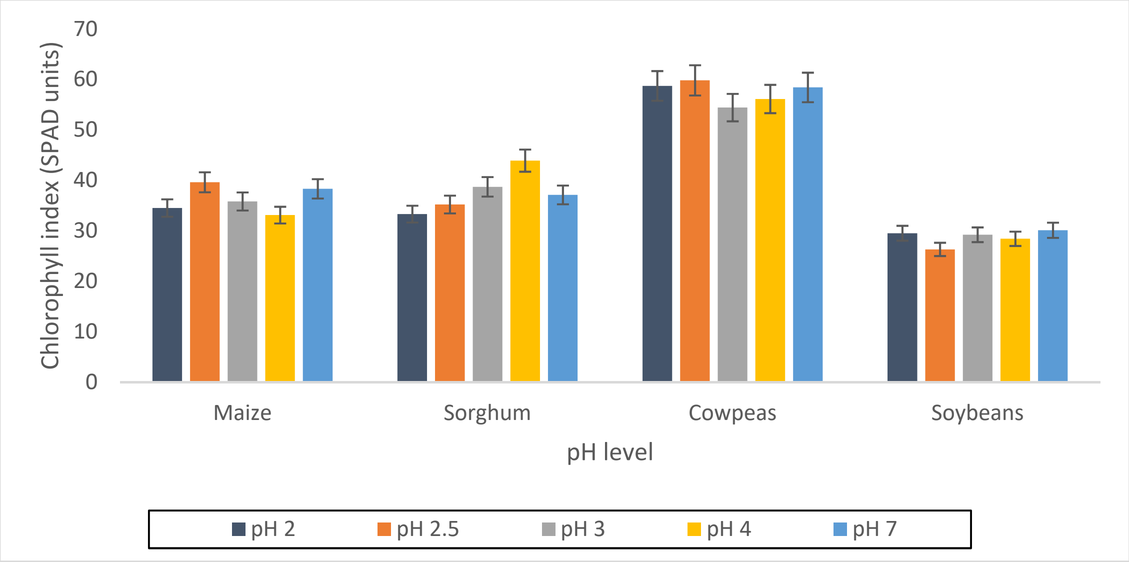

Figure 4. Chlorophyll content (SPAD units) at the termination of the experiment for crops wetted with synthetic acid mine drainage. Error bars indicate statistical significance at $P < 0.05$.

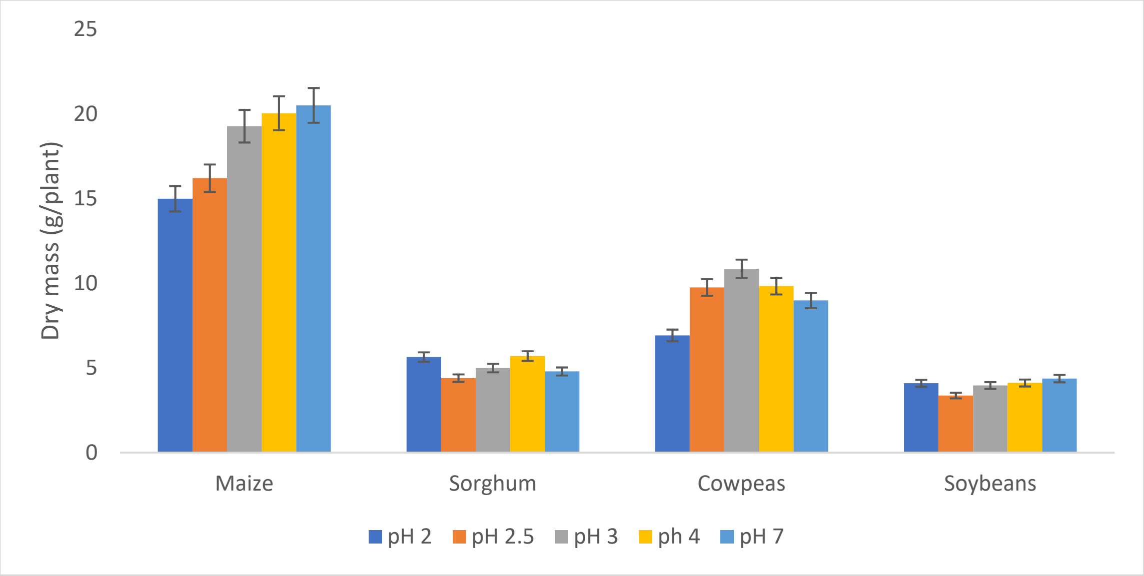

Figure 5. Total above-ground dry mass (g/pot) at the termination of the experiment for the four crops grown in the glasshouse trial as a function of simulated AMD. Error bars indicate statistical significance at $P < 0.05$.

