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Exposure of Vegetation to Anhydrous Ammonia Vapor in an Environmental Chamber

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The objectives of this work were to develop a method to study the response of vegetation upon exposure to anhydrous ammonia vapor and to identify potential influences plants may exert on each other under various environmental conditions in the laboratory. A method of processing plant tissues before and after exposure for use in proteomic studies is also described. Results suggest that ammonia vapor release can damage several critical physiological processes in vegetation. Recommendations include conducting further studies with vegetation exposed to ammonia vapor under various environmental conditions as well as identifying and comparing relevant biomarkers expressed at specified experimental time points.

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PREFACE

The work described in this report was authorized by the U.S. Department of Homeland Security, Science and Technology Directorate, Chemical Security Analysis Center (Aberdeen Proving Ground, MD). The work was started in February 2021 and completed in August 2021.

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1. INTRODUCTION

1.1 Background

The U.S. Army Combat Capabilities Development Command Chemical Biological Center (DEVCOM CBC; Aberdeen Proving Ground, MD) was tasked with researching and making recommendations on how to quantify and referee the release of anhydrous ammonia under various environmental conditions, including how an ammonia release could affect vegetation. The work took place on Aberdeen Proving Ground in the CBRNE Assessment, Science and Technology Laboratory (CASTLE) within the Advanced Chemistry Laboratory from February 2021 through August 2021. The overarching objective of this work by DEVCOM CBC was to support the U.S. Department of Homeland Security in an effort to better understand how a catastrophic release of ammonia could affect surrounding vegetation and, ultimately, the biomarkers within the vegetation that are present after an ammonia release. DEVCOM CBC researchers therefore simulated a large-scale ammonia release within a controlled environmental chamber to initiate a biological cascade within vegetation. After exposure to ammonia, the vegetation exhibited changes in physical appearance that represent shifts occurring in the molecular signaling pathways. Data and results from this effort will be used to inform future analysis.

Ammonia is an inorganic, alkaline compound found in nature and in fabricated materials. The majority of ammonia in the atmosphere is produced from the breakdown of organic matter such as manure, dead plants, and dead animals.[1] In recent years, atmospheric ammonia levels have been rising steadily.[2] Agricultural operations contribute the bulk of the ammonia present in the atmosphere, and the rest are from industrial and vehicular emissions.[2] In the atmosphere, ammonia reacts with acidic pollutants to form particulates in the air. These airborne particulates are a significant source of air pollution that negatively impacts human health, the climate, biodiversity, and the environment as a whole.[3]

Atmospheric and soil ammonia levels average around 1–5 ppb, depending on the region.[3] The lowest concentrations of atmospheric ammonia are in remote areas where there is little human and animal activity, such as the Arctic.[4] In contrast, higher levels of ammonia are found in regions of increased human activity. This is particularly true in localities where agricultural farming is abundant due to the widespread use of ammonia-based fertilizers.[5] Regardless of the source, all particles in the atmosphere will eventually fall onto the earth's surface through processes known as wet or dry deposition. In wet deposition, particles are washed out from the atmosphere via precipitation such as rain or snow. Conversely, the deposition of particles directly from the atmosphere to the surface without precipitation is referred to as dry deposition.[6] The deposition of atmospheric particles is influenced by many environmental factors, both natural and fabricated, which leads to challenges in accurately modeling these deposition processes.[7]

Once particles have left the atmosphere and deposited onto the earth's surface, their fate is further influenced by the environmental conditions where they land. The transport, adsorption, and desorption of ammonia in a microclimate depends on the context and influential components within the area of interest. For example, the wind velocity in an area can influence the amount of particles either reaerosolizing back into the atmosphere or depositing onto the earth's surface, which is composed mainly of bodies of water (including glaciers), land, and vegetation cover.[8] Interactions between the particles and environmental elements influence the transport and deposition behaviors of ammonia particles.[3] In water, the ammonium ion equilibrium is dependent upon the pH. Certain pH conditions can lead to the release of ammonia back into the atmosphere as a gas or vapor, which is also known as volatilization. Ammonia particles can also attach to other particles in the water. On land, if ammonia does volatilize out of the soil and into the atmosphere, it can eventually adsorb to particulate matter within the soil or be taken up by microorganisms and transformed to nitrate or nitrite anions.[3] To determine ammonia's fate and impact within an area requires accurate measurements of ammonia levels within a microenvironment.

The relationship between ammonia and vegetation is complex, and it may be the most challenging aspect of studies that aim to quantify ammonia levels in the atmosphere. Parameters have been developed and refined over time. These include methods for measuring the total emission of ammonia gas exchange over vegetation,[9] the resistance of the stomata and leaf surfaces, and the leaf area index.[10] Current methods for quantifying the emission and deposition of ammonia involve measurements, satellite remote-sensing, and modeling.[11] However, most of those methods are focused on assessing ammonia's impact on a larger scale and not within a certain region. A localized, catastrophic release of ammonia is not immediately influenced by the regional environment in the same way that naturally occurring and normal human centric releases are. To better predict how ammonia will behave when released in an environment—controlled or uncontrolled—will require a deeper understanding into the complicated chemistry of particle and gas exchange occurring in vegetation.[12] Traditional mechanisms described above begin influencing the impact of ammonia after initial release and potential damage has occurred. Herein, we report the observational behavior of ammonia vapor in the presence of a fern species, *Polystichum acrostichoides*, via a simulated catastrophic release of ammonia vapor.

