

HYDROGEN SULFIDE EFFECT RELEASED BY DIBUTYLDITHIOPHOSPHATES ON
SOLANUM TUBEROSUM, RAPHANUS SATIVUS AND PHASEOLUS VULGARIS.

by

Luis Fernando Esquivel-Cervantes

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Plant Pathology

MONTANA STATE UNIVERSITY
Bozeman, Montana

May 2025

©COPYRIGHT

by

Luis Fernando Esquivel-Cervantes

2025

All Rights Reserved

DEDICATION

I dedicate my work to my parents, Piedad Cervantes-Consuelos and Jose Esquivel-Ortiz, my siblings, Jesus, Lourdes, and Alejandra, and my close friends for all their support and help.

I also dedicate this work to my nephews Jose, Bryan, Christopher, and Alexis, and nieces Melissa, Gisselle, and Jasmine, who always bring me happiness and laughs.

ACKNOWLEDGEMENTS

This work would not have been possible without funding from the United States Department of Agriculture (USDA). Thanks to Montana State University and the PSPP Department for providing the infrastructure for this research project.

To my advisor, Dr. Michael Giroux, for his guidance and solid commitment to helping me improve and gain confidence in myself. His experience and supportive attitude serve as motivation for me to work harder.

I extend my thanks to my committee members: Dr. Nina Zidack, for her valuable feedback, support, cheering, and advice on my experimental design and data collection; Dr. John Martin, for his assistance with data analysis, interpretation, and experimental design; and Dr. Ned Bowden, for his insights and teaching regarding the compounds used in this research. I would like also to thank MS Alanna Oiestad for her help with data analysis, and experimental design, and for aiding in the setting up of my experiments.

Finally, I would like to express my gratitude to all the people I have met at Montana State University, particularly to my lab mates who assisted in collecting data and setting up experiments.

TABLE OF CONTENTS

1. INTRODUCTION	1
Hydrogen Sulfide (H ₂ S).....	3
Dibutyldithiophosphate (DBDTP).....	5
Radish	8
Green Beans	9
Potato	10
Reference	14
2. THE IMPACT OF HYDROGEN SULFIDE RELEASED BY DIBUTYLDITHIOPHOSPHATES ON POTATOES	21
Contribution of Authors and Co-Authors	21
Manuscript Information	22
Abstract	23
Introduction.....	24
Materials and Methods.....	27
Tissue culture trial.....	27
Field Trial.....	27
Phytotoxicity Trial	29
Heat Stress Trial.....	30
Statistical Analysis	31
Results.....	32
Effect of DBDTP on Potato under Tissue Culture Conditions	32
Impact of DBDTP on Potato under Field Conditions.....	34
Effect of DBDTP in height, vigor, biomass, tubers per plant, and grams per plant in 2023.....	36
Effect of DBDTP in height, vigor, biomass, tubers per plant, and grams per plant in 2024.....	37
Effect of DBDTP on potatoes in foliar applications	39
Effect of DBDTP on Potato under heat stress conditions	41
Discussion	43
Conclusion	55
References.....	56
3. THE EFFECTS OF TOXIC GAS HYDROGEN SULFIDE RELEASED BY DIBUTYL-DITHIOPHOSPHATES ON RADISHES AND GREEN BEANS	61
Contribution of Authors and Co-Authors	61
Manuscript Information	62
Abstract	63

TABLE OF CONTENTS CONTINUED

Introduction.....	63
Materials and Methods.....	65
DBDTP Treatment Preparation	66
Greenhouse Experimental Design.....	66
Greenhouse Phytotoxicity Trial	67
Radish and Bush Bean Field Experimental Design	68
Statistical analysis	69
Results and Discussion	70
Effect of DBDTP on radishes under greenhouse conditions at 0 ppm NPK fertilization.....	71
Effect of DBDTP on radishes under greenhouse conditions at 100 ppm fertilization.....	75
Phytotoxicity effect of DBDTP in foliar applications.....	78
Effect of DBDTP on radishes under field conditions	82
Effect of DBDTP on bush beans under field conditions.....	83
Conclusions.....	86
References.....	87
4. CONCLUSION AND FUTURE DIRECTIONS	91
CUMULATIVE REFERENCES CITED.....	94

LIST OF TABLES

Table	Page
1. Table 1.1 Post-hoc analysis of variance for potato tuber weight per plant in trial 2024 (grams per plant), comparing the 0 mM DBDTP control versus 0.5, 1, 2, and 10 mM DBDTP	39

LIST OF FIGURES

Figure	Page
1. Figure 1.1. Multiple environmental stressors can induce gasotransmitter production in plants. Abiotic stressors (drought, salt, heavy metal, temperature, light, and UV radiation) induce the gasotransmitter generation (H_2 , H_2S , NO , CO , and CH_4) (Yao et al., 2019)	3
2. Figure 1.2. Synthesis of dibutyldithiophosphates from butanol and tetraphosphorus decasulfide, produce 4 Diethyldithiophosphate in the presence of Potassium Hydroxide producing Dibutyldithiophosphate releases in water 2 H_2S ; phosphate fertilizer and butanol (Brown et al., 2021)	6
3. Figure 2.1. Effect of DBDTP on potato plantlets under tissue culture conditions on the 4 th week. (A) Potato root length and shoot height by DBDTP treatments, (B) Potato fresh and dry weight by DBDTP treatments, (C)-(G) Representative plantlets from treatments: 0.2 mM (C), 0.1 mM (D), 0.05 mM (E), 0.025 mM (F), and 0 mM (G, control). Each data point in the figure represents the estimated mean ($\pm$ SE) from two independent trials based on measurements from eight plants per treatment and five replicates per treatment. Error bars represent the standard error (SE) of the estimated marginal means. A single asterisk (*) indicates a significant difference from the control (0 mM DBDTP) at $P < 0.05$, double asterisks (**) indicate strong significance at $P < 0.01$, and triple asterisks (***) indicate highly significant differences at $P < 0.001$, based on post hoc analysis.	33
4. Figure 2.2. Plant height and vigor under field conditions for five concentrations of DBDTP in 2023. (A) Plant height measured one and two months after transplanting per DBDTP concentration, (B) Plant vigor assessed one and two months after transplanting per DBDTP concentration. (C) Plant biomass in grams per plant per DBDTP concentration. (D) Number of tubers per plant per DBDTP treatment. Each data point in the figure represents the estimated mean ($\pm$ SE) from the first trial conducted in 2023, based on measurements from eight plants per treatment across ten blocks. Error bars represent the standard error (SE) of the estimated marginal means. A single asterisk (*) indicates a significant difference from the control (0 mM DBDTP) at $P < 0.05$, double asterisks (**) indicate strong significance at $P < 0.01$, and triple asterisks (***) indicate highly significant differences at $P < 0.001$, based on post hoc analysis.	35

LIST OF FIGURES CONTINUED

Figure	Page
5. Figure 2.3. Potato tubers' weight per plant in 2023 and 2024 under field conditions at harvest after following DBDTP treatments. (A) Effect of four total DBDTP applications on tuber weight (grams per plant) in potatoes harvested in 2023, two months after transplanting. (B) Effect of four total DBDTP applications on tuber weight (grams per plant) in potatoes harvested in 2024, three months after transplanting. Each data point represents the estimated mean ($\pm$ SE) from separate trials conducted in 2023 and 2024, based on measurements from eight plants per treatment across ten blocks. Error bars represent the standard error (SE) of the estimated marginal means. A single asterisk (*) indicates a significant difference from the control (0 mM DBDTP) at $P < 0.05$, double asterisks (**) indicate strong significance at $P < 0.01$, and triple asterisks (***) indicate highly significant differences at $P < 0.001$, based on post hoc analysis.	36
6. Figure 2.4. Plant vigor, and biomass under field conditions in 2024. (A) Plant vigor measured one month and two months after transplanting per DBDTP concentration, (B) Plant biomass (grams per plant) per DBDTP concentration at harvest, (C) Effect of DBDTP on the number of tubers per plant at harvest. Each data point in the figure represents the estimated mean ($\pm$ SE) from the second trial conducted in 2024, based on measurements from eight plants per treatment across ten blocks. Error bars represent the standard error (SE) of the estimated marginal mean. A single asterisk (*) indicates a significant difference from the control (0 mM DBDTP) at $P < 0.05$, double asterisks (**) indicate strong significance at $P < 0.01$, and triple asterisks (***) indicate highly significant differences at $P < 0.001$, based on post hoc analysis.	38

LIST OF FIGURES CONTINUED

Figure	Page
7. Figure 2.5. Effect of DBDTP as foliar applications on potato plantlets at different application times. (A) Visual assessment of phytotoxicity at one, eight, and fifteen days after transplanting for five DBDTP treatments from two trials analyzed together. (B) Visual assessment of phytotoxicity one month after transplanting for both trials (April and June 2024) analyzed separately, (C) Fresh weight per plant for the two rounds of the experiment at one, eight, and 15 days after transplanting analyzed together, significance versus control and within same treatments, (D) Fresh weight per plant at one month after transplanting application for the two rounds of the experiment analyzed separately. Each data point in the figure represents the estimated mean ($\pm$ SE) from two trials. Error bars represent the standard error (SE) of the estimated marginal means. A single asterisk (*) indicates a significant difference from the control (0 mM DBDTP) at $P < 0.05$, double asterisks (**) indicate strong significance at $P < 0.01$, and triple asterisks (***) indicate highly significant differences at $P < 0.001$, based on post hoc analysis.....	41
8. Figure 2.6. Effect of daily DBDTP applications in a solution of 200 ppm 20-20-20 NPK on potato plantlets exposed to heat stress for three weeks. (A) Potato survival percentage by week for four DBDTP treatments, (B) Potato height average from one week, two weeks, and three weeks after heat stress across four DBDTP treatments, (C) Potato plantlets one week after heat stress DBDTP treatment 0, 0.025, 0.1, and 0.4 mM left to right, (D) Potato plantlets two weeks after heat stress, DBDTP treatment 0, 0.025, 0.1, and 0.4 mM left to right, (E) Potato plantlets three weeks after heat stress, DBDTP treatment 0, 0.025, 0.1, and 0.4 mM left to right. Each data point in the figure represents the estimated mean ($\pm$ SE) from one trial. Error bars represent the standard error (SE) of the estimated marginal means. A single asterisk (*) indicates a significant difference from the control (0 mM DBDTP) at $P < 0.05$, double asterisks (**) indicate strong significance at $P < 0.01$, and triple asterisks (***) indicate highly significant differences at $P < 0.001$, based on post hoc analysis.	43

LIST OF FIGURES CONTINUED

Figure	Page
9. Figure 2.7. Phytotoxicity damage due to foliar application of five DBDP treatments at one, eight, fifteen, and one month after transplanting on potato plants grown in plugs. (A) Phytotoxicity at one day after transplanting with doses 0.5, 0, 10, 1, and 2 mM (left to right), (B) Phytotoxicity eight days after transplanting with doses 0, 10, 1, 2, and 0.5 mM (left to right), (C) Phytotoxicity fifteen days after transplanting with doses 0, 1, 2, 0.5, and 10 mM (left to right).	51
10. Figure 2.8. Phytotoxicity damage due to foliar applications of five DBDTP treatments one month after transplanting on potato plants, (A) 0 mM DBDTP treatment, (B) 0.5mM DBDTP treatment, (C) 1 mM DBDTP treatment, (D) 2 mM DBDTP treatment, (E) 10 mM DBDTP treatment (control).....	52
11. Figure 3.1. Phytotoxicity scale, where 1 indicates no burns on the leaves; 2 represents small individual burns on some leaves; 3 indicates at least one leaf with spots that merged into a bigger spot but covering no more than $\frac{1}{4}$ of the leaf; 4 represents bigger spots in at least one leaf covering more than $\frac{1}{3}$ of the leaves but without dead leaves; and 5 indicates at least one true leaf dead and more leaves with scores 3 and 4 or dead plant	68
12. Figure 3.2. Emergence Percentage seven days after planting by five DBDTP seed treatments using 0 ppm NPK and 100 ppm NPK. Error bars represent the standard error (SE) of the estimated marginal means. Each data point in the figure represents the estimated mean ($\pm$ SE) from two trials. A single asterisk (*) indicates a significant difference from the control (0 mM DBDTP) at $P < 0.05$, double asterisks (**) indicate strong significance at $P < 0.01$, and triple asterisks (***) indicate highly significant differences at $P < 0.001$, based on post hoc analysis.	71