5. Experiment 3. Anti-transpirant glasshouse trial

Since it was apparent that foliage scorching occurred in the early stages of crop growth, the anti-transpirant study was undertaken in an effort to protect crops at their early and vulnerable growth stages against foliar scorching from acid mine waters.

5.1 Methodology

All agronomic practices and pH treatments were as outlined in section 3.2.1. Crops were sprayed with Wiltpruf, a ready-to-use anti-transpirant one week after emergence until just before the point of run-off. Two days later, crops were then sprayed daily with their respective acidity treatments.

5.2 Data collection

Data were collected and analysed as outlined in section 3.2.2 above.

5.3 Results

Fractional leaf area damage

It emerged in this study that the use of the anti-transpirant increased the propensity for leaf scorching. Wheat plants treated with Wiltpruf had their highest injury of 7.2 % at pH 2, while it was only 4.78 % at pH 2.5 in the absence of Wiltpruf. This was at 28 days after planting. As time progressed, the percentage of injured leaf area surface increased and then decreased again (Figure 6). A similar trend was noted in oats (Figure 7), where it is evident that higher injury was realized in the presence of Wiltpruf. Less than 0.2 % areal injury was recorded at pH 3 where Wiltpruf was applied. No injuries were recorded at pH 3 without Wiltpruf. Calculated areal injury for lucerne is shown in Figure 8, where it is also demonstrated that the anti-transpirant increased the occurrence of leaf scorching with a significantly ($P<0.05$) higher injury percentage (7.2 %) recorded 28 days after planting. No scorching was observed for field peas.

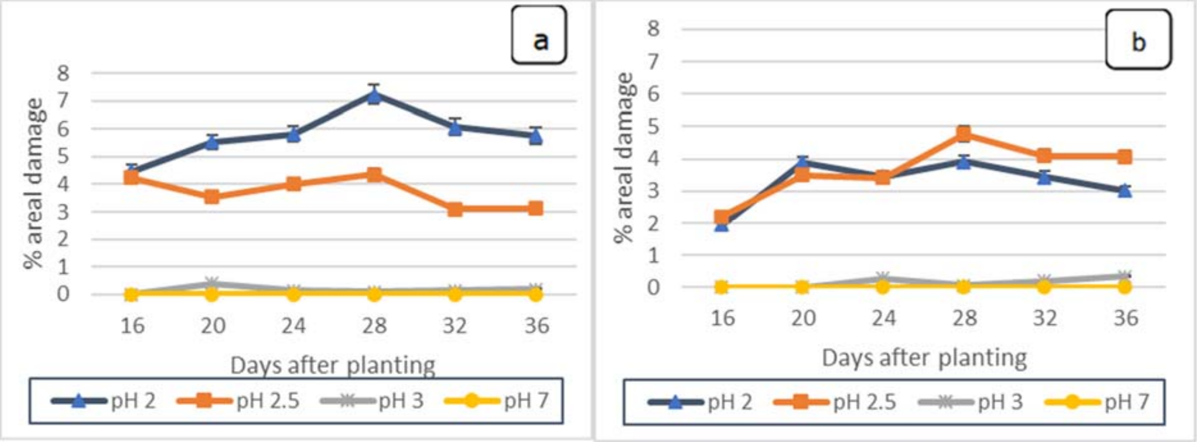

Figure 6. Percent of wheat leaf area damaged by spraying foliage with synthetic acid mine waters of pH 2, 2.5, 3, and 7 in the presence (a) or absence (b) of the anti-transpirant at 16, 20, 24, 28, 32, and 36 days after planting.