1.2 Ammonia Chemistry

Ammonia (NH_3) can exist as either a gas or a liquid, depending on its pressure and temperature. At room temperature, pure ammonia (also known as anhydrous ammonia) is a colorless gas with a distinctive, pungent odor.[13] Ammonia readily dissolves in water to form its ionized form, ammonium hydroxide (NH_4^+), which is corrosive at high concentrations. NH_3 and NH_4^+ exist and are in equilibrium; the ratio shifts to favor formation of one or the other, depending on the pH and temperature. An increase in pH and temperature will result in an increase of NH_3 and a decrease of NH_4^+. Conversely, a decrease in pH and temperature will favor the shift toward more NH_4^+ formation, as shown:[15]

$$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^- \quad (1)$$

1.3 Ammonia within the Environment

The majority of ammonia present naturally in the environment is generated by the waste of living organisms, particularly aquatic organisms. Other sources of ammonia in the environment are gas-exchange reactions occurring in the atmosphere, forest fires, organic matter decay, and nitrogen fixation processes.[15] Nitrogen is one of the three macronutrients required by plants for their successful growth and development. Plants cannot use nitrogen directly as an energy source; they must first convert it (via a nitrogen fixation process) into a usable form such as ammonia. Because ammonia is such a valuable source of nitrogen for plants, it is widely used as an agricultural fertilizer.

In the atmosphere, ammonia will react with sulfuric and nitric acids to form particulates. The majority of ammonia particles present in the air are associated with sulfate ions, since the reaction of ammonia with sulfuric acid is favored over its reaction with nitric acid.[12] In regions with lower levels of sulfate and higher levels of nitrogen oxide, the proportion of ammonium nitrate (eq 3) to ammonium sulfate (eq 2) will be greater.

$$H_2SO_4 + 2NH_3 \rightarrow (NH_4)_2SO_4 \quad (1)$$

$$HNO_3 + NH_3 \rightarrow NH_4NO_3 \quad (3)$$

The distribution and deposition of ammonia, along with its conversion products, is influenced by the interchange processes of ammonia and ammonium. Much of the research has focused on the deposition of ammonia in precipitation, but the dry deposition of ammonia and its interaction with regional components has not been as thoroughly explored.[12] To accurately characterize ammonia within an environment, it is important to consider the elements and their influence on ammonia deposition and distribution. The ways in which ammonia interacts with the surrounding vegetation, soil, and bodies of water affect transport and deposition of ammonia in the environment. Although outside the scope of this work, these effects can be partially taken into account via dense gas dispersion models. Similar scenarios are described in the literature.[16]

Ammonia oxidation processes commonly occur in organic-rich agricultural soils. However, the redox conditions that are present in topsoil are complex, which further complicates any accurate assessment of the behavior of ammonia in a region. Ground soil contains varied architectural features such as worm holes, cracks, and hollow stalks from dead vegetation.[14] In addition, there are fluctuations in moisture content, amount of organic matter, and concentrations of oxygen, carbon dioxide, and methane within the soil. These variables affect the behavior of ammonia within the environment by influencing whether the ammonia is metabolized into nitrite, nitrate, nitrous oxide, nitrogen gas, or microbial biomass.[3,17] These phenomena are important on the perimeter of release events and can be used to determine the extent of damage and influence that ammonia has on the local environment.

Past research on environmental ammonia has provided valuable data on how the behavior of ammonia is affected by industrial and agricultural processes in a region. However, there has been less research focused on characterizing the performance of ammonia within various types of soils. The effects of different soil properties on ammonia function have been

described in studies involving soil decontamination processes. In these studies, ammonia gas is introduced into the ground to elicit alkaline hydrolysis of soil contaminants. In one study, pH levels as high as 10.8 were measured.[14] Events such as ammonia-assisted soil decontamination or agricultural fertilizer application cause the ground to be directly permeated by ammonia. More common and widespread are the indirect routes that result in the ground presence of ammonia. Particular features of vegetation, such as roots, exert many nuanced effects upon the distribution and deposition of ammonia. Roots are responsible for the uptake and transport of water and minerals from the soil to the leaves of the plant. An abundance or restriction of available nutrients can influence gene expression and regulation, and such changes are capable of effecting physiological changes within the plant. In other words, environmental stressors are able to induce temporary or permanent changes in vegetation.[18,19] These changes could be significant enough to affect pathways involved in a plant's normal uptake of ammonia, and in turn, influence the behavior of ammonia associated with the vegetation within that area.