LIST OF FIGURES CONTINUED

Figure

Page

13. Figure 3.3. Radish Overall fresh weight and root fresh weight under greenhouse conditions for five DBDTP concentrations at 0 ppm 20-20-20 NPK fertilization for the first round of the experiment (May 2024) and the second round of the experiment (September 2024). The x-axis shows the doses used in seed treatments and the doses used in the drench applications. (A) Overall fresh weight in May 2024 by number of applications and DBDTP treatments. (B) Root fresh weight by number of applications and DBDTP treatments in May 2024. (C) Overall fresh weight by DBDTP treatments and number of applications in Sep 2024. (D) Root fresh weight in Sep 2024 by DBDTP treatments and the number of applications. Error bars represent the standard error (SE) of the estimated marginal means. Each data point in the figure represents the estimated mean ($\pm$ SE) from two trials analyzed individually. Statistical comparisons were made against the control in each group (0 mM DBDTP) and within each dose by comparing one application vs. four applications. Comparisons were not made between different concentrations. A single asterisk (*) indicates a significant difference at $P < 0.05$, double asterisks (**) indicate strong significance at $P < 0.01$, and triple asterisks (***) indicate highly significant differences at $P < 0.001$, based on post hoc analysis..... 73
14. Figure 3.4. Radish Overall fresh weight and root fresh weight under greenhouse conditions for five DBDTP concentrations at 100 ppm 20-20-20 NPK fertilization for the first run of the experiment (May 2024) and the second run of the experiment (September 2024). The x-axis shows the doses used in seed treatments and the doses used in the drench applications. (A) Overall fresh weight in May 2024 by number of applications and DBDTP treatments. (B) Root fresh weight in May 2024 by the number of applications and DBDTP treatments. (C) Overall fresh weight in Sep 2024 by number of applications and DBDTP treatments. (D) Root fresh weight in Sep 2024 by the number of applications and DBDTP treatments. Error bars represent the standard error (SE) of the estimated marginal means. Each data point in the figure represents the estimated mean ($\pm$ SE) from two trials analyzed individually. Statistical comparisons were made against the control in each group (0 mM DBDTP) and within each dose by comparing one application vs. four applications. Comparisons were not made between different concentrations. A single asterisk (*) indicates a significant difference at $P < 0.05$, double asterisks (**) indicate strong significance at $P < 0.01$, and triple asterisks (***) indicate highly significant differences at $P < 0.001$, based on post hoc analysis..... 77

LIST OF FIGURES CONTINUED

Figure	Page
15. Figure 3.5. Phytotoxicity effect and fresh weight of DBDTP in foliar applications on radish. (A) Phytotoxicity effect of DBDTP foliar application on radishes. (B) Overall fresh weight by DBDTP treatments at 8DAG foliar application. (C) Overall fresh weight by DBDTP treatments at 15DAG foliar application. Error bars represent the standard error (SE) of the estimated marginal means. Each data point in the figure represents the estimated mean ($\pm$ SE) from two trials analyzed together. Statistical comparisons were made against control in each group (0 mM DBDTP). A single asterisk (*) indicates a significant difference at $P < 0.05$, double asterisks (**) indicate strong significance at $P < 0.01$, and triple asterisks (***) indicate highly significant differences at $P < 0.001$, based on post hoc analysis.....	81
16. Figure 3.6. Phytotoxicity effect of DBDTP foliar applications on radish's foliage. (A) Phytotoxicity on radishes eight days after germination left to right 0 mM, 0.5 mM, 1 mM, 2 mM, and 10 mM. (B) Phytotoxicity on radishes fifteen days after germination left to right 0 mM, 0.5 mM, 1 mM, 2 mM, and 10 mM.	82
17. Figure 3.7. Radish emergence percentage, overall fresh weight, and root fresh weight for five DBDTP seed treatments in the field (data from 2023 and 2024). (A) Germination Percentage for radishes grown in the field using five DBDTP seed treatments. (B) Overall fresh weight and root fresh weight for radishes grown in the field using five DBDTP seed treatments. Error bars represent the standard error (SE) of the estimated marginal means. Each data point in the figure represents the estimated mean ($\pm$ SE) from two trials. Statistical comparisons were made against the control in each group (0 mM DBDTP). A single asterisk (*) indicates a significant difference at $P < 0.05$, double asterisks (**) indicate strong significance at $P < 0.01$, and triple asterisks (***) indicate highly significant differences at $P < 0.001$, based on post hoc analysis.	83

LIST OF FIGURES CONTINUED

Figure	Page
18. Figure 3.8. Emergence percentage, fresh weight, and number of pods in bush beans using DBDTP seed treatment in the field. (A) Germination percent of bush beans in the field in 2023 and 2024 using DBDTP seed treatments. (B) Overall fresh weight and pods fresh weight by DBDTP seed treatments in bush beans. (C) Number of pods in bush beans in the field using DBDTP seed treatments. Error bars represent the standard error (SE) of the estimated marginal means. Each data point in the figure represents the estimated mean ($\pm$ SE) from two trials. Statistical comparisons were made against the control (0 mM DBDTP). A single asterisk (*) indicates a significant difference at $P < 0.05$, double asterisks (**) indicate strong significance at $P < 0.01$, and triple asterisks (***) indicate highly significant differences at $P < 0.001$, based on post hoc analysis.	85

ABSTRACT

Agriculture production faces many climatic and economic challenges nowadays. Enhancing crop traits such as yield and plant growth by the application of external products is key. Dibutyldithiophosphates (DBDTPs) release Hydrogen Sulfide (H_2S) in the presence of water and promote plant growth. Therefore, this study aimed to investigate the effects of DBDTP rates and application methods on the growth and development of potatoes, radishes, and bush beans.

Results from tissue culture experiments on potatoes showed that DBDTP significantly increased root length at all tested concentrations. The phytotoxicity assessment indicated that foliar application of 0.5 mM DBDTP could be suitable for future trials on potatoes, as this concentration did not cause foliar damage. In contrast, the same concentration in radishes resulted in significant phytotoxicity, indicating that concentrations below 0.5 mM need to be tested. Moreover, the treatments 0.025 mM and 0.1 mM DBDTP showed significant improvements in survival rates after three weeks of heat stress in potatoes, suggesting that continuous application of these treatments can benefit plants under heat stress conditions.

Finally, greenhouse yield results for radishes indicated that applying DBDTP as a seed treatment at 12.5 mM, followed by a drench treatment at 0.5 mM, could increase fresh weight by over 4.4% under both 100 ppm and 0 ppm NPK conditions. Field experiments in potatoes also revealed that applying 0.5 mM DBDTP could enhance yield by more than 5%. In radishes and bush beans, yields increased by 7.2% and 10.48% at 25 mM DBDTP respectively.

This study demonstrates the potential of DBDTPs to enhance plant growth, yield, and survival under stress conditions in various crops. It highlights DBDTP as a multiple-functional crop booster, offering growers an option not only to increase yield or biomass but also to protect plants in challenging weather conditions.

CHAPTER ONE

INTRODUCTION

The effects of climate change on agriculture are being felt worldwide through rising ocean acidity, ecosystem damage, an increase in water stress, and falling agricultural yields (Schellnhuber & Cramer, 2006). Climate change alters weather conditions and thus has direct biophysical effects on agricultural production (Nelson et al., 2013). There is a pressing need to increase crop yields and adapt to climate change. It is crucial to develop crops that can endure drought, floods, and extreme temperatures and still produce the same or higher yields.

Additionally, selecting for or facilitating plants to have a better root system to enhance nutrient and water absorption along with better germination, and enhanced crop growth will be required to face emerging challenges in agriculture while improving yield. Solutions must be environmentally safe, nonpolluting, (Carter et al., 2018), affordable, safe for humans, and reachable.

There are many ways to improve crop quality, such as genetic improvements via selective breeding, adequate and timely cultural practices, pest and disease control, soil and seed management, crop rotation, efficient fertilizer applications, application of biostimulants, gasotransmitters, etc. (Subbian et al., 2000; Shrestha et al., 2021).

Both gasotransmitters and biostimulants have shown an impact on different processes in plants. Biostimulants are substances or microorganisms that enhance nutrient efficiency, stress tolerance, and crop quality traits, these substances are efficient, favoring the good performance of the plant's vital processes, and allowing improved yields (Sharma et al., 2019; Feitosa de Vasconcelos & Garófalo Chaves, 2019). On the other hand, gasotransmitters are endogenously

generated gaseous molecules that play crucial signaling roles in various biological applications that are more specific to certain physiological processes. (Wang, 2014). The study of gasotransmitters and biostimulants in plants has been widely researched. However, in recent years, molecules that can release gasotransmitters have gained importance. This has highlighted the need to study those molecules that can be easily applied in agriculture, be affordable, and still have positive effects on plants' processes. Dithiophosphates currently are one of these molecules.

Exploring the role of gasotransmitters offers new ways to improve crop quality.

Gasotransmitters are small, low boiling point chemicals that move freely through cell membranes, play a role in physiological processes, and can be applied exogenously or generated endogenously by plants (García Mata & Lamattina, 2013; Natarajan & G., 2023).

Gasotransmitters are involved in signal transduction and have specific cellular and molecular targets; they can exist in either gaseous form or be taken up as a precursor compound and released within plants. Gasotransmitters play important roles in cells and can have a wide range of effects including altering gene expression and influencing the level of proteins, lipids, and carbohydrates (Wang, 2018). Although the term gasotransmitters refers to gaseous signaling molecules produced enzymatically by unicellular or pluricellular organisms, organisms can perceive and respond physiologically to exogenously supplied gasotransmitters (Lamattina & García-Mata, 2016)

There are different types of gasotransmitters, including hydrogen (H_2), hydrogen sulfide (H_2S), nitric oxide (NO), carbon monoxide (CO), and methane (CH_4), each having unique properties enabling them to regulate specific biological functions. Extensive research has been conducted on the roles of these signaling molecules in biological applications (reviewed by Yao

et al., 2019). Many studies suggest that gasotransmitters can contribute to improving plant tolerance to stress conditions such as drought, heavy metals, salt, temperature, and pathogens (Yao et al., 2019 Figure .1) (Li et al., 2013; Hancock & Whiteman, 2014; Xu et al., 2017; Jin et al., 2017).



Figure 1.1. Multiple environmental stressors can induce gasotransmitter production in plants. Abiotic stressors (drought, salt, heavy metal, temperature, light, and UV radiation) induce the gasotransmitter generation (H_2 , H_2S , NO , CO , and CH_4) (Yao et al., 2019)

Hydrogen Sulfide (H_2S)

Hydrogen sulfide (H_2S) is a colorless gas soluble in organic solvents and water. For centuries, it has been recognized for its unpleasant rotten egg odor and its harmful effects on plants. It can be found in the atmosphere having been produced through sources such as volcanoes, salt marshes, wetlands, geothermal vents, livestock, industry, combustion of biomass and fossil fuels, as well as bacterial anaerobic respiration (Ahmed et al., 2021). In mammals, it is synthesized within their tissues through endogenous enzymes and non-enzymatic pathways (Li et

al., 2011). Plants produce H_2S enzymatically through sulfate reduction and reaction of cysteine. Activated sulfate is first reduced to sulfite, which is then further reduced to H_2S by sulfite reductase. These reactions occur within the plastids of plant cells. (Filipovic & Jovanović, 2017)

For a long time, hydrogen sulfide (H_2S) was considered harmful to plants but after being identified as a third endogenous gaseous transmitter (gasotransmitter) along with Nitric Oxide (NO) and Carbon Monoxide (CO), its effects on plants were further investigated. It has now been shown to be an important signaling molecule in various organisms, including bacteria, fungi, worms, animals, and plants (Kabil et al., 2014; Hancock & Whiteman, 2014). Over the past few years, extensive research has been conducted on the effects of exogenous applications of H_2S on plants. It has been linked to a wide range of physiological responses including seed germination, resistance to heavy metal and weather stress conditions, pathogen stress, and delaying senescence (Li & Zhu, 2014; Bloem et al., 2004; Hancock & Whiteman, 2014). But the main purpose for exogenous applications of H_2S relies on its role in the alleviation of stress (Hancock & Whiteman, 2014; Li & Zhu, 2014; Yao et al., 2019; Zhang, 2016). This is because H_2S can increase the activity of enzymes that are involved in nutrient transport by interacting with other signaling chemicals such as plant hormones and other gasotransmitters. H_2S balances antioxidant pools, influences the activities of antioxidative enzymes, and promotes the accumulation of osmo-protective metabolites (amino acids, sugar, etc.) (Xuan et al., 2020; Lisjak et al., 2015; Bhardwaj & Kapoor, 2021)

Compounds that degrade in response to a specific trigger to release H_2S are called H_2S donors (also known as releasing agents), they are important research tools in advancing the understanding of the biology and potential of H_2S in different fields, these donors have

advantages such as good water-solubility, slow and controllable H₂S release capability, and a variety of reported biological activities (Powell et al., 2018; Kang et al., 2017). Currently, H₂S donors include sodium hydrosulfide (NaSH), sodium sulfide (Na₂S), morpholin-4-ium 4-methoxyphenyl (morpholino) phosphinodithioate (GYY4137), *L*-thiovaline (TV), thioglycine and dibutyldithiophosphate (Yang et al., 2022; Carter et al., 2019; Lisjak et al., 1988).

Dibutyldithiophosphate (DBDTP)

Dibutyldithiophosphates (DBDTPs) were developed to positively affect plants' growth, survival, and yield by the controlled slow release of H₂S. DBDTPs have been tested in agricultural applications within the last five years, however, their use is well-known in other areas. For instance, zinc salts of dialkyl- and diaryldithiophosphates (ZDDPs) have been used in lubricants and oil additives for decades due to their ability to function as both antioxidant and anti-wear agents (Zhang & Spikes, 2016; Willermet et al., 1995; Burn et al., 1990). Millions of tons of ZDDPs are synthesized each year.

In agriculture, dithiophosphate salts could improve seed germination, plant growth, root development, and survival under stressful conditions. The salts are synthesized from the reaction of C₂ to C₁₂ fatty alcohols with P₄S₁₀ at an elevated temperature (Figure 1.2) (Brown et al., 2021). DBDTP can be used as fertilizer additives or in a solution to aid in plant growth and tolerance to environmental stressors by releasing H₂S. These improvements in agricultural systems represent an important development, because, when plants have a good root-shoot system following the seedling stage, they have better usage of water, nutrients, light, space, and stress tolerance (Bláha, 2019).

