

The impact of simulated nitric and sulfuric acid precipitation on five North American dandelion growth parameters

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ABSTRACT

Acid precipitation is a prevailing environmental problem caused by many man-made and naturally occurring processes. Growth characteristics of the North American dandelion (*Taraxacum officinale*) were analyzed after 8 weeks of exposure to either distilled water, simulated rainwater (pH 5.6) or simulated nitric, sulfuric, and hydrochloric acid rain at pH 4.4 and 4.2. Statistical analyses determined that acid treatment (HNO₃, H₂SO₄, or HCL) as well as pH (4.2, 4.4, 5.6, or 7.0) of the treatment had significant effects on number of leaves produced, longest leaf length, and leaf dry mass. Additionally, two-way interaction effects between acid treatment showed that plants exposed to H₂SO₄ at pH 4.4 produced more leaves, had a higher mean leaf mass and higher total plant biomass than plants produced in other pH 4.4 treatment groups. HNO₃ pH 4.2 treatment plants produced more leaves, had higher mean leaf, root, and total plant biomass than plants grown with different acid treatments at pH 4.2. Finally, dandelions treated with HCl pH 4.2 and pH 4.4 produced the longest living leaves compared to other pH and acid treatment combinations. These results demonstrate that in most circumstances dandelions treated with low pH acid solution (4.2 and 4.4) appear to outperform those treated with distilled (pH 7.0) or rainwater (pH 5.6). In addition, no visible morphological effects, such as necrosis or chlorosis were observed in plants treated with any of the simulated acid precipitation treatments while growing in the greenhouse for the duration of the study. It is possible that these results are in part due to safety mechanisms many plants possess to physiologically minimize damage arising from environmental stress, thus allowing them to survive when conditions are unfavorable.

Keywords: *Taraxacum officinale*, stress resistance, ATP synthase, cellular cation exchange

INTRODUCTION

Acid rain refers to precipitation, of any form, with acidic elements [1]. Sulfur dioxide and nitrous oxide are often cited as the primary causes of acid precipitation and originate from various human activities including: the burning of fossil fuels [1], use of vehicles and heavy machinery [1], and process industries [2]. Moreover, there are several other naturally occurring processes that contribute to the formation of acid precipitation, including sulfur pollutants present in the ocean and volcanic eruptions [2]. When pollutants are emitted into the atmosphere, they are transported by wind [2], interact with vapors and sunlight to form sulfuric (H₂SO₄) and nitric acid (HNO₃), and then mix with water and other natural matters before falling to the ground as precipitation [1, 2].

Acid precipitation is notably harmful because it can cause acidification of the soil, atmosphere, and water [3]. These alterations not only have many wide-ranging effects to the environment but also its biotic occupants, with plants being especially prone to physical and chemical change from acid rain exposure. For example, changes in soil/soil water pH may lead to drastic changes in cellular nutrient content, water balance, lipid peroxidation, and antioxidant system function in plants [3, 4]. Other negative effects of acid precipitation exposure include cell membrane damage, reduced epicuticular wax deposition on leaves, necrosis, changes to leaf morphology (that can negatively impact gas exchange), premature abscission, and delayed flowering phenology. [3-7].

Several studies have also examined the impact of simulated acid rain on leaf chlorophyll production. Du et al. [8] conducted a study exposing over 60 terrestrial plant species to simulated acid rain. Results of this study showed that direct simulated acid rain treatment (with varying pH) to plants (both woody and herbaceous) significantly reduces leaf chlorophyll levels [8]. Fan and Wang [6] provided further evidence that chlorophyll formation may be decreased due to acid exposure in their study examining the effects of acid precipitation on five different woody plant species. These findings are significant because leaf chlorophyll content can be used as an effective way to quantify the correlation between foliage damage and plant productivity [8]. All these studies investigate the impacts of acid precipitation on plant species with no known resistance to anthropogenic contamination, and it is therefore no surprise that these plants solicit a negative response to this environmental stressor. Very few studies have been performed to examine whether similar negative effects are seen in plant species known to exhibit resistance to environmental contamination (sunflowers, *Helianthus annuus*, being an exception) [9].