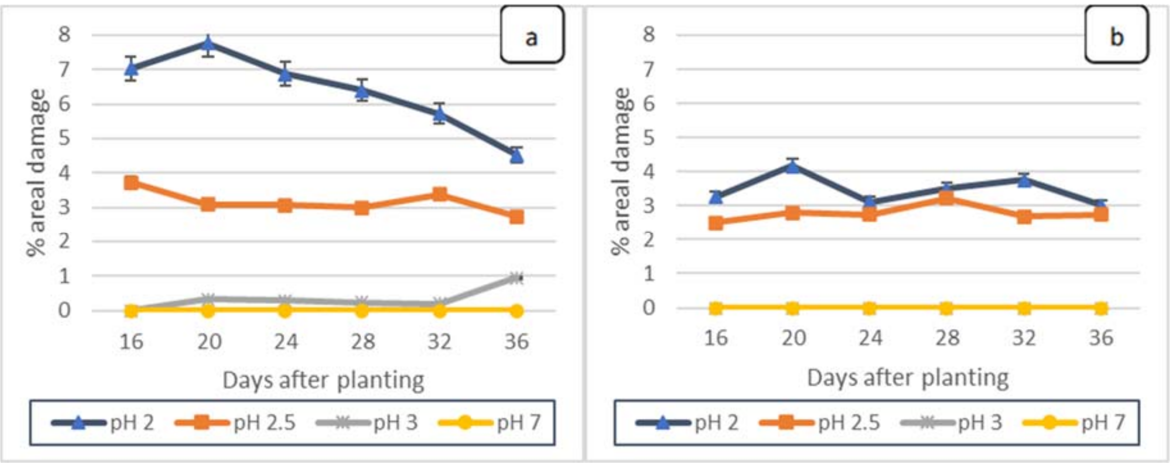

Figure 7. Percent of oat leaf area damaged by spraying foliage with synthetic acid mine waters of pH 2, 2.5, 3, and 7 in the presence (a) or absence (b) of the anti-transpirant at 16, 20, 24, 28, 32, and 36 days after planting.

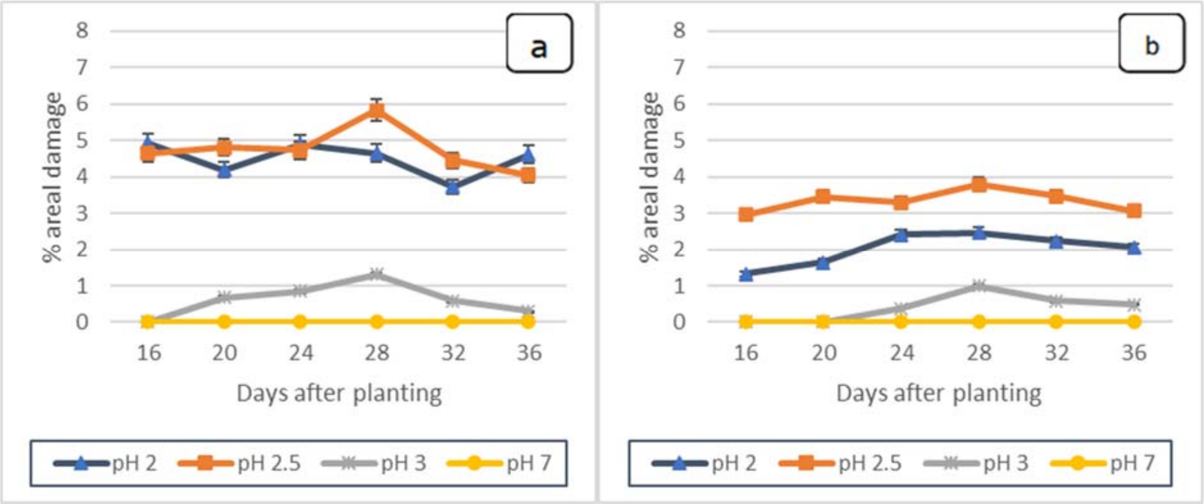

Figure 8. Percent of lucerne leaf area damaged by spraying foliage with synthetic acid mine waters of pH 2, 2.5, 3, and 7 in the presence (a) or absence (b) of the anti-transpirant at 16, 20, 24, 28, 32, and 36 days after planting.

Dry mass and chlorophyll content

The use of Wiltpruf also did not have a significant effect on chlorophyll content. However, the pH x anti-transpirant interaction showed a significant ($P<0.05$) difference for chlorophyll content. Chlorophyll content at pH 7 with the antitranspirant was significantly higher at 45.56 SPAD units, while at pH 2.5 with an antitranspirant it was lower at 40.36 SPAD units (Table 3). Data on dry biomass show that there was no significant effect of the anti-transpirant on biomass across all pH treatments (Table 4).

Table 3. Chlorophyll content (SPAD units) for all crops sprayed with synthetic acid mine water of pH 2, 2.5, 3 and 7 in the presence or absence of the antitranspirant.