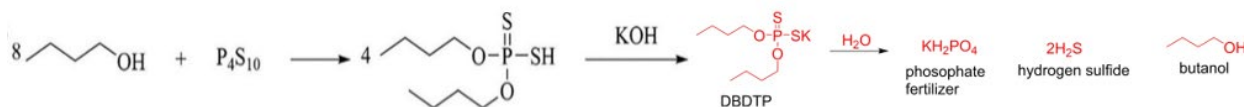


Figure 1.2. Synthesis of dibutyldithiophosphates from butanol and tetraphosphorus decasulfide, produces Dibutyldithiophosphate. In the presence of Potassium Hydroxide the salt is produced. In water Dibutyldithiophosphate releases H₂S, phosphate fertilizer, and butanol. (Brown et al., 2021)

After the initial discovery of H₂S as a plant gasotransmitter in plants, many agricultural H₂S studies used aqueous H₂S made by dissolving NaSH or Na₂S in water. Although simple to generate, these solutions released large quantities of H₂S into the atmosphere (likely 95%), and only a small and unknown amount was absorbed by the plants (Carter et al., 2019). The release of H₂S into the atmosphere was a problem because it is toxic at loadings as low as several parts per million and possesses a strong, unpleasant odor of rotten eggs (Carter et al., 2019; Occupational Safety and Health Administration, U.S. Department of Labor, n.d.).

To handle the difficulties of working with aqueous H₂S in agriculture, scientists used small chemicals that slowly release H₂S such as GYY-4137. This chemical slowly released H₂S over weeks to months in water and did not release toxic levels of H₂S in the atmosphere above plants. Furthermore, the amount of H₂S released near a plant could be controlled by the careful addition of GYY-4137. (Carter et al., 2018; Lisjak et al., 2011; Carter et al., 2019). However, GYY-4137 has three limitations; first, when it undergoes hydrolysis, GYY-4137 releases an amine and a phosphate that are not naturally present in the environment and could have unknown effects on plants. Second, the structure of GYY-4137 is not easily modifiable to alter its rate of release of H₂S. Third, it is expensive and costs \$20 per milligram. Dialkyl dithiophosphates offer a better alternative to GYY4137 since they have varying hydrolysis rates that are dependent on the alcohols used during their synthesis, dibutyldithiophosphates degrade to release safe and

natural chemicals such as fatty alcohols and monopotassium phosphate, and they are inexpensive and produced in millions of tons per year (Carter et al., 2019).

During the past few years, DBDTP has been tested by various authors in different studies on corn and radishes. Carter et al (2019) demonstrated the effect of DBDTP on corn plants. They used different concentrations of DBDTP (ranging from 1mg to 200mg) in aqueous solutions, using 500 mL of tap water and applying 10 mL via syringe around and on the seed. The results showed that dibutyldithiophosphate had a significant impact on the growth of corn plants. The weight of the plants showed statistically significant at 1, 10, 50, and 75 mg loadings of dibutyldithiophosphate compared to 0 control, and there were improvements in their weight for all loadings except 100 and 200 mg.

Brown et al. (2021) conducted a field trial on corn using dibutyldithiophosphate. They found that when added to the starter fertilizer at concentrations of 0, 0.5, 1.0, or 2.0 kg per acre (4046.86 m²) before planting the seeds, it resulted in a 6.4% increase in the harvest yield of corn. The study suggests that dibutyldithiophosphates have potential applications in agriculture, particularly at a dose of 2 kg per acre.

In 2022 Ranasinghe-Arachchige et al. tested the effect of DBDTP in the growth of radish plants using 1, 3, 10, 30, 100, 300, and 600 mg, and 30 seeds were planted at each dose. After the seeds were added to the soil, chemicals were added to the top of the seed and covered with the soil. The weight of the radish plants showed statistically significant improvement in growth when Di(*t*-butanol) dithiophosphates (tBDPA) loaded into PLA were added at doses of 10, 30, 100, 300, and 600 mg compared to the control plants not exposed to any chemicals.

The identification of inexpensive fertilizer additives that can increase yield or survival of specialty crops to environmental stressors is crucial to benefit farmers who grow potatoes and radishes by ensuring yields even in weather-challenging years.

Specialty crops are high-value crops, with distinct characteristics, and intensive cultivation practices that involve various aspects, including cultivation, marketing, and research to enhance competitiveness and sustainability. Specialty crops include fruits, tree nuts, berries, vegetables, potatoes, melons, pulse crops, nursery, greenhouse, floriculture, cultivated Christmas trees, short rotation woody crops, and other specialty crops (Perdue & Hamer, 2019) that play a significant role in American agriculture and contribute significantly to the country's economy.

Radish

Raphanus sativus (Radish) belongs to the *Brassicaceae* family and is a widely consumed root vegetable all around the world. It is a dicotyledonous herbaceous plant, annual or biennial with a height between 0.5 up to 1.20 meters. Leaves are finely pubescent with irregularly toothed edges, flowers in inflorescence in a terminal raceme violet to pink to white. Fruits are indehiscent siliques, glabrous, thick, with several longitudinal veins, cylindrical-lanceolate, or oblong-conical, with no or very slight transverse contractions between seeds, with 2 to 3 seeds per fruit. Seeds are globose, opaque, reddish to ochre to reddish-brown, finely reticulated, approximately 3 to 3.5 mm in diameter. Roots are fleshy, deep, taproot with colors between red, purple, or white. When plants decompose, they give off a foul rotten egg-like odor (Weil et al., 2006; Jacobs, 2012; Perdomo-Roldán et al., 2009)

Radishes have been domesticated since ancient times and were originally cultivated in China (Navazio, 2007). Radishes are widely used in the US as fresh salad vegetables and

garnishes. They grow best on light mineral soils, muck soils, or high organic matter soils, along with adequate moisture and fertility, but radishes may be grown on a wide range of soils. Radish plants are not tolerant of shade, standing water, or severely nitrogen-deficient soils. Soil pH should be 6.5 or higher (Weil et al., 2006; Oregon State University, 2010; Navazio, 2007). Often, planting occurs after manure, sludge, or fertilizer application, seeds are sown 1/4 to 1/2 inch (0.5-1.2 cm) deep. The recommended spacing between plants for spring varieties ranges from 1/2 to 1 inch, depending on the label variety. For winter radishes, the recommended spacing is 2 to 4 inches or even farther apart to allow for proper root development, radish is normally grown as a sole crop. The early spring or garden types mature in 25-40 days and 50–60 days for winter types, radishes have round or long roots that are usually white, red, or bicolored. (Jacobs, 2012; Andersen, n.d.; CABI, 2022). Up to 800 cartons of red radishes can be harvested per acre per crop, with the potential for three to four crops per season on the same land. Harvest can be done by hand or mechanical. Red radishes are pulled and tied in bunches. The bunches are washed and should be moist and cooled immediately after harvest for the best shelf life. Bunches are packed in either 2 or 4 dozen/cartons (Jacobs, 2012; Oregon State University, 2010).

Green Beans

Green beans (*Phaseolus vulgaris* L.) are a very recognized crop worldwide, native to Mesoamerica known by various common names including snap beans, bush beans, common beans, and garden beans. They belong to the legume family Fabaceae, important for their role in nitrogen fixation, where Rhizobacteria takes nitrogen from the air and converts it into an assimilating form for plants. Bush beans are an herbaceous annual plant with lateral and adventitious roots, oval trifoliate leaves alternately arranged, inflorescence in an axillary or

terminal false raceme up to 15–35 cm long, with flowers arranged along the rachis in pairs and mostly self-pollinated. Plant is consumed as dry seed or unripe pods, occasionally the leaves are eaten as a vegetable, and the straw is used to feed livestock (PubChem, 2025; Bitocchi et al., 2012; Dida & Etisa, 2018). Green beans are rich in protein, calories, vitamins, minerals, and fiber (Broughton et al., 2003).

Green beans are a very diverse crop that have been adapted to different regions (Broughton et al., 2003), growing well in drained loamy and sandy clay soil types with an optimal pH of 6.0 – 7.0. Sowing should start when soil temperatures are around 55-60°F (12.8–15.6°C), with optimal seed germination at 70°F (21.1°C). Seeds are planted 1 to 2 inches (2.54–5.08 cm) deep and 2 to 4 inches (5.08-10.16 cm) apart at rates of 75 to 120 lbs per acre (84.2–134.5 kg/ha). Fertilizer is normally applied in bands, applications of nitrogen, potassium, and phosphorus are based on soil tests, and the use of manure in combination with NPK increases the productivity. Irrigation is crucial for beans during flowering and approximately 1 to 1½ inches (2.54–3.81 cm) of water weekly is required. Harvest occurs approximately 8 weeks after planting, good commercial yield depends on the region and seeding rates, yielding between 4, 800 to 10, 000 pounds per acre (5,380 - 11,208 kg/ha) (Welty & Doohan, 2000; Alhrout et al., 2016; OECD, 2016; Ernest, 2024)

Potato

Western South America is the primary center of the origin and diversity of the potato crop and its wild relatives in the Bolivian-Peruvian Andes region (de Haan & Rodriguez, 2016; Datiles & Acevedo-Rodríguez, 2022). Potatoes are an important crop around the world. It

represents an important source of food for humans; however, growing potatoes is hard labor due to water availability, diseases, environmental stresses, soil deficiency nutrition, etc.

Solanum tuberosum (potato) is part of the Solanaceae family, it is an herbaceous plant up to 0.4-1.4 meters tall and produces subterranean tubiform stolons. The root system is usually 40-50 cm deep, up to 1m without obstructions. Tubers develop at the tip of the stolons, globose to ellipsoid, and are very variable in size, weight, and color. Stems up to 1.5 cm in diameter may be green, purple, or mottled green and purple. Leaves are pinnate with a single terminal leaflet and three or four pairs of large, ovoid leaflets with smaller ones in between. The inflorescence is a cymose panicle; flowers vary in colors white, yellow, pink, or violet. The berries are globular, with two carpels, and may contain many seeds. They are green or green-tinged with white or purple spots or bands when ripe and the berries are toxic due to the presence of glycoalkaloids (Spooner and Knapp 2013; Struik 2007; Datiles & Acevedo-Rodríguez, 2022).

Potatoes grow best in loose, well-drained, non-crusting, sandy loam or loam soils with high organic matter content and pH between 5.5 and 6.5 (Leap et al., 2017). In general, potatoes should be planted when the soil temperature is higher than 45°F (6°C) and lower than 70°F (21°C) (Nair et al., 2017). Soil amendments such as compost could be applied to the field, as needed (5 tons/acre) (Leap et al., 2017). Nitrogen can be applied at 1/5 of the recommended rate at planting, 2/5 at emergence, and the remaining 2/5 at hilling, excess of N can decrease yield, make haulm destruction more difficult, and reduce quality as well as increase N residues susceptible to leaching. Phosphorus and potassium applications can be applied fully at the time of planting. Soil testing is crucial to calculate the correct amount and proportion of nutrients to

the plant based on the test and the type of fertilizer recommended. (Nair et., al, 2017; Firman & Allen, 2007)

Tillage is performed in the fall and then again in the spring before planting. Only certified disease-free seed stock must be used for planting. Seed tubers may be pre-cut in advance of planting, they should be 1½ to 2 ounces (42-57 gr) in size and should contain at least 1-2 eyes/buds. A storage temperature of 60°F (15.5°C) for 7-10 days before planting will help initiate sprout activity and encourage more rapid emergence. Seed pieces are planted in furrows that are 30-36 inches (76-91 centimeters) apart. Seed tubers are spaced 8-12 inches (20-30 centimeters) apart in the furrow and the seeding depth is around 3-5 inches (7.5-12.5 centimeters) (Nair et al., 2017). Disease-free seed potatoes are initiated from aseptic plant tissue culture. Plantlets are multiplied in vitro from disease-free cuttings. Subsequently, in vitro plantlets are transplanted in insect-free greenhouses to produce high-quality seed potatoes. Alternatively, mini tubers are produced from in vitro plants planted at high plant densities. These mini tubers are then planted in isolated fields protected from insects to produce normal-sized seed potatoes. The next phase is for seed potato farms and private seed growers to conventionally multiply these seed tubers (Datiles & Acevedo-Rodríguez, 2022).

Potato plants emerge from the ground 2-6 weeks after planting, depending on weather, location, and time of year. The plants grow quickly and will begin to grow tubers just a few weeks after emergence. Potatoes require high volumes of water during the growing season. Growers must be very careful to reduce water stress because stressed plants produce lower quality and yield. (Washington State Potato Commission, 2023).

The time of harvesting of potatoes varies with cultivar, cultural practices, climate, and price. During the harvesting process, the above-ground parts of the plant are destroyed, and the tubers are lifted and collected. The vines are destroyed either by manual or mechanical pulling, cutting, or chemicals. Vines need to be killed two to three weeks before harvest, chemicals are sprayed approximately three weeks before harvest because sometimes vines are difficult to kill when soil moisture is high, or plants are large and vigorous. (Datiles & Acevedo-Rodríguez, 2022; Petroff, 2002; Bohl & Johnson, 2010). Potatoes are transported from the field to packing sheds, processing plants, or warehouses for different purposes. In the northern potato production areas, a significant portion of the crop is stored throughout the winter, spring, and summer months for processing and fresh market purposes. The temperature used for storage ranges from 50° to 58°F (10 to 14.4°C), depending on the eventual use of the tubers, the cultivar, the relative maturity, and the growth conditions. Storage capacity can vary greatly, from 500 tons to over 20,000 tons. It is crucial to construct a storage facility that meets the necessary holding and ventilation capacity requirements. While most potatoes are stored in bulk piles, some areas make use of box storage. (Bohl & Johnson, 2010).

The main goal of this study is to investigate the effect of DBDTP on the growth of the Russet Burbank (RB) and Clearwater Russet (CW) potato in field and growth chamber conditions, Rover radishes under greenhouse and field conditions, and Provider bush beans under field conditions only.