1.3.1 Relationship between Ammonia and Vegetation

In plants, vascular structures are responsible for transporting nutrients from the root system through the plant. If the normal functioning of these processes is disrupted, physiological and biochemical events are triggered. A change in a plant's physical appearance can indicate a change in the nearby environment, either in the atmosphere or in the soil. The toxicity threshold in vegetation varies among species. Although low levels of ammonia typically produce positive effects in plants, large concentrations of ammonia are toxic. In response, plants modulate their biochemical and physiological processes. Generally, the point at which ammonia becomes detrimental to the plant is when the amount of ammonia uptake surpasses the plant's assimilation and detoxification abilities.[19,20]

The driving forces for atmospheric ammonia uptake or emission are dependent on the concentration gradient between the ammonia that is present in the ambient air and within the plants. This relationship can be described in terms of the plant's compensation point, which refers to the concentration at which ammonia is absorbed or emitted by the plant. Ammonia levels above this compensation point result in uptake of ammonia by the plant, and the emission of ammonia from vegetation occurs when the concentration of ammonia in the air falls below the compensation point. Additional factors such as the amount of light in the area, pH conditions, and carbon dioxide in the atmosphere can also influence ammonia uptake.

Gas absorption or emission is mostly carried through stomatal processes. Stomata, which are pore-like structures present within most plant leaves, are responsible for gas and water exchange through a process called transpiration.[20,21] The stomatal structures open or close in response to their surroundings to optimize photosynthesis processes and internal water levels. This impacts the rate of transpiration in the plant, which in turn affects the plant's water and nutrient transport.[22]

Ammonia concentrations in plants can accumulate in levels critical enough to induce visual changes. One of these observable changes is the browning of plant leaves.[23] Damage can also occur in the plant roots and xylem tissues, which are important for water uptake and transport. As a result, normal water levels can be disrupted, leading to desiccation,

wilting, and stunting. Symptoms of ammonia toxicity include a dulling and darkening of the leaves, wilting, and desiccation. The leaf margins may start to turn brown, and this effect can eventually spread throughout the plant.[24,25] The underlying cause of this browning is the accumulation of naturally occurring phenolic compounds and their subsequent oxidation. These phenolic compounds are produced by plants as a defense response, particularly when the tissue is injured or stressed. In plants, biosynthesis of phenolic compounds is catalyzed by phenylalanine ammonia lyase enzyme (PAL). In proteomic studies on plant stress, an increase in PAL activity before and during tissue browning was identified.[25]

1.3.2 *P. acrostichoides*

The evergreen fern *P. acrostichoides* is capable of tolerating freezing temperatures and can maintain its vibrant, green-colored fronds all year round (including during winter). The fronds on *P. acrostichoides* are tapered at the top and typically grow about 30–80 cm long and 5–12 cm wide, as shown in Figure 1. Each frond has a dark-brown stipe that runs one-third of the frond's length and is covered in light, translucent, brownish-tan scales that are about 5 mm long. Each frond has 20–35 pairs of green, leathery textured pinnae. Fine, flexible spines are present along the edge of each pinna, oriented toward the pinna's tip.[26]

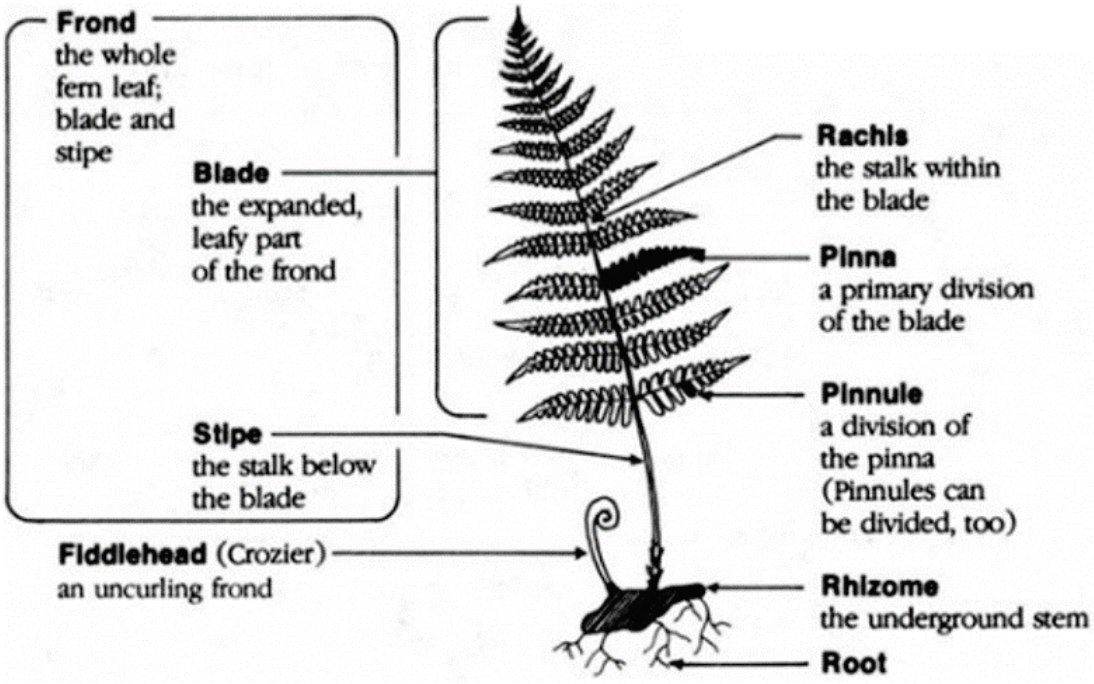

Figure 1. General anatomy of a fern.[27]