Dandelions (*Taraxacum officinale*; Weber Asteraceae) are widely considered a model organism for serving as biomonitors of pollutants and other environmental stressors for several reasons. First, these plants have a wide global distribution and are prevalent in a variety of habitats including alpine, arctic, and tropical environments [10]. Second New World dandelions are triploid ($3n = 24$) agamospermous apomicts [11, 12] and therefore produce large quantities of seed which can easily be grown in the laboratory or the field [13, 14]. Lastly, dandelions are known to resist, and in some circumstances bioaccumulate, several types of anthropogenic contaminants including acute and chronic radiation [15, 16], polycyclic aromatic hydrocarbons (PAHs) [17, 18], and atmospheric particulate and soil heavy metals [19, 20]. The ability of dandelions to resist such a variety of environmental stressors make them an ideal candidate species for studying the effects of acid precipitation exposure in plants. Given their innate ability to survive conditions many other plant species cannot, we hypothesized that exposure to sulfuric and nitric acid precipitation will have minimal impact on dandelion growth.

MATERIALS AND METHODS

Field sampling

Dandelion seeds were collected from populations growing in Springfield, Ohio (39.9190° N, 83.8084° W). Upon collection, seeds from individual plants (N = 100 flowers, ~200 seeds/flower) were sealed in separate marked plastic bags, placed on ice, and transported to the laboratory and stored at 4 °C.

Seed germination/simulated acid precipitation

Dandelion seeds were planted in 300, 8 x 9 cm pots each containing 2-3 seeds and PROMIX® potting soil. Germinated seeds were grown for 4 weeks in a temperature-controlled greenhouse (mean daily temperature of 30.4 °C) where plants were watered daily (with a hose) during the initial growth phase. Greenhouse temperature was monitored daily, and window panels were opened when the temperature exceeded 35 °C. Insects and pests were controlled prior to the beginning of this study using a commercial insect fogger.

Simulated acid precipitation was made using sulfuric (36N), nitric (15N), and hydrochloric (12N) acid (Certified ACS Plus, Fisher Chemical | Fisher Scientific) and distilled water. Three simulated acid precipitation solutions (per treatment group) were used in this study: pH 4.2 and pH 4.4, representative of the high and low pH range for acid rain, along with pH 5.6 to represent the pH of normal rainwater [1]. The hydrochloric acid treatment group served as a negative control for this study (hydrochloric acid is used as the negative control because it is not a naturally occurring form of acid precipitation like nitric and sulfuric acid which mimic acid rain conditions), while distilled water (pH 7.0) was applied to one treatment group of plants as a positive control.

At the conclusion of the 4-week growth period, pots were split into 3 groups of 100 and each group received a different simulated acid precipitation treatment (nitric, sulfuric; or hydrochloric acid). Each of these treatment groups were further divided into groups of 25 (N= approximately 200 plants/group). Using micropipettes, one group was treated (at the soil / leaf rosette interface) with 2 ml of distilled water (positive control), another group with 2 ml of simulated rainwater (pH 5.6), while the third and fourth groups were treated with 2 ml of pH 4.2 and pH 4.4 simulated acid rain respectively. Plant leaves were also treated with a foliar spray using the same solutions as described above. All treatments were applied twice weekly for a duration of 8 weeks.

Observed dandelion growth parameters

All plants (N=630) were monitored for changes in color and visible signs of acid precipitation damage (e.g., necrosis and chlorosis) and these changes were recorded and photographed prior to harvesting. At the conclusion of 8 weeks of growth under simulated acid treatment, the total number of leaves and the longest living leaf on each plant were measured (cm) and recorded. All plants

were then dried and weighed individually, and whole plant dry mass (g), root dry mass (g), and leaf dry mass (g) were recorded. All of the above variables were used as measures of dandelion health because each is associated with fecundity and/or viability [20, 21].

Statistical analysis

Statistical analysis was performed using Minitab® (version 21.2) for Windows. Analyses of variance (ANOVA) [22] were utilized to examine the effects of simulated acid precipitation treatment (pH 4.2, 4.4, 5.6 and the distilled water control) on dandelion growth characteristic data (number of leaves, longest leaf, whole plant dry mass, leaf dry mass, and root dry mass). Growth characteristic datasets were log₁₀-transformed so that measured values would have normal distributions. An unbalanced ANOVA design was used because the total number of plants grown and harvested in each group were unequal. Bonferroni post-hoc tests were performed to compare means within an acid precipitation/growth character comparison if a significant difference was detected. All tests included Minitab® identified outliers and were deemed significant if $P \leq 0.05$.