Many critical aspects of DBDTP use will be addressed in this study, such as the level and method of applying DBDTP and when to apply DBDTP to best stimulate growth. In order to understand the use of DBDTP, these will be tested on different stages of the potatoes' growth,

starting as node cuttings in tissue culture media containing various levels of DBDTP, followed by another application during growth of transplants in the greenhouse, and a final application during planting in the field. Since fertilization and foliar fertilizers for plant nutrient supplementation in annual crops are typically applied multiple times during the growing season, it is reasonable to apply DBDTP at different intervals during the potato crop season.

In the case of radishes, DBDTP will be tested under different fertilizer levels in the greenhouse, along with foliar application and field studies. Additionally, to ensure the widespread use of DBDTP in agriculture, this will also be tested under field conditions for bush beans.

Reference

- Ahmed, M., Fahad, S., Ali, M. A., Hussain, S., Tariq, M., Ilyas, F., Ahmad, S., Saud, S., Hammad, H. M., Nasim, W., Wu, C., & Liu, H. (2021). Hydrogen Sulfide: A Novel Gaseous Molecule for Plant Adaptation to Stress. *Journal of Plant Growth Regulation*, 40(6), 2485–2501. <https://doi.org/10.1007/s00344-020-10284-0>
- Alhrout, H. H., Khalifeh, H., in, H. A., Haddad, M. A., & Al-Dalein, S. Y. (2016). The impact of organic and inorganic fertilizer on yield and yield components of common bean (*Phaseolus vulgaris*). *Advances in Environmental Biology*, 10(9), 8–13. https://www.researchgate.net/publication/310828606_The_impact_of_organic_and_inorganic_fertilizer_on_yield_and_yield_components_of_common_bean_Phaseolus_vulgaris
- Andersen, C. (n.d.). *Agriculture and Natural Resources Home Gardening Series Radishes Environment*. University of Arkansas. Retrieved October 23, 2023, from <https://www.uaex.uada.edu/publications/PDF/FSA-6075.pdf>
- Bhardwaj, S., & Kapoor, D. (2021). General view on H₂S and abiotic stress tolerance in plants. *Elsevier EBooks*, 113–132. <https://doi.org/10.1016/b978-0-323-85862-5.00010-5>
- Bitocchi, E., Nanni, L., Bellucci, E., Rossi, M., Giardini, A., Zeuli, P. S., Logozzo, G., Stougaard, J., McClean, P., Attene, G., & Papa, R. (2012). Mesoamerican origin of the common bean (*Phaseolus vulgaris* L.) is revealed by sequence data. *Proceedings of the National Academy of Sciences*, 109(14), E788–E796. <https://doi.org/10.1073/pnas.1108973109>

- Bláha, L. (2019). Importance Of Root-Shoot Ratio For Crops Production. *Agronomy & Agricultural Science*, 2(2), 1–7. <https://doi.org/10.24966/aas-8292/100012>
- Bohl, W. H., & Johnson, S. B. (2010). Commercial Potato Production in North America The Potato Association of America Handbook Second Revision of American Potato Journal Supplement Volume 57 and USDA Handbook 267 by the Extension Section of The Potato Association of America. https://potatoassociation.org/wp-content/uploads/2014/04/A_ProductionHandbook_Final_000.pdf
- Broughton, W. J., Hernández, G., Blair, M., Beebe, S., Gepts, P., & Vanderleyden, J. (2003). Beans (*Phaseolus* spp.) – model food legumes. *Plant and Soil*, 252(1), 55–128. <https://doi.org/10.1023/a:1024146710611>
- Brown, E. M., Ranasinghe Arachchige, N. P. R., Paudel, A., & Bowden, N. B. (2021). Synthesis, Stability, and Kinetics of Hydrogen Sulfide Release of Dithiophosphates. *Journal of Agricultural and Food Chemistry*, 69(43), 12900–12908. <https://doi.org/10.1021/acs.jafc.1c04655>
- Burn, A. J., Dewan, S. K., Gosney, I., & Tan, P. S. G. (1990). Phosphorus-31 nuclear magnetic resonance study of the mechanism and kinetics of the hydrolysis of zinc(II)O,O-diethyl dithiophosphate and some related compounds. *Journal of the Chemical Society, Perkin Transactions 2*, 5, 753–758. <https://doi.org/10.1039/P29900000753>
- CABI. (2022). *Raphanus sativus* (radish). *CABI Compendium*, *CABI Compendium*. <https://doi.org/10.1079/cabicompendium.46796>
- Carter, J. M., Brown, E. M., Grace, J. P., Salem, A. K., Irish, E. E., & Bowden, N. B. (2018). Improved growth of pea, lettuce, and radish plants using the slow release of hydrogen sulfide from GYY-4137. *PLOS ONE*, 13(12), e0208732. <https://doi.org/10.1371/journal.pone.0208732>
- Carter, J. M., Brown, E. M., Irish, E. E., & Bowden, N. B. (2019). Characterization of Dialkyldithiophosphates as Slow Hydrogen Sulfide Releasing Chemicals and Their Effect on the Growth of Maize. *Journal of Agricultural and Food Chemistry*, 67(43), 11883–11892. <https://doi.org/10.1021/acs.jafc.9b04398>
- Chatzianastasiou, A., Bibli, S.-I., Andreadou, I., Efentakis, P., Kaludercic, N., Wood, M. A., Whiteman, M., Di Lisa, F., Daiber, A., Manolopoulos, V. G., Szabó, C., & Papapetropoulos, D. A. (2016). Cardioprotection by H₂S Donors: Nitric Oxide-Dependent and -Independent Mechanisms. *J Pharmacol Exp Ther*, 358(3), 431–440. <https://doi.org/10.1124/jpet.116.235119>
- Daniels, C. (2013). *Growing Radishes in Home Gardens Crop at a Glance Selecting Types to Plant*. Washington State University. <https://s3.wp.wsu.edu/uploads/sites/2071/2014/04/Radishes-FS127E.pdf>

- Datiles, M. J., & Acevedo-Rodríguez, P. (2022). *Solanum tuberosum* (potato). *CABI Compendium*, *CABI Compendium*. <https://doi.org/10.1079/cabicompendium.50561>
- de Haan, S., & Rodriguez, F. (2016). Potato Origin and Production. *Advances in Potato Chemistry and Technology*, 1–32. <https://doi.org/10.1016/b978-0-12-800002-1.00001-7>
- Dida, G., & Etisa, D. (2018). Common bean (*Phaseolus Vulgaris* L.): A Review. *International Journal of Nutritional Science and Food Technology*, 4(7), 54–62. https://www.researchgate.net/publication/332179103_Common_bean_Phaseolus_Vulgari_s_L_A_Review?enrichId=rgreq-9354211c390d0586f004e136a0d65664-XXX&enrichSource=Y292ZXJQYWdlOzMzMjE3OTEwMztBUzoxMDI0MzQyMDUzOTA0Mzg1QDE2MjEyMzM1ODUyNjk%3D&el=1_x_2&_esc=publicationCoverPdf
- Ernest, E. (2024, April 19). *High Plant Populations Needed for Best Snap Bean Yields*. University of Delaware. <https://sites.udel.edu/weekllycropupdate/?p=24181>
- Feitosa de Vasconcelos, A. C., & Garófalo Chaves, L. H. (2019). Biostimulants and Their Role in Improving Plant Growth under Abiotic Stresses. In *www.intechopen.com*. IntechOpen. <https://www.intechopen.com/chapters/69956>
- Filipovic, M. R., & Jovanović, V. M. (2017). More than just an intermediate: hydrogen sulfide signaling in plants. *Journal of Experimental Botany*, 68(17), 4733–4736. <https://doi.org/10.1093/jxb/erx352>
- Firman, D. M., & Allen, E. J. (2007). Agronomic Practices. In *Potato Biology and Biotechnology Advances and Perspectives* (pp. 719–738). Elsevier. <http://base.dnsgb.com.ua/files/book/Agriculture/Cultures/Potato-Biology-and-Biotechnology.pdf>
- García Mata, C., & Lamattina, L. (2013). Gasotransmitters are emerging as new guard cell signaling molecules and regulators of leaf gas exchange. *Plant Science*, 201–202, 66–73. <https://doi.org/10.1016/j.plantsci.2012.11.007>
- Gotor, C., García, I., Aroca, A., Laureano-Marín, A. M., Arenas-Alfonseca, L., Jurado-Flores, A., Masero Moreno, I., & Rico Romero, L. (2019). Signaling by hydrogen sulfide and cyanide through post-translational modification. *Journal of Experimental Botany*, 70(16), 4251–4265. <https://doi.org/10.1093/jxb/erz225>
- Hancock, J. T., & Whiteman, M. (2014). Hydrogen sulfide and cell signaling: Team player or referee? *Plant Physiology and Biochemistry*, 78, 37–42. <https://doi.org/10.1016/j.plaphy.2014.02.012>
- Jacobs, A. (2012a). *Plant Guide OILSEED RADISH *Raphanus sativus* L. Plant Symbol = RAS42*. https://plants.usda.gov/DocumentLibrary/plantguide/pdf/pg_rasa2.pdf

- Jacobs, A. (2012b). *Plant Guide for oilseed radish (Raphanus sativus L.)*. USDA-Natural Resources Conservation Service.
https://plants.usda.gov/DocumentLibrary/plantguide/pdf/pg_rasa2.pdf
- Jin, Z., Wang, Z., Ma, Q., Sun, L., Zhang, L., Liu, Z., Liu, D., Hao, X., & Pei, Y. (2017). Hydrogen sulfide mediates ion fluxes inducing stomatal closure in response to drought stress in *Arabidopsis thaliana*. *Plant and Soil*, 419(1-2), 141–152.
<https://doi.org/10.1007/s11104-017-3335-5>
- Kabil, O., Vitvitsky, V., & Banerjee, R. (2014). Sulfur as a Signaling Nutrient Through Hydrogen Sulfide. *Annual Review of Nutrition*, 34(1), 171–205. <https://doi.org/10.1146/annurev-nutr-071813-105654>
- Kang, J., Neill, D. L., & Xian, M. (2017). Phosphonothioate-Based Hydrogen Sulfide Releasing Reagents: Chemistry and Biological Applications. *Frontiers in Pharmacology*, 8.
<https://doi.org/10.3389/fphar.2017.00457>
- Lamattina, L., & García-Mata, C. (2016). *Gasotransmitters in Plants*. Springer.
<https://doi.org/10.1007/978-3-319-40713-5>
- Li, L., Rose, P., & Moore, P. K. (2011). Hydrogen Sulfide and Cell Signaling. *Annual Review of Pharmacology and Toxicology*, 51(1), 169–187. <https://doi.org/10.1146/annurev-pharmtox-010510-100505>
- Li, Z. B., & Zhu, L. (2014). Hydrogen sulfide donor sodium hydrosulfide-induced accumulation of betaine is involved in the acquisition of heat tolerance in maize seedlings. 38(1), 31–38. <https://doi.org/10.1007/s40415-014-0106-x>
- Li, Z.-G., Ding, X.-J., & Du, P.-F. (2013). Hydrogen sulfide donor sodium hydrosulfide-improved heat tolerance in maize and involvement of proline. *Journal of Plant Physiology*, 170(8), 741–747. <https://doi.org/10.1016/j.jplph.2012.12.018>
- Lisjak, M., Špoljarević, M., Wood, M., Whiteman, M., Wilson, I., Hancock, J., & Teklić, T. (2015). H₂S and NO signalling in plants. *Semantic Scholar*.
<https://www.semanticscholar.org/paper/H2S-and-NO-signalling-in-plants-Lisjak-%C5%A0poljarevi%C4%87/c8fd18c43bc24469bc9cb918bc248ac7a1b72642>
- Lisjak, M., Teklic, T., Wilson, I. D., Wood, M. E., Whiteman, M., & Hancock, J. T. (1988). Abstracts of Eleventh Annual Meeting, Japanese Society of Biorheology. *Biorheology*, 25(6), 893–915. <https://doi.org/10.3233/bir-1988-25610>
- Lisjak, M., Teklić, T., Wilson, I. D., Wood, M., Whiteman, M., & Hancock, J. T. (2011). Hydrogen sulfide effects on stomatal apertures. *Plant Signaling & Behavior*, 6(10), 1444–1446. <https://doi.org/10.4161/psb.6.10.17104>