2. MATERIALS AND METHODS

2.1 Plant Preparation

A total of seven fern fronds with their roots attached were collected on the morning of 3 August 2021. The fronds averaged 10–13 in. in height. The pinnae on the fronds were uniformly green, glossy, and flexible. Before the experiment was started, the fern fronds were planted in square aluminum trays. The planting soil pH was measured by mixing a 1:1 ratio of soil and deionized water and then testing the solution with pH paper. The soil pH was measured to be around 7.0–7.5.

The ferns were divided into three groups (Table 1). Test 1 group (T1) was designated for short-term ammonia exposure, and Test 2 group (T2) was designated for long-term ammonia exposure. The third group of two ferns served as the control; it remained under ambient room conditions for the entirety of the experiment. All of the fern fronds were of similar size and appearance.

Table 1. Intended Testing Conditions for Fern Fronds Exposed to Ammonia Vapor

Test Group	Duration of Exposure (min)	Ammonia Concentration (ppm)	Temperature (°C)	Relative Humidity (%)
T1	2	25,000	5	75
T2	30	25,000	5	75
Control	N/A	N/A	20.8	59

N/A, not applicable.

2.2 Generation of Ammonia Vapor

Liquid NH_3 was collected via an anhydrous ammonia gas cylinder (Praxair; Danbury, CT). The gas cylinder had an internal educator tube and was directly connected to a jacketed addition funnel (without a regulator) to allow the flow of the liquid NH_3. An acetone–dry ice bath was added to the bottom half of the jacket of the Chemglass (Vineland, NJ) 25 mL jacketed addition funnel and equilibrated for at least 2 min. The liquid NH_3 in the addition funnel was poured into a small (25 mL) beaker that was sitting in a shallow acetone–dry ice bath in a Petri dish, directly below the jacketed addition funnel. The volume estimated from the 25 mL beaker was used in the experiments.

The beaker containing 10–12 mL of liquid ammonia was then transferred into the environmental chamber. A stainless steel tray was placed inside the environmental chamber and positioned in front of the plant to be exposed, as shown in Figure 2. The internal dimensions of the chamber were 38.5 × 30.5 × 21 in. (width × depth × height), which resulted in an internal volume of 404.1 L. The beaker containing 10–12 mL of liquid ammonia was then transferred into the environmental chamber. To create vapor, the liquid ammonia was poured from the beaker into the stainless steel tray. An estimation of the ammonia concentration for each test was calculated. Internal recirculating fans were operating during the exposure periods.

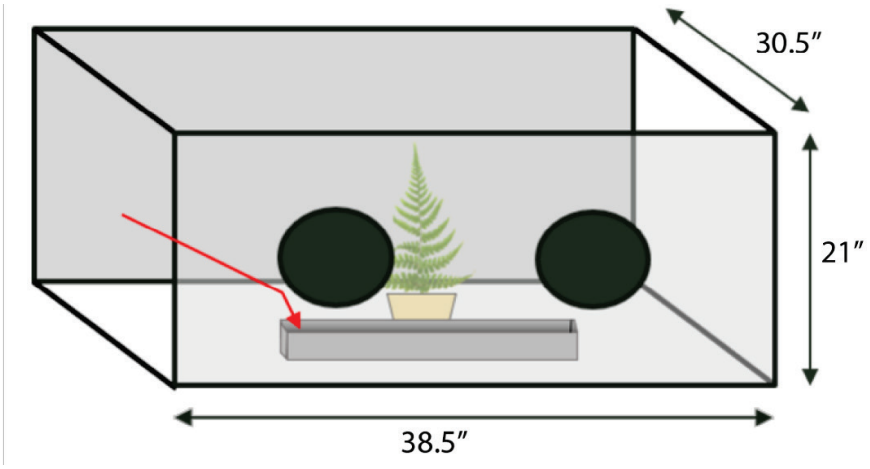

Figure 2. Schematic diagram of the experimental setup. Red arrow indicates ammonia placement within the chamber.

2.3 Processing Plant Tissue for Protein Extraction

Once the ammonia vapor exposure was complete, the fern fronds were removed from the chamber and placed in ambient room conditions for plant tissue processing. Plant tissue was harvested from the control and exposed ferns in biological triplicates. Pinnules were collected from each frond at predetermined time points throughout the experiment: pre-exposure to ammonia vapor, 0 min post-exposure, 30 min post-exposure, 1 h post-exposure, and 24 h post-exposure. To create the biological triplicates, three pinnules of similar size were cut away using needle-nose scissors. Pinnules were removed from the top, middle, and bottom of each fern frond. To avoid cross-contamination with ammonia, a laboratory wipe was wetted with water and used to wipe the blades of the scissors after each pinnule was cut. Each pinnule sample was weighed and individually transferred to a 2 mL centrifuge tube. An average weight of 20 mg was recorded for each pinnule sample.