RESULTS AND DISCUSSION

Growth Characteristic Descriptive Data

HNO₃ Treatment Group

Dandelions treated in nitric acid at varying pH levels produced between 1 and 40 leaves with 4.2 pH HNO₃ producing the plant with the greatest total number of leaves. 4.2 pH HNO₃ also produced plant with that greatest total dry mass, which ranged between 0.003 and 7.62 g, and the plant with the greatest total dry leaf mass, ranging from 0.001-2.873 g for plants in this treatment. Longest leaf ranged from 0.300 and 26.10 cm, distilled water (7.0 pH) produced the plant with the longest leaf length. Total root dry mass ranged from 0.002-5.059 g with 4.4 pH HNO₃ producing the largest total dry root mass of all plant within this treatment (Table 1). Leaf morphology of plants treated with HNO₃ simulated acid rain showed little to know signs of distress and necrosis. Means of plants treated with HNO₃ indicates that 4.2 pH treatment produced the largest total number of leaves, whole plant dry mass, and leaf dry mass, while 4.4 pH treatment produced the greatest root dry mass, and distilled water produced the greatest mean longest leaf length [Table 1].

H₂SO₄ Treatment Group

H₂SO₄ produced a total number of leaves from 1-70 with the 4.2 pH H₂SO₄ treatment group producing the greatest number of leaves on a single plant. The 4.2 pH H₂SO₄ treatment also produced the greatest leaf mass which ranged from 0.001 to 4.649 g for all plants treated with H₂SO₄, regardless of pH. Longest leaf length ranged from 0.5-21.80 cm for all plants within this treatment group, simulated rainwater (5.6 pH) produced the longest length of a leaf among the other acid levels. Total plant dry mass for plants within the H₂SO₄ treatment ranged from 0.014-8.911 g where 4.4 pH solution produced the plant with the greatest mass. 4.4 pH acid treatment also produced the plant with the greatest root mass which ranged from 0.004-4.929 g for all plants in this treatment (Table 2). Leaf morphology of plants treated with H₂SO₄ simulated acid rain showed little to know signs of distress and necrosis. Means of plants treated with H₂SO₄ indicates that 4.4 pH treatment produced the largest total number of leaves, whole plant dry mass, and leaf dry mass while the rainwater treatment (pH 5.6) produced plants with the greatest root dry mass and mean longest leaf length [Table 2].

HCl Treatment Group

The negative control, HCl, produced a total number of leaves from 2-32 with the distilled water (7.0 pH) treatment group producing the greatest number of leaves on a single plant. The 7.0 pH HCl treatment also produced the greatest leaf mass which ranged from 0.002 to 3.1900 g for all plants treated with HCl (regardless of pH). Additionally, distilled water produced the plant with the greatest total dry mass ranging from 0.004-7.349 g for all plants within the HCl treatment group. HCl also produced the plant with the largest root dry mass, which was between 0.001 and 4.4330 g. Longest leaf length ranged from 0.60-26.8 cm for all plants

Table 1. Growth Characteristic Descriptive Data for HNO₃ Treatment. Range, mean, and standard error mean for dandelions treated with varying pH levels of HNO₃.

pH	Total Number of Leaves			Longest Leaf (cm)			Total Dry Mass (g)			Total Leaf Dry Mass (g)			Total Root Dry Mass (g)		
	Range	Mean	SE	Range	Mean	SE	Range	Mean	SE	Range	Mean	SE	Range	Mean	SE
4.2	1-40	10.00	1.02	0.300-21.60	8.896	0.909	0.003-7.62	1.507	0.248	0.001-2.873	0.625	0.107	0.002-5.059	0.853	0.155
4.4	2-22	8.946	0.611	1.20-21.9	8.304	0.674	0.051-5.871	1.382	0.817	0.007-2.446	0.4466	0.0685	0.027-4.221	0.948	0.131
5.6	2-30	8.11	1.19	1.10-26.10	8.59	1.19	0.011-7.147	0.950	0.234	0.002-2.2630	0.3634	0.0895	0.009-4.407	0.563	0.138
7.0	2-17	7.548	0.750	2.40-20.40	10.75	1.16	0.007-5.355	0.849	0.207	0.002-2.335	0.2769	0.0858	0.004-2.93	0.531	0.131