- Natarajan, A., & G., J. W. (2023). Gasotransmitters: A Review. *International Journal of Basic & Clinical Pharmacology*, 12(4), 607–615. <https://doi.org/10.18203/2319-2003.ijbcp20231900>
- Navazio, J., Colley, M., & Dillon, M. (2007). Organic Seed Alliance Supporting the ethical development and stewardship of seed Principles and Practices of Organic Radish Seed Production in the Pacific Northwest This publication made possible through a grant from Western SARE. In *Organic Seed Alliance*. https://projects.sare.org/wp-content/uploads/1623radish_seed_manual.pdf
- Nelson, G. C., Valin, H., Sands, R. D., Havlík, P., Ahammad, H., Deryng, D., Elliott, J., Fujimori, S., Hasegawa, T., Heyhoe, E., Kyle, P., Von Lampe, M., Lotze-Campen, H., Mason d’Croz, D., van Meijl, H., van der Mensbrugghe, D., Müller, C., Popp, A., Robertson, R., & Robinson, S. (2013). Climate change effects on agriculture: Economic responses to biophysical shocks. *Proceedings of the National Academy of Sciences*, 111(9), 3274–3279. <https://doi.org/10.1073/pnas.1222465110>
- Occupational Safety and Health Administration, U.S. Department of Labor. (n.d.). *Hydrogen Sulfide*. www.osha.gov. Retrieved September 10, 2023, from <https://www.osha.gov/hydrogen-sulfide>
- OECD. (2016). *Safety Assessment of Transgenic Organisms in the Environment, Volume 6*. OECD Consensus Documents, Harmonisation of Regulatory Oversight in Biotechnology; OECD Publishing, Paris. https://www.oecd.org/en/publications/safety-assessment-of-transgenic-organisms-in-the-environment-volume-6_9789264253421-en.html
- Oregon State University. (2010, July 21). *Radish*. College of Agricultural Sciences. <https://horticulture.oregonstate.edu/oregon-vegetables/radish-0>
- Perdomo-Roldán, F., Mondragón-Pichardo, J., & Vibrans, H. (2009, July). *Raphanus sativus - ficha informativa*. www.conabio.gob.mx. <http://www.conabio.gob.mx/malezasdemexico/brassicaceae/raphanus-sativus/fichas/ficha.htm#3.%20Identificaci%C3%B3n%20y%20descripci%C3%B3n>
- Perdue, S., & Hamer, H. (2019). Specialty Crops Volume 2 • Subject Series • Part 8 United States Department of Agriculture. In *USDA*. https://www.nass.usda.gov/Publications/AgCensus/2017/Online_Resources/Specialty_Crops/SCROPS.pdf
- Petroff, R. (2002). *Crop Profile for Potato in Montana*. Montana State University. <https://ipmdata.ipmcenters.org/documents/cropprofiles/MTpotato.pdf>
- Powell, C. R., Dillon, K. M., & Matson, J. B. (2018). A review of hydrogen sulfide (H₂S) donors: Chemistry and potential therapeutic applications. *Biochemical Pharmacology*, 149, 110–123. <https://doi.org/10.1016/j.bcp.2017.11.014>

- PubChem. (2025). *Phaseolus vulgaris*. Nih.gov; PubChem.
<https://pubchem.ncbi.nlm.nih.gov/taxonomy/3885>
- Ranasinghe Arachchige, N. P. R., Brown, E. M., & Bowden, N. B. (2022). Sustained Release of Hydrogen Sulfide from Di(*t*-butanol)dithiophosphate Phenethylamine Salt Encapsulated into Poly(lactic acid) Microparticles to Enhance the Growth of Radish Plants. *ACS Agricultural Science & Technology*, 2(5). <https://doi.org/10.1021/acsagscitech.2c00179>
- Schellnhuber, H. J., & Cramer, W. P. (2006). Avoiding Dangerous Climate Change. In *Google Books*. Cambridge University Press.
https://books.google.com/books?hl=es&lr=&id=7Ui8fBmNlm4C&oi=fnd&pg=PA10&dq=climate+change&ots=YAs5DrcMHO&sig=Xa24BEF1RuKQy_qZ6yMcvItamsk#v=onepage&q&f=false
- Sharma, P., Abrol, A., Qureshi, A., & Sharma, S. (2019). Role of biostimulants with special reference to Panchgavya and Jeevamrit in floriculture-a review. *AgricINTERNATIONAL*, 6(1). <https://www.semanticscholar.org/paper/Role-of-biostimulants-with-special-reference-to-and-Sharma-Abrol/65e765e38805b5f855e944ccb816f0c136ca63c4>
- Shrestha, S., Dhungana, M., Sahani, S., & Bhattarai, B. (2021). Seed Quality Improvement to Approach Sustainable Yield Of Field Crops by Various Preparation Techniques: Seed Priming, Treatment and Inoculation_ A Review. *Plant Physiology and Soil Chemistry*, 1(1), 12–20. <https://doi.org/10.26480/ppsc.01.2021.12.20>
- Spooner, D. M., & Knapp, S. (2013, November). *Solanum tuberosum* | *Solanaceae Source*. Solanaceaesource.myspecies.info.
<https://solanaceaesource.myspecies.info/content/solanum-tuberosum>
- Struik, P. C. (2007). Above-Ground and Below-Ground Plant Development. In *POTATO BIOLOGY AND BIOTECHNOLOGY ADVANCES AND PERSPECTIVES* (pp. 219–236). Elsevier. <http://base.dnsgb.com.ua/files/book/Agriculture/Cultures/Potato-Biology-and-Biotechnology.pdf>
- Subbian, P., Lal, R., & Subramanian, K. S. (2000). Cropping Systems Effects on Soil Quality in Semi-Arid Tropics. *Journal of Sustainable Agriculture*, 16(3), 7–38.
https://doi.org/10.1300/j064v16n03_03
- Wang, R. (2014). Gasotransmitters: growing pains and joys. *Trends in Biochemical Sciences*, 39(5), 227–232. <https://doi.org/10.1016/j.tibs.2014.03.003>
- Wang, R. (2018). Overview of Gasotransmitters and the Related Signaling Network. In *Gasotransmitters* (pp. 1–28). The Royal Society of Chemistry.
<https://doi.org/10.1039/9781788013000-00001>
- Weil, R., White, C., & Lawley, Y. (2006). *Forage Radish: New Multi-Purpose Cover Crop for the Mid-Atlantic*. https://covercrop.org/wp-content/uploads/Weil_radish.pdf

- Welty, C., & Doohan, D. (2000, September). *Crop Profile for Green Beans in Ohio*. The Ohio State University Extension Program; The Ohio State University .
<https://ipm.osu.edu/sites/ipm/files/imce/OHgreenbean.pdf>
- Willermet, P. A., Dailey, D. P., Carter, R. O., Schmitz, P. J., & Zhu, W. (1995). Mechanism of formation of antiwear films from zinc dialkyldithiophosphates. *Tribology International*, 28(3), 177–187. [https://doi.org/10.1016/0301-679x\(95\)98965-g](https://doi.org/10.1016/0301-679x(95)98965-g)
- Xu, D., Cao, H., Fang, W., Pan, J., Chen, J., Zhang, J., & Shen, W. (2017). Linking hydrogen-enhanced rice aluminum tolerance with the reestablishment of GA/ABA balance and miRNA-modulated gene expression: A case study on germination. *Ecotoxicology and Environmental Safety*, 145, 303–312. <https://doi.org/10.1016/j.ecoenv.2017.07.055>
- Xuan, L., Li, J., Wang, X., & Wang, C. (2020). Crosstalk between Hydrogen Sulfide and Other Signal Molecules Regulates Plant Growth and Development. *International Journal of Molecular Sciences*, 21(13), 4593. <https://doi.org/10.3390/ijms21134593>
- Yang, Z., Wang, X., Feng, J., & Zhu, S. (2022). Biological Functions of Hydrogen Sulfide in Plants. *International Journal of Molecular Sciences*, 23(23), 15107. <https://doi.org/10.3390/ijms232315107>
- Yao, Y., Yang, Y., Li, C., Huang, D., Zhang, J., Wang, C., Li, W., Wang, N., Deng, Y., & Liao, W. (2019). Multiple environmental stressors can induce gasotransmitter production in plants. Abiotic stressors (drought, salt, heavy metal, temperature, light, and UV radiation) induced the generation of gasotransmitters (H₂, H₂S, NO, CO, and CH₄). In *Research Progress on the Functions of Gasotransmitters in Plant Responses to Abiotic Stresses*. <https://www.mdpi.com/2223-7747/8/12/605>
- Yao, Y., Yang, Y., Li, C., Huang, D., Zhang, J., Wang, C., Li, W., Wang, N., Deng, Y., & Liao, W. (2019). Research Progress on the Functions of Gasotransmitters in Plant Responses to Abiotic Stresses. *Plants*, 8(12), 605. <https://doi.org/10.3390/plants8120605>
- Zhang, H. (2016). Hydrogen Sulfide in Plant Biology. In *Gasotransmitters in Plants*. Springer. <https://link.springer.com/book/10.1007/978-3-319-40713-5>
- Zhang, J., & Spikes, H. (2016). On the Mechanism of ZDDP Antiwear Film Formation. *Tribology Letters*, 63(2). <https://doi.org/10.1007/s11249-016-0706-7>
- Zhou, Z., von Wantoch Rekowski, M., Coletta, C., Szabo, C., Bucci, M., Cirino, G., Topouzis, S., Papapetropoulos, A., & Giannis, A. (2012). Thioglycine and l-thiovaline: Biologically active H₂S-donors. *Bioorganic & Medicinal Chemistry*, 20(8), 2675–2678. <https://doi.org/10.1016/j.bmc.2012.02.028>
- Ziogas, V., Molassiotis, A., Fotopoulos, V., & Tanou, G. (2018). Hydrogen Sulfide: A Potent Tool in Postharvest Fruit Biology and Possible Mechanism of Action. *Frontiers in Plant Science*, 9(1375). <https://doi.org/10.3389/fpls.2018.01375>

CHAPTER TWO

THE IMPACT OF HYDROGEN SULFIDE RELEASED BY
DIBUTYLDITHIOPHOSPHATES ON POTATOES

Contribution of Authors and Co-Authors

Manuscript in Chapter 2

Author: Luis Esquivel-Cervantes

Contributions: conceptualization, methodology, validation, formal analysis, investigation, data curation, writing – original draft, visualization.

Co-Author: Nina Zidack

Contributions: conceptualization, resources, supervision, writing – review and editing, funding acquisition.

Co-Author: Alanna Oiestad

Contributions: conceptualization, writing – review and editing

Co-Author: Jack Martin

Contributions: conceptualization, supervision, formal analysis, writing – review and editing

Co-Author: Ned Bowden

Contributions: writing – review and editing, funding acquisition

Co-Author: Michael Giroux

Contributions: conceptualization, methodology, investigation, resources, supervision, project administration, writing – review and editing, funding acquisition.

Manuscript Information

Esquivel-Cervantes, L., Zidack N., Oiestad A., Bowden N., Martin J., & Giroux M. (2025). The Impact of Hydrogen Sulfide Released by Dibutyldithiophosphates on Potatoes

Status of Manuscript:

- ☒ Prepared for submission to a peer-reviewed journal
- ☐ Officially submitted to a peer-reviewed journal
- ☐ Accepted by a peer-reviewed journal
- ☐ Published in a peer-reviewed journal

Abstract

The effects of climate change on agriculture are increasingly leading to economic hardship for farmers due to heat and drought stress. Focus on enhancing crop traits such as yield, root length, height, and biomass by the application of external products when an environmental stressing event occurs is a possible solution to protect crops from stress related damage. Hydrogen sulfide is a gasotransmitter in plants. Application of slow-release hydrogen sulfide products has emerged in agriculture as a way to protect crops from environmental stress and improve harvest yields. This study investigated how dibutyl dithiophosphate (DBDTP), which releases hydrogen sulfide (H_2S), impacted the growth and development of potatoes. In this study, two potato varieties, Russet Burbank (RB) and Clearwater (CW), were grown under tissue culture, field, and growth chamber conditions. Observations revealed that DBDTP increased root length at all tested concentrations under tissue culture, where 0.025 and 0.1 mM had a significant positive effect compared to the 0 mM control. Plant height was increased, though not significantly, at 0.05mM DBDTP, while applying 0.1 and 0.2 mM DBDTP significantly reduced plant height. Under field conditions, data indicated that yield was significantly higher when applying DBDTP at 0.5 mM. Treated plants yielded 57.9 g per plant, compared to 33.5 g per plant for control plants. Phytotoxicity results suggest 0.5 mM DBDTP is the most appropriate concentration for foliar applications, as it did not cause visible damage when applied eight days after transplanting. Under heat stress conditions, continuous application of 0.025 and 0.1 mM DBDTP, in combination with 200 ppm fertilization, significantly improved plant survival rates. This study demonstrates the versatile ways in which DBDTP may be applied to improve plant growth a under varying growth conditions and in the presence of heat stress.

Introduction

Potatoes are considered an important crop worldwide for their impact on human history and nutritional content. Potatoes are an important source of carbohydrates, vitamin C, vitamin B1, vitamin B3, iron, and essential minerals like potassium, phosphorus, and magnesium. They also play a significant function in the antioxidant defense system, making them a vital food security crop (Sharma, Jaiswal, & Jaiswal, 2020; Ortiz & Mares, 2017). However, climate change directly impacts potato productivity by negatively affecting growth, adaptability, and yields. Thus, improving characteristics such as root growth, height, biomass, yield, and adaptation to stress conditions is fundamental for addressing these challenges (Nelson et al., 2013)

Currently, different approaches have been proposed to address production challenges in agriculture. The use of growth enhancers to improve metabolic processes of plants, and thus improve growth, is a relatively new area of research. This approach includes the use of exogenous applications of gasotransmitters that interact with enzymes, phytohormones, and other gasotransmitters inside plants to improve different biological processes and the health of crops. These compounds can positively influence crop development processes, including root growth, plant height, yield, and stress tolerance. (Dai et al. 2024; Ali et al., 2013)

Gasotransmitters are gaseous molecules that boil at low temperatures, easily pass through cell membranes, and are synthesized by living organisms. These chemicals can be applied externally to plants to affect their growth by interacting with up to hundreds of different enzymatic pathways. They play a role in numerous physiological processes and signaling functions that target specific cellular and molecular mechanisms. Although these chemicals exist

as gases at room temperature, they can also be administered to plants as precursor chemicals that are liquids or solids and react within plants to release a gasotransmitter. This method of delivery is generally favored due to ease of handling and the release of gasotransmitters within plants rather than into the atmosphere around plants (Kolupaev et al., 2022; Wang, 2018; García Mata & Lamattina, 2013).