To prepare the harvested plant samples, each sample was placed in a 2 mL centrifuge tube. Needle-nose scissors were used to cut up the samples inside the tube. The plant tissue was suspended in 1 mL of methanol containing a protease inhibitor cocktail (stock number P9599; Sigma-Aldrich; St. Louis, MO) to help mitigate proteolysis and preserve protein integrity. A dilute stock solution of the protease inhibitor cocktail (1000×) was prepared in advance, then 50 µL of this cocktail was diluted in 50 mL of methanol and mixed thoroughly. The dilute protease inhibitor cocktail stock solution was on dry ice when in use, and it remained in the freezer at all other times. The tube containing the plant tissue and protease inhibitor cocktail was vigorously shaken for 10 s. This process was performed on all three plant samples of each biological triplicate set.

3. RESULTS AND DISCUSSION

The results described herein show that high concentrations of ammonia do affect vegetation. The authors surmise that the extent to which the ammonia affects the vegetation will be realized when the biomarkers are investigated.

3.1 Chamber Exposure

The environmental chamber had known internal dimensions that resulted in an internal volume of 404.1 L. Therefore, an upper bound of the ammonia vapor concentration could be determined for each exposure. At least 10 mL of liquid ammonia was collected for each exposure. The conditions for each test are shown in Table 2.

Table 2. Experimental Conditions for Each Test of Ammonia Exposure to Ferns

Test	Ammonia Concentration (ppm)*	Temperature (°C)	Relative Humidity (%)
T1	23,000–28,000	3.8	78.5
T2	25,000–33,000	4.8	81.7

*Ammonia concentration range estimated based on uncertainty from reading ammonia level on the beaker.

The fern fronds collected for this experiment were similar in size and color. Figure 3 shows the plants in groups before they were exposed to ammonia. In Figure 4, the ferns are shown inside the environmental chamber prior to generation of ammonia vapor.

Figure 3. Designation of fern groups prior to exposure.

Figure 4. Fern fronds inside the chamber prior to exposure.

3.2 Post-Exposure

The fern fronds were exposed to ammonia for 2 or 30 min. Both exposure times resulted in visibly evident changes in the fern fronds with respect to their original color pre-exposure as well as in comparison to the unexposed control fern. The time-series images in Figure 5 show the ferns before and after exposure. Figure 5 (top) shows that the pre-exposed ferns had similar colorings and sizes. Figure 5 (middle) shows the appearance of the ferns at 10 min after ammonia exposure. Here, color differences between T1 and T2 ferns are evident. The browning that occurred in the longer-exposed T2 ferns was noticeably darker, suggesting that the damage sustained by the ferns in this experiment was influenced by the length of the exposure. After 1 h, differences in the brown coloring were more pronounced between the T1 and T2 ferns, as is evident in the Figure 5 (bottom) images.