Table 2. Growth Characteristic Descriptive Data for H₂SO₄ Treatment. Range, mean, and standard error mean for dandelions treated with varying pH levels of H₂SO₄.

pH	Total Number of Leaves			Longest Leaf (cm)			Total Dry Mass (g)			Total Leaf Dry Mass (g)			Total Root Dry Mass (g)		
	Range	Mean	SE	Range	Mean	SE	Range	Mean	SE	Range	Mean	SE	Range	Mean	SE
4.2	1-70	8.42	1.19	0.50-19.0	6.871	0.707	0.014-7.914	1.222	0.212	0.001-4.649	0.504	0.102	0.004-3.230	0.689	0.117
4.4	4-33	14.96	1.06	0.70-20.90	10.616	0.707	0.047-8.911	1.655	0.242	0.005-3.985	0.966	0.130	0.017-4.929	0.678	0.122
5.6	3-33	12.233	0.801	3.40-21.80	12.367	0.601	0.031-8.388	1.569	0.194	0.010-3.665	0.892	0.113	0.020-4.442	0.6973	0.0963
7.0	3-20	9.393	0.468	1.00-21.30	10.710	0.620	0.021-5.363	1.202	1.61	0.006-2.718	0.5382	0.0765	0.014-2.841	0.6778	0.0952

within this treatment group, 4.2 pH treatment produced the greatest length of the longest leaf among the other acid levels (Table 3). Leaf morphology of plants treated with H₂SO₄ simulated acid rain showed little to know signs of distress and necrosis. Means of plants treated with HCl indicates that 4.4 pH treatment produced the greatest leaf and root dry mass and the longest leaf length while distilled water (7.0 pH) produced plants with the greatest total number of leaves and whole plant dry mass [Table 3].

Table 3. Growth Characteristic Descriptive Data for HCl Treatment. Range, mean, and standard error mean for dandelions treated with varying pH levels of HCl.

pH	Total Number of Leaves			Longest Leaf (cm)			Total Dry Mass (g)			Total Leaf Dry Mass (g)			Total Root Dry Mass (g)		
	Range	Mean	SE	Range	Mean	SE	Range	Mean	SE	Range	Mean	SE	Range	Mean	SE
4.2	2-24	8.429	0.647	0.70-26.8	10.465	0.791	0.028-3.078	0.98	0.105	0.002-1.218	0.3135	0.0393	0.007-2.3990	0.6789	0.0764
4.4	2-20	7.083	0.745	0.70-22.5	11.236	0.969	0.043-4.718	1.316	0.201	0.008-2.6290	0.5122	0.0973	0.023-2.506	0.816	0.118
5.6	4-23	9.358	0.475	2.50-17.30	7.822	0.428	0.0450-3.5980	0.8332	0.0855	0.009-1.6390	0.3225	0.0393	0.0180-2.5080	0.5387	0.0584
7.0	2-32	10.048	0.759	0.60-18.50	6.794	0.548	0.004-7.349	1.039	0.152	0.002-3.1900	0.4240	0.0660	0.001-4.4330	0.6045	0.0961

Statistical Analyses

A two-way ANOVA of the entire dataset revealed the effects of acid treatment (HNO₃, H₂SO₄, or HCL) and pH (4.2, 4.4, 5.6, and 7.0) on total number of leaves, longest leaf length, whole plant dry mass, root dry mass, and leaf dry mass. Acid treatment had a significant effect on number of leaves produced ($p = 0.000$), longest leaf length ($p = 0.035$), and leaf dry mass ($p = 0.000$). Significant effects of pH were seen on number of leaves produced ($p = 0.041$), longest leaf length ($p = 0.001$), whole plant dry mass ($p = 0.001$), leaf dry mass ($p = 0.001$), and root dry mass ($p = 0.011$). Further, there were significant interactions between acid treatment*pH for number of leaves produced ($p = 0.000$), length of longest leaf ($p = 0.000$), whole plant dry mass ($p = 0.009$), leaf dry mass ($p = 0.003$), and root dry mass ($p = 0.010$). Given the above, all variables in the two-way ANOVA to exhibit significant differences were further examined with post hoc analyses to determine the nature of these differences.