The primary gasotransmitters in plants include nitrogen monoxide (NO), carbon monoxide (CO), and hydrogen sulfide (H₂S) (Kolupaev et al., 2022). H₂S has been associated with a variety of physiological responses, including seed germination, plant growth, root development, resistance to heavy metal and salt stress, drought stress, temperature stress, pathogen defense, and the delay of senescence (Dai et al. 2024; Li & Zhu, 2014; Bloem et al., 2004). Currently, there are various compounds that can release H₂S (also known as H₂S donors), such as sodium hydrosulfide (NaSH), sodium sulfide (Na₂S), morpholin-4-ium 4-methoxyphenyl (morpholino) phosphinodithioate (GY4137), L-thiovaline (TV), thioglycine, and dithiophosphates (DDTPs) (Yang et al., 2022; Carter et al., 2019; Lisjak et al., 1988). These chemicals have shown a positive effect on plant growth and tolerance to biotic and abiotic stresses when used appropriately. However, between them, only dithiophosphates have been formulated for their use in agriculture.

In the past five years, dithiophosphates have been tested on corn in aqueous solutions or by applying them directly to the seeds demonstrating a beneficial impact on plant growth and harvest yield. Dithiophosphates are readily synthesized by reacting C₂ to C₁₂ fatty alcohols with P₄S₁₀ at elevated temperatures and over two million tons of these chemicals are synthesized each year as precursors for mining and engine oils. Dithiophosphates degrade with water to safe and

natural chemicals, including fatty alcohols and monopotassium phosphate (Ranasinghe Arachchige et al., 2022; Brown et al., 2021; Carter et al., 2019).

The innovation in affordable additives able to improve plant growth, yield, or survival of plants in response to environmental stressors is essential for supporting farmers and agriculture development. This study specifically focused on the DTP dibutyl dithiophosphate (DBDTP). Five concentrations of DBDTP were tested using drench applications in the field to evaluate its effects on potato yield, height, vigor, and biomass in Russet Burbank (RB) and Clearwater Russet (CW) varieties. Additionally, the phytotoxicity of the same concentrations in foliar applications was assessed in RB under growth chamber conditions. Five separate concentrations were examined in tissue culture to measure their impact on root length, height, fresh and dry weight of RB and CW potatoes.

A final experiment was conducted under heat stress conditions using similar doses to those used in the tissue culture experiment, focusing on evaluating the survival percentage and height of the two potato varieties mentioned above. Additionally, an RNA sequencing experiment was carried out using plants grown in tissue culture under standard growth conditions. In this experiment, the control group was compared to plants grown on media containing an ideal concentration of DBDTP to identify biological processes activated by DBDTP application.

To date, there have been no reports of the effects of any chemicals that release H_2S on potatoes grown under field, growth chamber, or tissue culture conditions. Therefore, this research aimed to explore the potential effects of DBDTP to expand its commercial applications. This study addressed several critical questions regarding uses of DBDTP to improve potato growth,

including optimal concentrations, method of application, and timing for best stimulation of growth, yield, and survival.

Materials and Methods

Tissue culture trial

An experiment under tissue culture conditions was conducted to evaluate the effects of 0, 0.025, 0.05, 0.1, and 0.2 mM DBDTP. Potato plantlets were grown from same-age nodes in MS medium within 7.6 x 7.6 x 10 cm magenta containers, following a Completely Randomized Design (CRD). Plant height, root length, overall fresh weight, and dry weight were measured in the fourth week. Each plantlet was removed, cleaned, measured, weighed, and placed in a labeled envelope in a drying room for one week to determine dry weight. There were five replications for each DBDTP concentration, with nine nodes in each magenta container. This was replicated for two varieties (Russet Burbank and Clearwater), and the experiment was repeated twice. Tissue culture media was prepared using 20g sucrose, 4.4g MS 519, and 2.3g phytagel per liter of water. After autoclaving the medium, this was then cooled to 65°C for 45 minutes in a water bath.

Following the cooling process, DBDTP solutions were filter sterilized using a 22 µm filter. Immediately after the chemical addition, ~60 ml medium was poured into magenta vessels. Nine potato nodes were cut and added to each magenta. Cuttings were placed into a CONVIRON growth chamber CMP 6010 and grown under 16 hours of light at 22°C and 8 hours of darkness at 19 °C .

Field Trial

Russet Burbank (RB) and Clearwater (CW) potato plantlets were grown for four weeks in tissue culture following the same methodology previously described, with the minor adjustment that plastic tubs were used with 80 ml medium, and 20 potato nodes were cut per tub.

After four weeks, plantlets were transplanted into trays in the greenhouse using 1.25" x 1.25" plugs made from a blend of peat moss, coco coir, and bio char (Gro Indoor, 2024). Before transplanting, DBDTP concentrations were applied a second time, applying the same concentrations as in tissue culture, by dipping the plugs in a solution containing DBDTP treatments and transplanting one plantlet per plug. A total of 80 plantlets were used for each treatment. All treatments received the same fertilization treatment, with 20-20-20 (NPK) fertilizer applied daily at a concentration of 200 ppm. The potatoes were grown in these plugs for four weeks. When plants reached a height of 12.7 to 15.2cm, they were transplanted in the field.

The field trial was conducted at the Horticulture Farm in Bozeman, Montana where a 8.83 x 29.26 meter plot was used to sow the potatoes in a randomized block split-plot design where variety was the main plot and DBDTP dose was the subplot. There were 8 plants per plot, with 26.7cm spacing between plants, 2.3m long rows, 0.76m spacing between rows, and 10 blocks. Five new doses were used in the field. Before planting, the trays were dipped in a DBDTP solution using the new concentrations. New doses consisted of 0, 0.5, 1, 2, and 10 mM, where 0 mM corresponded to 0 mM in the tissue culture, 0.5 mM to 0.025 mM, 1 mM to 0.05 mM, 2 mM to 0.1 mM, and 10 mM to 0.2 mM. The doses were modified to supply adequate DBDTP to plants as they grew in field soil. One month after planting, a drench was applied using the same concentrations as described above. The height and vigor were evaluated one and two months after transplanting. In 2023, the plot was harvested after 2 months due to Potato Virus Y

(PVY) infection and after 3 months in 2024. Plant biomass, tuber weight per plant, and number of tubers per plant were evaluated in both years.

Phytotoxicity Trial

The phytotoxicity of DBDTP was evaluated in foliar applications on RB potatoes at four different application times, one day after transplanting (1DAT), eight days after transplanting (8DAT), fifteen days after transplanting (15DAT) and one month after transplanting (1MAT). The plantlets used for this trial were grown for four weeks in tissue culture and then transplanted into trays using 1.25" x 1.25" plugs. The same doses evaluated in the field were but as a foliar application instead of a drench application (0, 0.5, 1, 2, 10mM).

In this trial, different sets of potatoes were used. Each set of potatoes was treated with DBDTP concentrations at different application times after transplanting. Explicitly, one set was treated one day after transplanting, another set was treated eight days after transplanting, a third set was treated fifteen days after transplanting, and the final set was treated 1 month after transplanting. The first three sets were transplanted simultaneously into trays, and a completely randomized design was employed at the time point level (one, eight, and 15 days after transplanting), with four replications for each treatment time. Each row within a tray served as an experimental unit. Within the tray, five rows of seven plantlets each were used to randomize the five doses. For the last set, a single plantlet was sown in 4" pots in a Completely Randomized Design with twenty-five pots per treatment. The amount of water sprayed in this experiment was obtained based on a previous calibration on the zero treatment. This calibration was done every time that DBDTPs were applied, and heavy applications were done to ensure the full cover of the foliage. All the potatoes were watered every day with 100 ppm 20-20-20 fertilizer.

Shoot fresh weight was measured one month after transplanting in the case of the first three application times, and one month after application in the case of the 1MAT. Phytotoxicity was evaluated on the top five leaves with a visual scale of 1 to 5, where 1 indicated no burns on the leaves; 2 represented small individual burns on some leaves; 3 indicated at least one leaf with spots that merged into a bigger spot but covering no more than $\frac{1}{4}$ of the leaf; 4 implied bigger spots in at least one leaf covering more than $\frac{1}{3}$ of the leaves but without dead leaves; and 5 represented at least one true leaf dead and more leaves with scores 3 and 4 or dead plant

Heat Stress Trial

Heat stress conditions in potatoes can be induced in a greenhouse or growth chambers at 35 ± 1 °C during the day and 28 ± 1 °C at night under a photoperiod of 14 h light and 10 h darkness. Under normal conditions, potatoes can grow at 22 ± 1 °C during the day and 18 ± 1 °C at night under the same photoperiod with 70% humidity (Zhang et al., 2019; Tang et al., 2020). Russet Burbank and Clearwater potatoes were initially grown in tissue culture for three weeks before being transplanted into trays using 1.25" x 1.25" plugs. In this experiment, three DBDTP treatments (0.025, 0.1, 0.4 mM), along with a control group (0 mM) were tested. After transplanting, trays were placed into a growth chamber set at 22°C during the day and 19 °C at night for two weeks before heat treatment. Treatment applications started in tissue culture following the same methodology as above. Subsequent applications of DBDTP were applied daily, alongside 200 ppm NPK fertilization, beginning two days after transplanting. A Randomized Complete Block (RCB) design was used within the growth chamber, containing five blocks. Within each block, each treatment combination was presented once in a two-row tray, with twelve plants per treatment. Flat trays were placed below each block, with plants

getting daily watering in the morning using three liters of a solution (consisting of mixed DBDTP treatment and fertilizer).

To apply the treatments, the two-row trays were first removed from their respective blocks and placed in a new tray. After the application process, once the plugs no longer dripped, the two-row trays were returned to their original blocks.

The plantlets were acclimated for two weeks before heat stress was induced, with temperatures set at 35 ± 1 °C during the daytime and 28 ± 1 °C at night, and a photoperiod of 14 hours of light and 10 hours of darkness. After onset of heat stress, plantlets were watered twice a day. Data collection of survival percentages and plant height were recorded at one, two, and three weeks after heat stress onset.

Statistical Analysis

The data were analyzed using analysis of variance (ANOVA) in R (R Foundation for Statistical Computing, Version 4.0.5, Vienna, Austria). The values are presented as the means of two independent experiments, analyzed either individually or collectively based on the specific objectives of each trial, comparing control versus the DBDTP treatments. Differences with a p-value of less than 0.05 were considered significant. Figures were created using Microsoft Excel.

In the case of data from the field experiment, a mixed-effects model for a randomized block split plot design using the lme4 package in R (Bates et al., 2015) was used each year to analyze data due to the huge difference in yield because of the anticipated harvest in 2023. Results were averaged over variety. In 2023, only yield data was square-root-transformed before analysis to meet assumptions and estimated marginal means were back transformed for interpretability. In 2024, assumptions were met and no need for transformation was necessary.

For the tissue culture and heat stress experiments, the data were analyzed by combining the two independent experiments in a mixed-effects model and averaging results over variety. The assumptions were met with all the response variables. In the phytotoxicity experiment, the data were analyzed using a linear mixed effect model for both trials, and assumptions were met.

Results

Effect of DBDTP on Potato under Tissue Culture Conditions

The effect of DBDTP H₂S donor was evaluated in tissue culture conditions to ensure no external factors such as soil, environment, or microbial interactions affected the physiological response of H₂S on potatoes. The results of this experiment were obtained from the analysis of two independent experiments in a combined mixed model averaging over varieties.

The following results correspond to the impact of five DBDTP treatments (0 mM, 0.025 mM, 0.05 mM, 0.1 mM, and 0.2 mM) on important response variables: root length, shoot height, total fresh weight, and total dry weight.