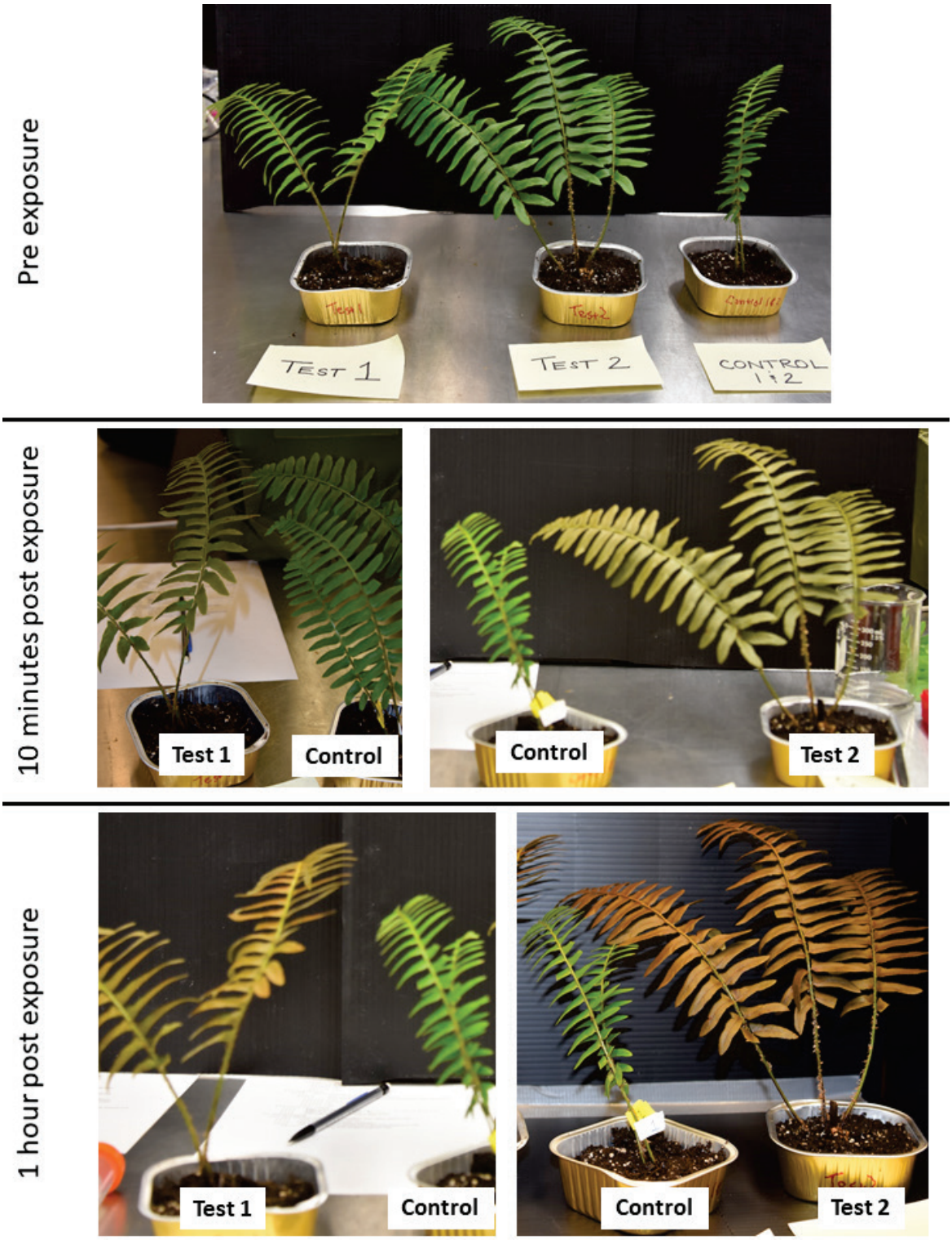

Figure 5. Time-series images for ferns pre- and post-exposure to ammonia.

Immediately post-exposure, the ferns were removed from the chamber, and the pinnules from each of the three groups were harvested (in biological triplicates) at predetermined time points over the next 24 h. Particular attention was given during harvesting to maintaining consistency in size among the plant tissues. Their weights were measured and recorded. The recorded weights at the designated time points for collection are summarized in Table 3.

Table 3. Weights of the Harvested Plant Tissues

Time Point	**Replicate**	**Weight (mg)**			
		Control 1	**T1**	**Control 2**	**T2**
Pre-exposure	1	21.7	20.96	19.77	19.05
	2	19.14	19.6	20.9	18.84
	3	19.55	20.51	18.61	22.15
	Average	20.13	20.36	19.76	20.01
10 min post-exposure	1	20.08	20.61	18.62	20.01
	2	20.55	21.9	18.89	21.14
	3	19.49	20.75	20.4	20.35
	Average	20.04	21.09	19.30	20.5
1 h post-exposure	1	18.72	17.7	19.87	21.64
	2	19.54	20.47	20.65	18.63
	3	19.58	21.55	19.01	18.01
	Average	19.28	19.91	19.84	19.43
24 h post-exposure	1	20.37	19.37	19	20.78
	2	22.63	20.64	20.25	21.58
	3	21.95	19.38	20.37	21.83
	Average	21.65	19.80	19.87	21.40

Cells were lysed via the freeze–thaw method. After the plant-tissue samples were chopped and suspended in the 1000× protease cocktail, all three samples of a biological triplicate set were simultaneously subjected to a 4 min freeze cycle in an acetone–dry ice bath followed by a 4 min thaw cycle in room-temperature water. The freeze–thaw cycle was repeated three more times for each set, resulting in a total of four freeze–thaw cycles per set. Afterward, the samples were placed in a –5 °C freezer for storage. On 16 August 2021, the samples were sent to Lawrence Livermore National Laboratory (Livermore, CA) for storage and additional analysis.

Within 1 h post-exposure, the plants started to brown, as shown in Figure 5. The degree of browning was noticeably darker on leaves in the T2 group as compared to leaves in the T1 group. Browning on the fronds at the 1 and 24 h time points is shown in Figures 6 and 7. A slight increase in the degree of darkening throughout the plants was noted at the 24 h time point. In addition, several small patches within individual pinnules appeared to have retained their green coloring. These green areas were mostly confined to regions closest to the stalk, as shown in Figure 7.