Acid treatment only appeared to impact leaf growth characteristic data. Post hoc analysis showed that the number of leaves produced by plants exposed to H₂SO₄ ($\mu = 11.08$) treatments differed significantly from those produced in HNO₃ ($\mu = 8.83$) and HCl treatments ($\mu = 8.93$; $p = 0.000$). The longest leaves produced by plants exposed to H₂SO₄ ($\mu = 10.13$ cm) treatments also differed significantly from those exposed to both the HNO₃ ($\mu = 8.97$ cm) and HCL treatments ($\mu = 8.80$ cm; $p = 0.035$). Lastly, leaf dry mass of plants exposed to H₂SO₄ ($\mu = 0.71$ g) treatments differed significantly from those produced in HNO₃ ($\mu = 0.45$ g) and HCl treatments ($\mu = 0.37$ g; $p = 0.000$).

pH of the simulated acid precipitation influenced leaf, root, and total plant growth characteristic data. Post hoc analyses revealed that a significant difference in the number of leaves produced was present between plants treated with pH 4.4 ($\mu = 10.56$) and pH 4.2 solutions ($\mu = 8.88$; $p = 0.041$). No significant differences were seen in leaf number between plants grown in all other pairwise pH solution comparisons. Differences in longest leaves produced were seen between plants in pH 5.6 ($\mu = 9.67$ cm) and pH 4.2 ($\mu = 8.78$ cm; $p = 0.001$) treatment groups, and between plants treated with pH 4.4 ($\mu = 9.86$ cm) and pH 4.2 ($\mu = 8.78$ cm; $p = 0.001$) solutions. Significant differences in longest leaves were not apparent in all other pairwise pH solution comparisons. Similar differences across all pairwise pH treatment comparisons were seen in all plant mass measurements. Leaf dry mass was significantly different when comparing plants treated with distilled water ($\mu = 0.44$ g) and the pH 4.4 ($\mu = 0.64$ g) solutions and when comparing pH 4.2 ($\mu = 0.47$ g) and pH 4.4 ($\mu = 0.64$ g; $p = 0.001$) treatment groups. Similarly, root dry mass of plants exposed to distilled water ($\mu = 0.62$ g) differed from those exposed to the pH 4.4 solutions ($\mu = 0.82$ g) while plants treated with pH 4.2 ($\mu = 0.73$ g) also differed from those treated with the pH 4.4 solutions ($\mu = 0.82$ g; $p = 0.010$). Lastly, total plant dry mass

was significantly different when comparing plants treated with distilled water ($\mu = 1.06$ g) and the pH 4.4 ($\mu = 1.46$ g) solutions and when comparing pH 4.2 ($\mu = 1.20$ g) and pH 4.4 ($\mu = 1.46$ g; $p = 0.001$) treatment groups.

Significant pH*acid treatment effects were shown for total number of leaves produced, longest leaf, leaf dry mass, root dry mass, and total plant dry mass ($p \leq 0.05$ for all tests). Plants exposed to the H_2SO_4 pH 4.4 treatment produced more leaves and had a higher mean leaf mass and total plant biomass than plants in the other pH 4.4 treatment groups (although HNO_3 pH 4.4 treatment plants produced a higher root dry mass). HNO_3 pH 4.2 treatment plants produced more leaves and had higher mean leaf, root, and total plant biomass than plants grown in the other pH 4.2 treatment groups. Dandelions grown HCL pH 4.2 and HCL 4.4 treatment groups produced the longest living leaves compared to all other pH*acid treatment combinations. Differences in rainwater and distilled water treatments across acid treatment groups may be due to greenhouse environmental conditions (i.e., greenhouse microclimate variation)