Treatment		Crop				Average
pH level	Antitranspirant/No antitranspirant	Wheat	Oats	Peas	Lucerne	
2.0	Antitranspirant	40.53	49.87	38.33	47.37	44.02^a
2.5	Antitranspirant	41.67	43.27	37.0	39.50	40.36^a
3.0	Antitranspirant	42.83	49.77	36.10	43.37	43.02^a
7.0	Antitranspirant	44.63	52.83	36.60	48.17	45.56^b
Average		42.42	48.93	37.01	44.60	*
2.0	No antitranspirant	43.77	48.90	38.10	44.0	43.69
2.5	No antitranspirant	43.40	53.07	37.30	42.0	43.94
3.0	No antitranspirant	43.0	51.77	39.23	45.20	44.80
7.0	No antitranspirant	40.50	45.90	36.0	47.37	42.44
Average		42.67	49.91	37.66	44.64	ns

Means in columns followed by the same letters are not significantly different at 5% level of significance according to LSD test; * = Significant at 5%; and ns-not significant.

Table 4. Dry mass (g) per pot for all crops sprayed with synthetic acid mine water of pH 2, 2.5, 3, and 7 with presence or absence of the antitranspirant at all pH levels at the presence and absence of the antitranspirant.

Treatment		Crop				Average
pH level	Antitranspirant/No antitranspirant	Wheat	Oats	Peas	Lucerne	
2.0	Antitranspirant	5.91	4.81	4.75	4.98	5.11
2.5	Antitranspirant	5.78	4.45	5.25	4.46	4.98
3.0	Antitranspirant	5.65	4.74	4.61	5.10	5.03
7.0	Antitranspirant	5.33	4.14	5.19	4.39	4.76
Average		5.67a	4.53	4.95	4.73a	ns
2.0	No antitranspirant	6.16	3.90	4.33	5.62	5.00
2.5	No antitranspirant	8.26	4.78	4.46	4.43	5.48
3.0	No antitranspirant	7.80	4.32	5.97	6.34	6.11
7.0	No antitranspirant	6.60	4.13	4.64	7.33	5.67
Average		7.21b	4.28	4.85	5.93b	ns

Means in columns followed by the same letters are not significantly different at 5% level of significance according to LSD test, ns-not significant.

6. Discussion

6.1 Response of field planted crops to foliar wetting with AMD

All parameters measured in the field trial; chlorophyll content and dry mass were not significantly affected by foliar wetting with synthetic AMD. Keevar and Jacobson (1983), made a similar observation after spraying soybeans with simulated acid rain, where they reported no effect on chlorophyll content and dry mass. It was noted in our trial that foliar absorption rate may have been influenced by both the degree of cuticular development and by the quantity, chemical composition, and physical form of epicuticular waxes, which all vary with leaf maturity and species. This was not expected, as foliar wetting with acid waters is usually associated with chlorosis, scorching, and necrosis in plant leaves, which should lower chlorophyll content and dry mass. Contrary to our study, Odiyi and Eniola (2015) reported that plants that were sprayed with simulated acid rain of pH 2.0 and 3.0 a week after emergence had a relative growth rate (RGR), chlorophyll content, and a harvest index that was significantly lower ($P < 0.05$) than the control of pH 7.0. In the present study, foliar spraying only began in the third week after crop emergence. At this point the crops may already have developed some tolerance or resistance to acid injury, which explains the lack of any significant differences between treatments.

6.2 Glasshouse leaf scorching trial

It was noted that foliar injury was concentrated on earlier formed leaves. However, as fraction of damaged leaves declined as plants grew older with time. Leaf injury began with the development of white lesions which are similar to those caused by sun-scorching. Undeveloped trifoliate leaves were not injured for both cowpeas and soybeans. The differences in the degree of injury among the different crops may be attributed to ease of wettability, rate of cuticular permeation, and resistance or tolerance of exposed leaves to altered extracellular characteristics (Keevar, 1982). Leaf curling or cupping was evident in soybean, and in all crops except maize, white lesions as well as necrosis developed. Similar observations were made by Silva et al. (2005) who noted that the green colour of leaves faded with increasing acidity.