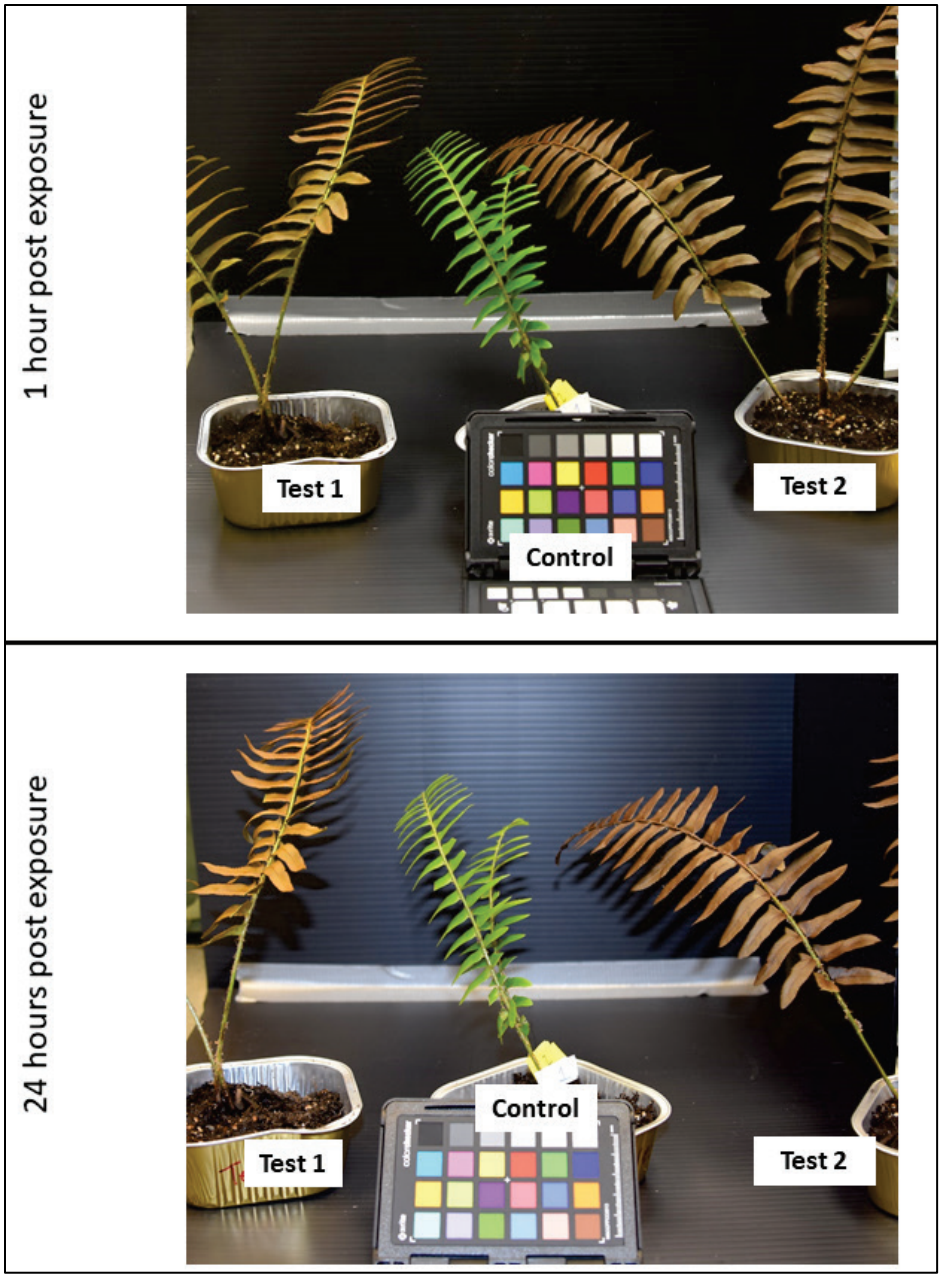

Figure 6. Ferns after ammonia exposure at 1 and 24 h.

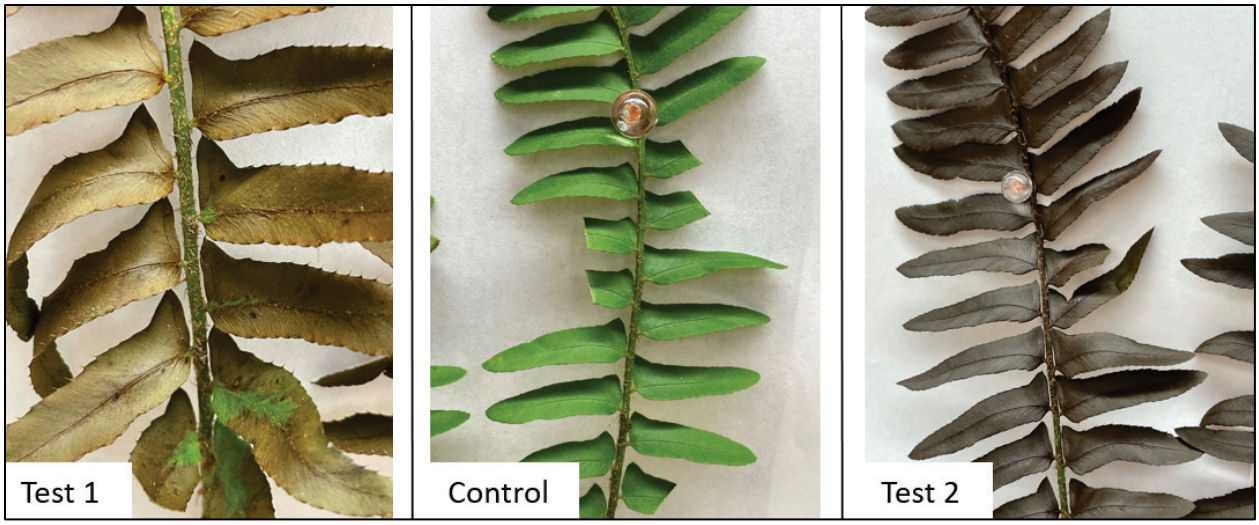

Figure 7. Appearance of stalks and leaves 24 h after ammonia exposure.