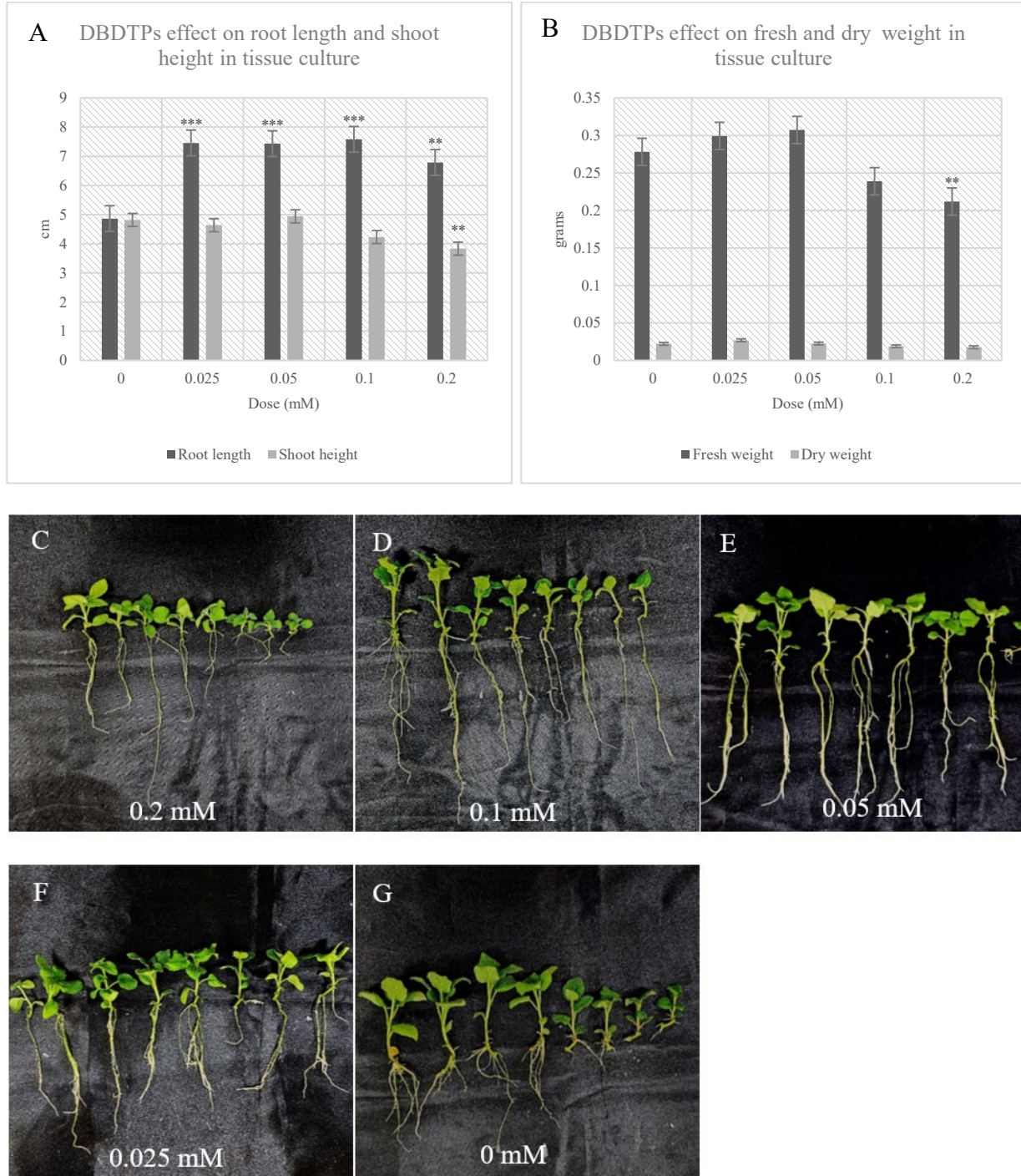


Figure 2.1. Effect of DBDTP on potato plantlets under tissue culture conditions on the 4th week. (A) Potato root length and shoot height by DBDTP treatments, (B) Potato fresh and dry weight by DBDTP treatments, (C)-(G) Representative plantlets from treatments: 0.2 mM (C), 0.1 mM (D), 0.05 mM (E), 0.025 mM (F), and 0 mM (G, control). Each data point in the figure represents the estimated mean ($\pm$ SE) from two independent trials based on measurements from eight plants

per treatment and five replicates per treatment. Error bars represent the standard error (SE) of the estimated marginal means. A single asterisk (*) indicates a significant difference from the control (0 mM DBDTP) at $P < 0.05$, double asterisks (**) indicate strong significance at $P < 0.01$, and triple asterisks (***) indicate highly significant differences at $P < 0.001$, based on post hoc analysis.

As illustrated in Figure 2.1A, root length significantly increased under all DBDTP treatments at four weeks after cutting when compared to the control group. These results suggest that DBDTP effectively promotes root development. However, shoot height did not follow the same trend; this was especially apparent at 0.2 mM DBDTP, where a significant negative effect was observed. At this concentration, fresh weight was also significantly lower than the 0 mM DBDTP control (Figure 2.1B). The impact of DBDTP on root length was visually distinguishable between the treatments and the control (Figure 2.1C-G)

Impact of DBDTP on Potato under Field Conditions

The impact of DBDTPs on RB and CW potato plants was evaluated in 2023 and 2024 by measuring plant height and vigor at one and two months after transplanting. Number of tubers per plant, tuber weight per plant, and above ground biomass at harvest were also recorded. Potatoes were harvested two months after transplanting in 2023. In 2024 they were harvested three months after transplanting. This timing difference significantly affected the response variables, potatoes at two months after transplanting were at the tuber filling stage and three months after transplanting were already at the maturity stage. The results of the experiments were analyzed separately for each year and averaged over variety.

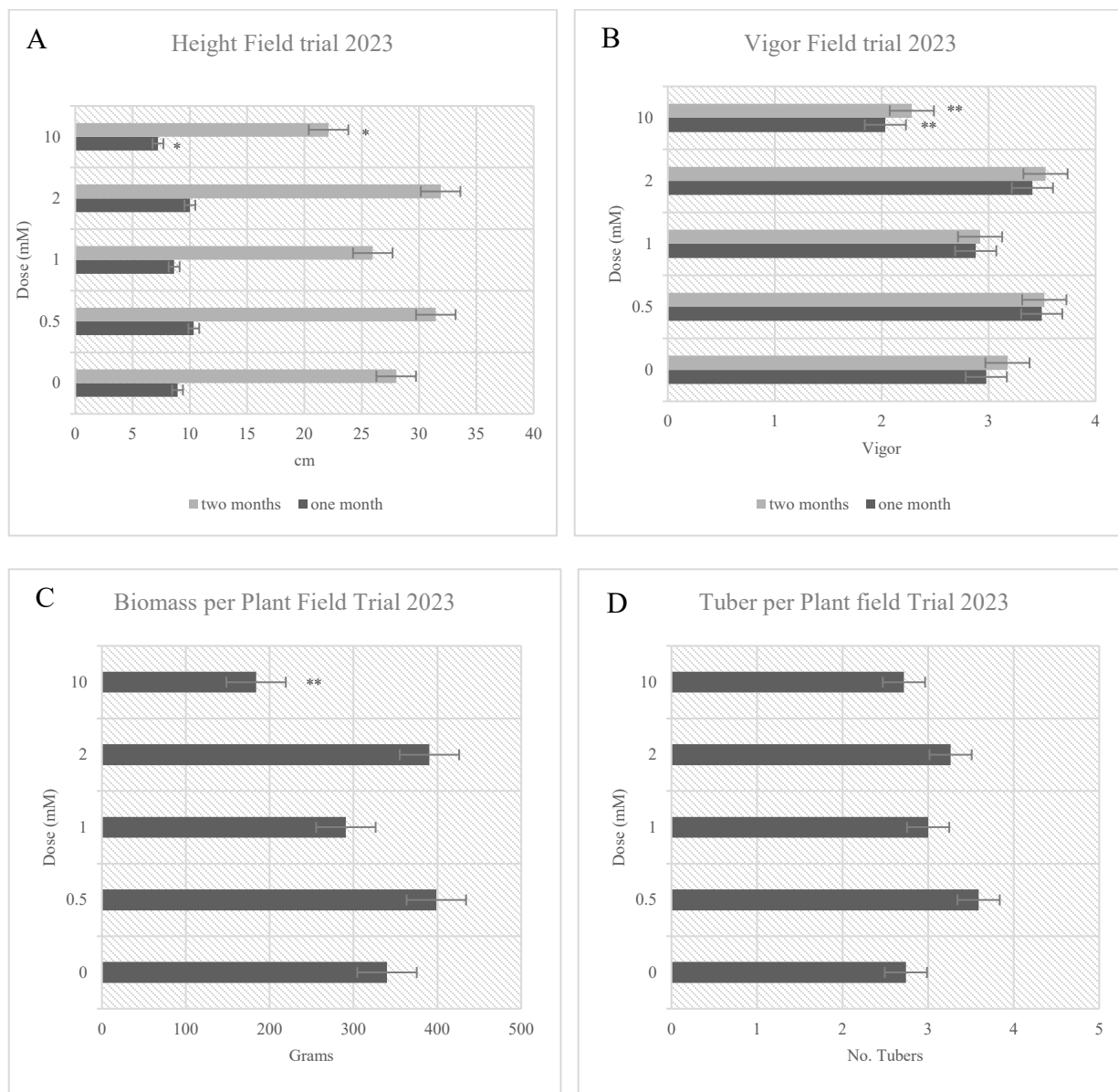


Figure 2.2. Plant height and vigor under field conditions for five concentrations of DBDTP in 2023. (A) Plant height measured one and two months after transplanting per DBDTP concentration, (B) Plant vigor assessed one and two months after transplanting per DBDTP concentration. (C) Plant biomass in grams per plant per DBDTP concentration. (D) Number of tubers per plant per DBDTP treatment. Each data point in the figure represents the estimated mean ($\pm$ SE) from the first trial conducted in 2023, based on measurements from eight plants per treatment across ten blocks. Error bars represent the standard error (SE) of the estimated marginal means. A single asterisk (*) indicates a significant difference from the control (0 mM DBDTP) at $P < 0.05$, double asterisks (**) indicate strong significance at $P < 0.01$, and triple asterisks (***) indicate highly significant differences at $P < 0.001$, based on post hoc analysis.

Effect of DBDTP in height, vigor, biomass, tubers per plant, and grams per plant in 2023.

The results showed that the application of DBDTP at 10 mM significantly reduced plant height and vigor of field grown plants at one and two months after transplanting compared to the 0mM control (Figure 2.2A, B). In contrast, the 0.5 mM and 2 mM concentrations increased plant height and vigor, although these effects were not statistically significant. As shown in Figure 2.2C, the addition of 10 mM DBDTP led to a notable (but not significant) reduction of biomass per plant compared to the 0 mM treatment. On the other hand, the 2 mM and 0.5 mM treatments increased biomass compared to the control (0 mM), although these increases were also not statistically significant. Analysis of variance indicated a non-significant difference in the number of tubers for dose; however, the 0.5 mM treatment produced nearly one additional tuber per plant (Figure 2.2D). In 2023, tuber weight per plant was significantly increased at 0.5 mM DBDTP by 24 grams versus the control (Figure 2.3A).

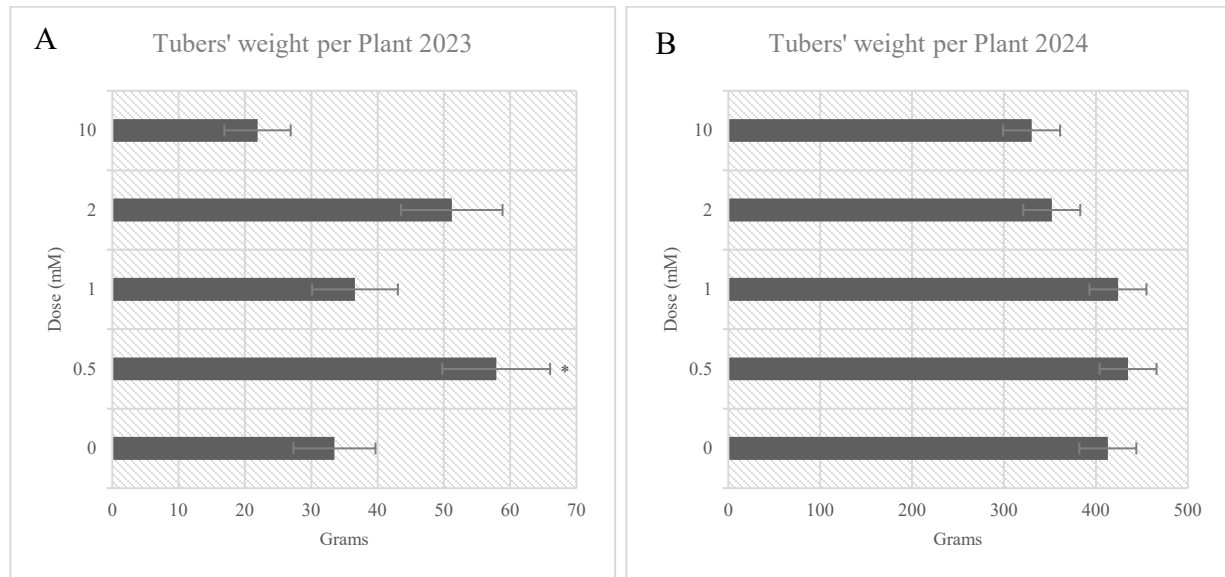


Figure 2.3. Potato tubers' weight per plant in 2023 and 2024 under field conditions at harvest after following DBDTP treatments. (A) Effect of four total DBDTP applications on tuber weight (grams per plant) in potatoes harvested in 2023, two months after transplanting. (B) Effect of four total DBDTP applications on tuber weight (grams per plant) in potatoes harvested in 2024,

three months after transplanting. Each data point represents the estimated mean ($\pm$ SE) from separate trials conducted in 2023 and 2024, based on measurements from eight plants per treatment across ten blocks. Error bars represent the standard error (SE) of the estimated marginal means. A single asterisk (*) indicates a significant difference from the control (0 mM DBDTP) at $P < 0.05$, double asterisks (**) indicate strong significance at $P < 0.01$, and triple asterisks (***) indicate highly significant differences at $P < 0.001$, based on post hoc analysis.

Effect of DBDTP in height, vigor, biomass, tubers per plant, and grams per plant in 2024.

In 2024, the response patterns differed from those in 2023 for some of the variables. One notable contrast between the two years was the presence of Potato virus Y (PVY) in 2023. To prevent the spread of and minimize disease effects, potatoes were harvested earlier in 2023 than in 2024, potentially contributing to the differences in the yield response.

In 2024, the use of DBDTP did not show a significant effect on height compared to the control at either one or two months after transplanting. The only significant observation was for plant vigor at one month after transplanting at 10 mM DBDTP (Figure 2.4A). The biomass exhibited a positive trend at 0.5 mM DBDTP (Figure 2.4B).

In terms of the impact of DBDTP concentration on number of tubers per plant in 2024 the effect of 2 mM and 0.5 mM (Figure 2.4C) was similar to the effect observed in the previous year (Figure 2.2D), where these two concentrations slightly increased the number of tubers per plant.

According to the analysis of variance, there was a significant effect on tuber weight in 2024 (p -value= 0.046), though this significant effect was between treatments and not between control and treatments (Table 1). Nonetheless, DBDTP at 0.5 mM and 1 mM showed a trend in increasing tuber weight compared to the control (Figure 2.3B).