Information on the physiological state of plants is normally provided by the chlorophyll content of leaves (Pham et al. 2021). There was no significant reduction in chlorophyll content due to foliar application of synthetic AMD. These results, however, contradict those of other authors who reported up to 6.7 % reduction in chlorophyll content when simulated acid rain was sprinkled on crops (Keever and Jacobson, 1983; Odiyi and Eniola, 2015; Du et al. 2017). These authors attributed the reduction in chlorophyll to scorching which compromised the activity of the chlorophyll pigment in leaves, which consequently affected biomass production. In this trial, there was no evidence of acidity effects due to AMD exposure on plant dry biomass in all crops but maize. This is consistent with the findings of other authors (Keever and Jacobson, 1983; Lee et al. 1981). In a similar study by Keevar and Jacobson (1983), they reported that varieties of soybean responded similarly to different levels of simulated acid rain synthesized with sulphuric acid. In their study, biomass increased from pH 5.6 to a maximum at pH 4.6 and decreased between pH 4.6 and 2.7 as acidity increased. The authors attributed the reductions in growth at pH 3.4 and 2.7 to the visible foliar injury in those treatments. In the present trial, we believe that foliar absorption rate was influenced by both the degree of cuticular development and by the quantity, chemical composition, and physical form of epicuticular waxes, which all vary with leaf maturity and with species.

6.3 Anti-transpirant glasshouse trial

It was noted in this study that crop species responded differently to acid treatment. A study by Kohno (2017) showed that plants displayed different acid sensitivities below pH 3.0 in terms of visible acute injury or growth and yield reduction. In our study, it was noted that Wiltpruf increased leaf scorching. It is more likely, that the acid water was able to erode the anti-transpirant layer, so it was not effective in protecting leaves, but made leaf scorching worse. While we are not sure of the mechanism at play, it appears as if the acidic waters easily get through the anti-transpirant layer. Percy and Barker (1987) made a similar observation and reported that the waxiest species they considered (rape), showed the highest damage when crops were sprinkled with simulated acid rain. Therefore, waxiness, either natural or artificial may not always give protection against foliar damage in some species. The physicochemical characteristics of the adaxial leaf surface that control wettability are the cause of this discovery (Percy and Barker, 1987). Droplets that land on a readily wetted surface with an amorphous wax coating are caught on initial impaction and spread out instantly over the surface of the leaf until the leaflet's water-holding capacity is reached and run-off occurs, often after a short period of time.

In this study, no scorching was found in field peas, which was probably due to a lack of pubescence on the leaf surface which limits pooling of the acid waters on the leaf surface, a similar observation was made by Curry (1978).

Wiltpruf did not affect chlorophyll content. However, Sagta and Nautiyal (2002) reported that anti-transpirants reduced chlorophyll content in *Dalbergia* seedling leaves. It was reported by Elfving et al. (1976) that chlorophyll was destroyed by acids, while the spongy mesophyll and palisade cells collapsed after their protoplasm disintegrated. In our trial, collapsed epidermal cells in the leaves were apparently insufficient to affect chlorophyll content. This means that the biomass of wheat and lucerne was significantly reduced by the anti-transpirant. In a similar study, Davenport et al. (1974) reported that anti-transpirants had a negative effect on the growth of snap beans. This is contrary to the assertion that Wiltpruf neither improves nor compromises crop growth rate. Anti-transpirants like Wiltpruf can reduce photosynthesis by forming a physical barrier on the leaves and increasing leaf water potential (Chalker-Scott, 2015). The effect of anti-transpirants is reduced leaf expansion and stem elongation over a short period of time. Mphande et al. (2020) reported that anti-transpirants can reduce yield due to depression of transpiration and carbon dioxide exchange. It is noteworthy that both oats and field pea biomass were not negatively affected by the application of Wiltpruf in the present study. This suggests that Wiltpruf offers minimum resistance to the movement of gases, apart from water vapour (Davenport et al. 1974) and that there was also no decline in the cell metabolic activity after the application of Wiltpruf (El-Sayed et al. 2013).

7. Conclusion

The findings from this study suggest that leaf scorching with sulphuric acid solutions emulating AMD is likely to be minimal on most crops, even with high levels of acidity (pH 2). The synthetic mine waters used in this study did not include acid-generating metal cations commonly found in AMD, and future studies should assess whether or not these elements need to be taken into account. Crops are slightly sensitive in the seedling stage and there are differences in species susceptibility to leaf scorching after wetting with acidic waters. Treating crop seedlings with Wiltpruf before foliar wetting with acid mine drainage aggravated the incidence of leaf scorching, and this practice is therefore not recommended. It is expected that greater challenges may be encountered in the rootzone of crops irrigated with untreated AMD, and hence it will be prudent to apply additional lime to the soil to neutralize the acidic irrigation water. It is also recommended that crops be planted on a wet profile and irrigated to facilitate emergence. Thereafter, if possible, the first post-emergence irrigation should be withheld until the crop can withstand foliage wetting with acidic irrigation water.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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