The remaining ferns were kept under ambient conditions for an additional week, but no further characterization methods were performed. Over a span of seven days, the water status of the plants continued to decrease, and further darkening of the pinnules was observed. By one week, the pinnules on T1 ferns were a medium-brown color, while the pinnules on T2 ferns were a darker brown. The pinnules were severely desiccated and curled, as shown in Figure 8. Upon dissection of the T1 and T2 ferns, the majority of the leaves were completely brown. However, about 75% of the stalks on T1 ferns retained their original green, and the area incorporating the top 25% was brown. The stalks of the T2 ferns were completely brown, and any brown coloring that was previously present was much darker at this point.

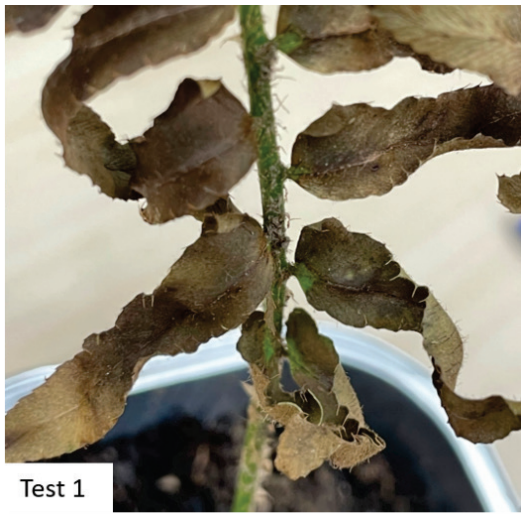

Figure 8. Appearance of curled pinnules on day 7.

Given the desiccated status of the plants one week after the experiment, biological mechanisms related to water transport may have been critically impaired as a result of the ammonia vapor exposure. In plants, the stomata play a crucial role in photosynthesis, respiration, and transpiration. Transpiration is a process that drives water transport in plants. Water from the interior of the leaves evaporates through the opening and closing of the stomata, creating a negative pressure within the xylem. This causes water and other nutrients to be "pumped up" to parts of the plant by capillary action. Damage to the stomata can result in abnormal functioning. If the stomata are open but unable to close as usual, then the plant may still be able to take up carbon dioxide and oxygen, but the rate of water transpiring out of the plant will increase as the plant attempts to overcome the stresses.[17,20,21] This impairment in transpiration processes may be a contributing factor in the dry and desiccated state of the pinnules. The upward movement of water from the roots through the plant could explain the retention of green throughout the stalk as the plant attempts to continue its normal processes. It is possible that the ferns were unable to compensate for the damage incurred, and they eventually succumbed to browning in their pinnules and stalk, which started at the top of the frond and eventually overtook the entire plant. By the end of the second week, no improvement was observed in the ammonia-exposed ferns. This suggests that the ferns were unable to overcome the damage and recover. The damaged ferns were subsequently pulled out of the soil and discarded. An interesting note is that after a few weeks, young and furled fiddleheads could be seen in the same soil that previously held the exposed ferns. This suggests that the damage was constrained to the upper stalk, and the roots were not as critically impacted, if they were affected at all.

4. CONCLUSIONS

The methods developed in this experiment illustrate the responses of vegetation to a large and sudden release of ammonia vapor. The results suggest that a correlation exists between the length of ammonia exposure and the degree of damage sustained by the vegetation. In our study, both groups of plants displayed visible browning shortly after removal from exposure conditions, and both were unable to recover afterward. The physiology of the wintergreen fern *P. acrostichoides* is sufficiently robust to withstand freezing temperatures and extreme shifts in soil moisture and light intensity. Therefore, one could make the case that the conditions inside the chamber during exposure may not have been a significant factor in the post-exposure damage observed in the ferns. It is possible that the ammonia concentration was high enough to damage the plants' biological functions and thereby impair their ability to properly regulate internal water levels, respiration, and transpiration. Tissues from the exposed and control plants were harvested throughout the experiment and processed for protein extraction. Identification of the biomarkers at various time points may provide valuable insight into the metabolic processes of plants that are impacted upon exposure to ammonia. Understanding and characterizing these effects, especially in local and common vegetative species, could prove useful in early detection of sudden and large releases of ammonia vapor.

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ACRONYMS AND ABBREVIATIONS

CASTLE	CBRNE Assessment Science and Technology Laboratory
CBRNE	Chemical, Biological, Radiological, Nuclear, and Explosives
DEVCOM CBC	U.S. Army Combat Capabilities Development Command Chemical Biological Center
PAL	phenylalanine ammonia lyase
T1	Test 1 group (short-term ammonia exposure)
T2	Test 2 group (long-term ammonia exposure)

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