Dandelions treated with sulfuric, nitric, and hydrochloric acid in this study showed little growth impairment and, in some cases, appeared to grow better than those treated with distilled water and rainwater. Most of the growth variation in this study was seen in the measured dandelion leaf parameters. Analysis of variance determined that acid treatments (HNO_3 , H_2SO_4 , or HCL) had significant effect on number of leaves produced, longest leaf length, and leaf dry mass. Plants exposed to H_2SO_4 produced more leaves ($\mu = 11.08$) than those treated with HNO_3 ($\mu = 8.83$) or HCL ($\mu = 8.93$) and distilled ($\mu = 8.99$) or rainwater ($\mu = 9.90$). HCL treated plants produced the longest living leaves ($\mu = 10.85$ cm), followed by those exposed to H_2SO_4 ($\mu = 8.74$ cm) and HNO_3 ($\mu = 8.60$ cm). Plants exposed to distilled water ($\mu = 9.42$ cm) produced longer leaves than those in the H_2SO_4 and HNO_3 treatments while rainwater treated plants produced the shortest leaves overall ($\mu = 7.02$ cm). Lastly leaf dry mass was greatest in plants exposed to H_2SO_4 ($\mu = 0.71$ g) followed by HNO_3 ($\mu = 0.45$ g) and HCL ($\mu = 0.37$ g). Distilled water ($\mu = 0.41$ g) and rainwater ($\mu = 0.43$ g) treated plants produced dry weight measurements similar to those exposed to HNO_3 and greater than those treated with HCL.

pH of the treatment solution (4.2, 4.4, 5.6, and 7.0) yielded similar statistical outcomes to those seen with acid treatment. pH of simulated acid precipitation influenced leaf, root, and total plant growth. Analyses indicated a significant difference in the number of leaves produced when plants were treated with a pH of 4.4 and pH of 4.2 ($4.4 > 4.2$). Differences in the longest living leaf produced were seen between plants treated with a pH 5.6 and pH 4.2 ($5.6 > 4.2$) when compared to plants treated with pH 4.4 and pH 4.2 ($4.4 > 4.2$). Leaf dry mass was significantly different for plants treated with distilled water and the pH 4.4 (DI water < 4.4) solution compared to pH 4.2 and pH 4.4 ($4.2 < 4.4$) treatment groups. Likewise root dry mass of plants treated with distilled water as well as with pH 4.2 solutions differed from those treated with pH 4.4 solutions ($4.4 > 4.2 > DI$ water). Total plant dry mass was significantly different for plants treated with distilled water and the pH 4.4 treatment ($4.4 > DI$ water) compared to pH 4.2 and pH 4.4 treatments ($4.2 < 4.4$).

Finally, significant two-way interaction effects between ACID TREATMENT and pH were shown when comparing total number of leaves produced, longest leaf, leaf dry mass, root dry mass, and total plant dry mass. Plants exposed to H_2SO_4 at pH 4.4 produced more leaves and had a higher mean leaf mass and total plant biomass than plants produced in other pH 4.4 treatment groups. HNO_3 pH 4.2 treatment plant produced more leaves and had higher mean leaf, root, and total plant biomass than plants grown with different acid treatments at pH 4.2. Dandelions treated with HCL pH 4.2 and pH 4.4 produced the longest living leaves compared to other pH and acid treatment combinations.

The above results demonstrate that in most circumstances dandelions treated with low pH acid solution (4.2 and 4.4) appear to outperform those treated with distilled (pH 7.0) or rainwater (pH 5.6). Dandelions grown in this study also produced more leaves (and therefore more leaf mass) in the H_2SO_4 treatment group when comparing all three acid treatments ($H_2SO_4 > HNO_3 > HCL$). Plants grown in the H_2SO_4 treatment even outperformed those exposed only to distilled or rainwater. All plants, regardless of acid treatment or pH showed no signs of distress (e.g., chlorosis or necrosis) while growing in the greenhouse for the duration of the study. One possible explanation for these results is that many plants have safety mechanisms in place to physiologically minimize the damage that can occur from environmental stressors, allowing them to survive when conditions are unfavorable. For example, studies have indicated that dandelions are able to withstand a lack of water without significant effect on their photosynthetic rate [23] and show increased stability and the ability to recover and regrow after drought occurrence [24]. Dandelions have also been found to increase seed production and normally develop seeds in the presence of increased traffic intensity [25]. These plants have been implicated in having a higher tolerance to herbivory and show the competitive ability to perform successfully in environments of varying climatic conditions, meaning they are able to colonize in fluctuating temperatures [26]. Lastly, dandelions can tolerate high concentrations of metals in their root and leaf tissues that have been sequestered from the environment [14, 19].

There are several possible physiological explanations for dandelion resistance to acid precipitation damage. For example, at acidity levels of pH 4.5 or higher, the chloroplast organelle can remain uncharged and increase the expression of six chloroplast ATP synthase subunits [27]. Enhanced ATP synthase activity can lead to an increased rate of photosynthesis, and growth in plants. At a lower pH (< 4.0), the chloroplast structure is destroyed, and therefore growth can be inhibited [28]. In fact, crops like rice and soybeans have been noted for their ability to tolerate low acidity stress (pH 4.5) by increasing the activity of PM H⁺-ATPase, resulting from an upregulation of PM H⁺-ATPase gene at transcriptional level [27]. Plasma membrane H⁺-ATPase

is an enzyme protein located in the plasma membrane of plants and fungi. It is the most abundant membrane protein as a single strand polypeptide that is about 100 kDa and is classified as a P-type ATPase [29]. Using ATP, this ATPase is integral in forcing protons from plant cells to generate an electrochemical proton gradient. This gradient is important for providing energy for secondary ion transport across the plasma membrane thus enabling physiological functions like nutrient uptake, intracellular pH regulation, stomatal opening, and cell growth [29]. By removing protons from the cell and generating membrane potential, this pump is able to energize the plasma membrane a prerequisite for growth [30]. The primary function of this enzyme is to regulate intracellular pH and nutrient uptake, implicating its involvement in regulating plant response to environmental stressors such as extreme temperature, salt, drought, phosphorus deficiency, and acid rain [27]. Dandelions exposed to simulated acid precipitation were likely associated with higher activity of the PM H⁺-ATPase, leading to increased gene expression and protein abundance. Additionally, this would mean that dandelions were able to maintain physiological H⁺ and charge gradients across the plasma membrane contributing to the more rapid growth of the dandelions under our experimental conditions.

Cellular cation exchange may be another possible explanation as to why dandelions did not show diminished growth in the 4.2 and 4.4 acid pH treatment groups in this study. Cation exchange is a soil property that describes the ability of a positively charged ion (e.g., H⁺) to be exchanged for another positively charged ion (e.g., Al³⁺, Ca²⁺, K⁺, Mg²⁺, Na⁺, etc.) attached to the surface of negatively charged soil components such as clay, organic matter, or humus [31]. This natural phenomenon normally occurs when plant roots actively release hydrogen ions or CO₂ (which dissolves in soil water to become HCO₃⁻) from their root hairs, thus lowering the pH of the soil. The hydrogen ions in the soil are strongly attracted to negatively charged soil particles and “exchange” places with any attached cations, making them available for root uptake by the plant. This process is stimulated by acid precipitation as it leaches through the soil and enters into exchange reactions with cations present in the soil cation exchange complex, essentially stripping the soil of its mineral elements [32]. A plant cell wall possesses a strong net negative charge based on its structural components [33] and thus binds mineral cations much like negatively charged soil particles. In this study, it is possible that the pH of dandelion root and leaf tissues decreased as the simulated acid precipitation was taken up by the plants. This would in turn stimulate internal cellular cation exchange, thus releasing positively charged mineral elements to be used by the plant for stimulated growth and development.

Analysis of the growth characteristics (total plant dry mass, root dry mass, leaf dry mass, number of leaves, and longest leaf length) indicated that under acidic conditions dandelions treated in lower acid concentrations had increased growth rates compared to those treated with deionized and rainwater. The minimal impact of acid precipitation was also verified by a lack of visible plant damage (e.g., necrosis or chlorosis) after six weeks of treatment exposure. These observations do not corroborate with what was expected in our hypothesis as dandelions treated with H₂SO₄ outperformed dandelions treated with HNO₃ and HCl, and HCl (our negative control) did not appear to cause any adverse effects to dandelion growth. These results further support the ability of dandelions to survive significant exposure to environmental toxins like acid precipitation likely due to their ability (in this circumstance) to perform internal cation exchange and/or use plasma membrane ATPase pumps that function to counteract nonoptimal varying environmental/cellular conditions. These findings provide additional information about the role dandelions play in the environment, and further support their ability to thrive in environmental conditions of distress, both of which are valuable resources for future research and plant conservation efforts.

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