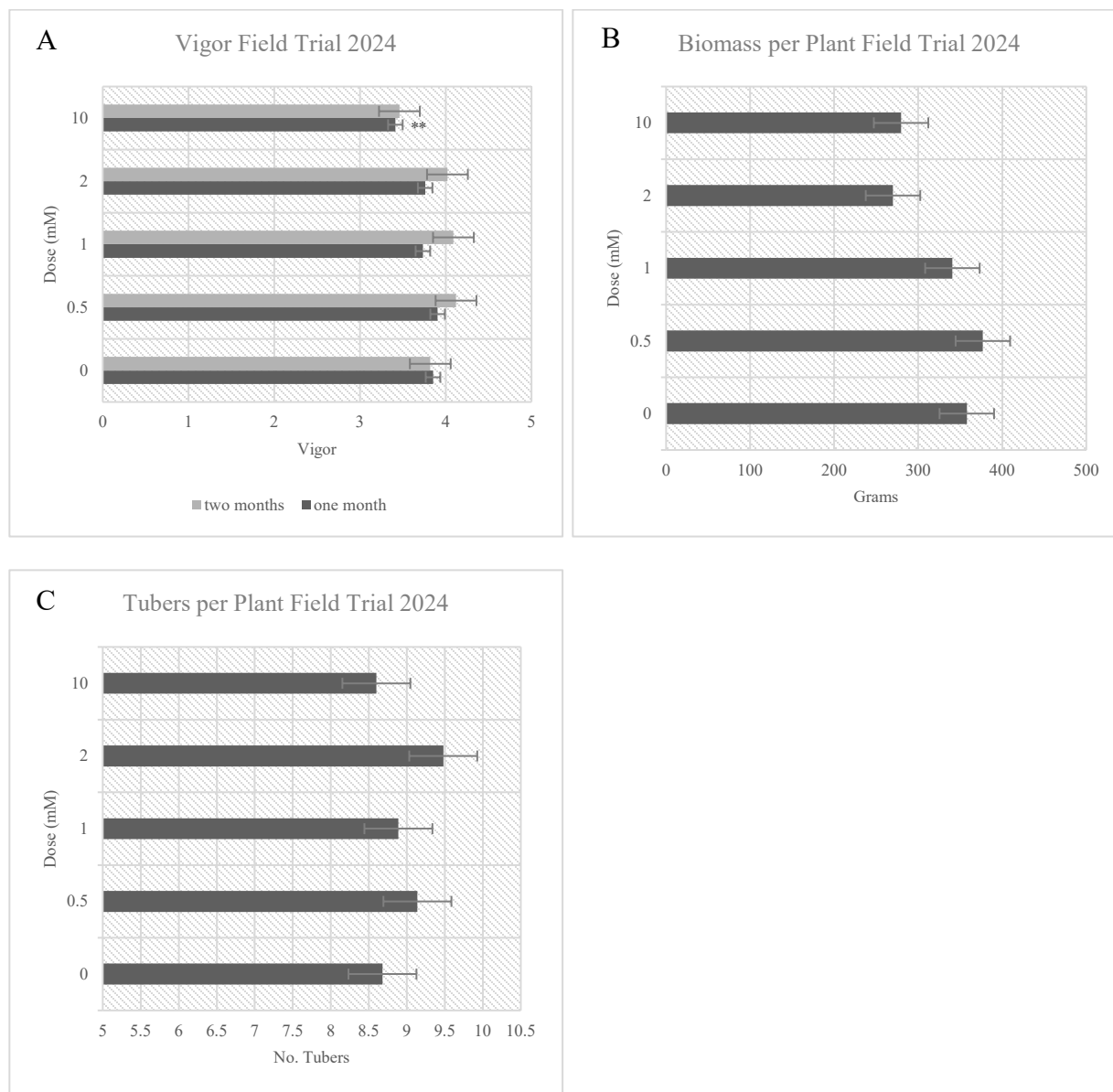


Figure 2.4. Plant vigor, and biomass under field conditions in 2024. (A) Plant vigor measured one month and two months after transplanting per DBDTP concentration, (B) Plant biomass (grams per plant) per DBDTP concentration at harvest, (C) Effect of DBDTP on the number of tubers per plant at harvest. Each data point in the figure represents the estimated mean ($\pm$ SE) from the second trial conducted in 2024, based on measurements from eight plants per treatment across ten blocks. Error bars represent the standard error (SE) of the estimated marginal mean. A single asterisk (*) indicates a significant difference from the control (0 mM DBDTP) at $P < 0.05$, double asterisks (**) indicate strong significance at $P < 0.01$, and triple asterisks (***) indicate highly significant differences at $P < 0.001$, based on post hoc analysis.

Table 1.1 Post-hoc analysis of variance for potato tuber weight per plant in trial 2024 (grams per plant), comparing the 0 mM DBDTP control versus 0.5, 1, 2, and 10 mM DBDTP

Contrast	Mean Difference	Standard error difference	T value	P value
Dose 0 vs Dose 0.5 mM	22.24	41.244	0.54	0.97
Dose 0 vs Dose 1 mM	10.89	41.244	0.26	0.99
Dose 0 vs Dose 2 mM	-60.31	41.244	-1.46	0.47
Dose 0 vs Dose 10 mM	-82.82	41.244	-2.01	0.19

Effect of DBDTP on potatoes in foliar applications

The phytotoxicity effect of DBDTP was tested on RB potato plantlets in growth chambers, utilizing the concentrations evaluated in the field drench applications. This was done to ensure that the doses showing positive effects in field tests would not have negative impacts when applied in fertigation. At Montana State University, potatoes are often irrigated using fertigation, making it an efficient method for applying DBDTP. This trial will serve as a preliminary assessment for further experiments on DBDTP's foliar application, focusing on various responses such as yield, growth, and survival in weather stress conditions.

Results indicate that there is a significant interaction between the DBDTP dose and the application time, suggesting that the same dose has different phytotoxicity effects at different application times. Thus, applying the same DBDTP dose earlier or later may reduce or increase damage. Results presented in Figure 2.5A indicate that DBDTP foliar treatment induced significant phytotoxicity at one, eight, and fifteen days after transplanting at concentrations of 1, 2, and 10 mM compared to the 0 mM control. For the 0.5 mM concentration, a notable phytotoxic effect was observed only one day after transplanting but not at eight and fifteen days after transplanting compared to the control. After one month, the analysis revealed a significant interaction between the two rounds of the experiment. Figure 2.5B illustrates that DBDTP produced effects similar to those observed at one, eight, and fifteen days after transplanting, with

concentrations of 1, 2, and 10 mM showing significant phytotoxicity. Interestingly, the 0.5 mM concentration did not exhibit phytotoxicity.

In the case of the response variable shoot fresh weight, the analysis showed a marginally significant interaction between dose and application (p-value=0.070). Figure 2.5C illustrates the effect of heavy foliar application of DBDTP on shoot fresh weight per plant. DBDTP had a significant negative impact on shoot fresh weight only at 10 mM at the one day after transplanting application in contrast to the control. In Figure 2.5D, the effect of DBDTP on shoot fresh weight is observed after one month of the application. A similar trend to the previous data from one, eight, and fifteen days is seen here, where only the highest concentration impacted fresh weight in both rounds of the experiment.

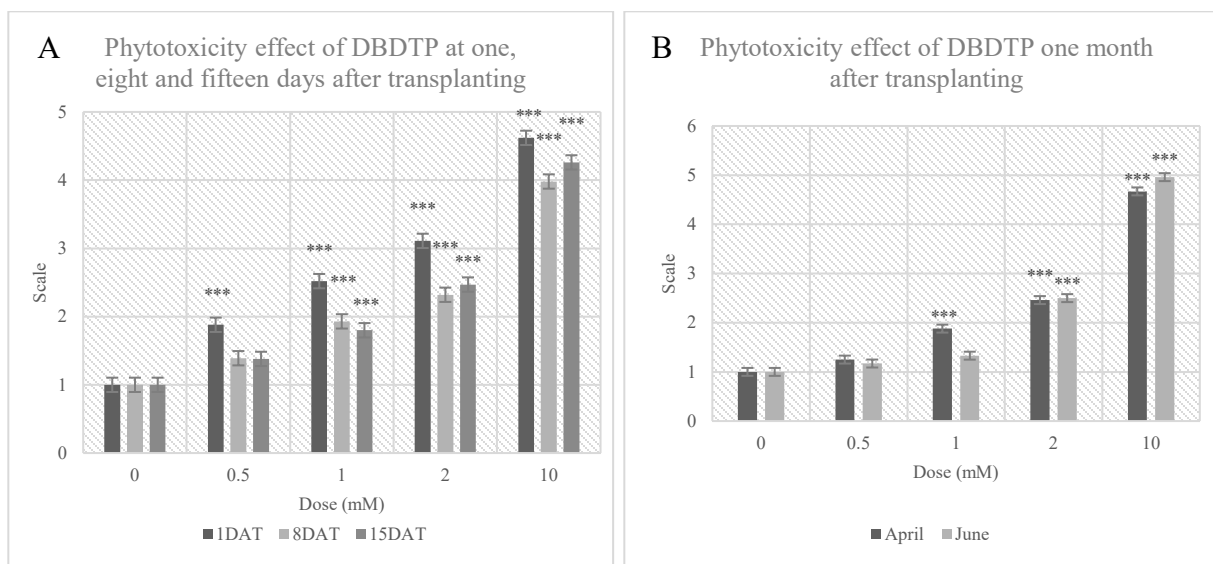


Figure 2.5 Continued

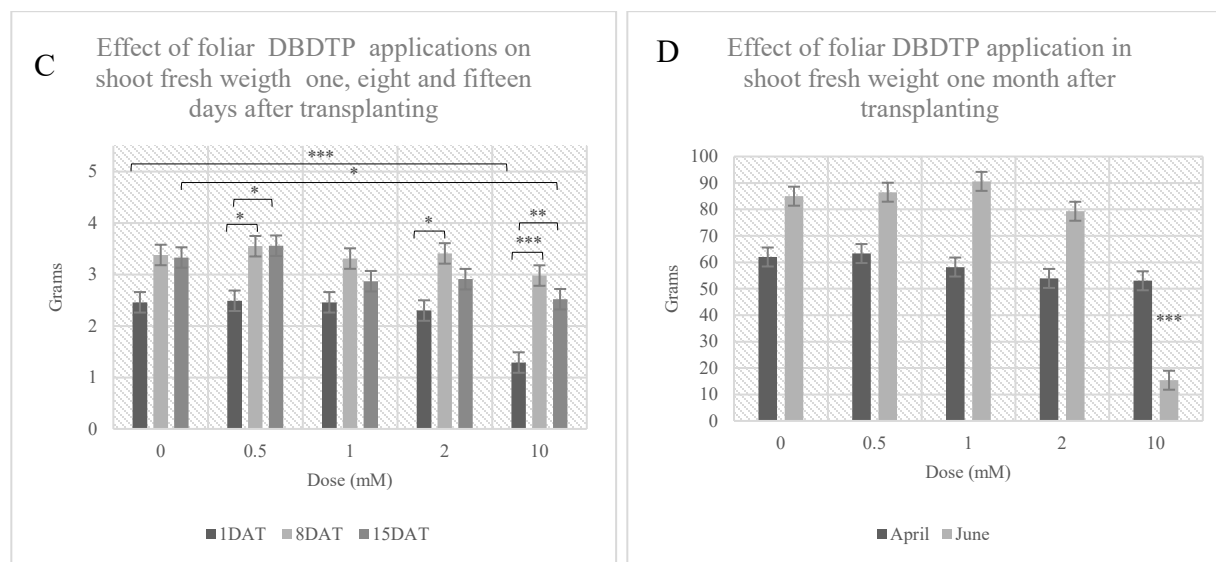


Figure 2.5. Effect of DBDTP as foliar applications on potato plantlets at different application times. (A) Visual assessment of phytotoxicity at one, eight, and fifteen days after transplanting for five DBDTP treatments from two trials analyzed together. (B) Visual assessment of phytotoxicity one month after transplanting for both trials (April and June 2024) analyzed separately, (C) Fresh weight per plant for the two rounds of the experiment at one, eight, and 15 days after transplanting analyzed together, significance versus control and within same treatments, (D) Fresh weight per plant at one month after transplanting application for the two rounds of the experiment analyzed separately. Each data point in the figure represents the estimated mean ($\pm$ SE) from two trials. Error bars represent the standard error (SE) of the estimated marginal means. A single asterisk (*) indicates a significant difference from the control (0 mM DBDTP) at $P < 0.05$, double asterisks (**) indicate strong significance at $P < 0.01$, and triple asterisks (***) indicate highly significant differences at $P < 0.001$, based on post hoc analysis.

Effect of DBDTP on Potato under heat stress conditions

The effect of DBDTP on survival percentage and height was evaluated on RB and CW potatoes under heat stress conditions in a growth chamber at 35 ± 1 °C during the daytime and 28 ± 1 °C at night, with a photoperiod of 14 hours of light and 10 hours of darkness. The effect of dose on survival varied significantly by week, as noted by the significant interaction between

dose and week justifying an analysis by week. However, for height, no such interaction was observed, and the main effects were analyzed in a combined model.

Results from the ANOVA showed that 0.025 and 0.1 mM DBDTP treatments affected the survival percentage from week one to week three versus the control with a significant effect at weeks two and three (Figure 2.6A). The DBDTP effect on height is shown in Figure 2.6B and indicates that the 0.1 mM DBDTP concentration has a significant, positive impact on plant growth under these growth conditions. The impact of DBDTP on survival percentage was notable, with a clear positive trend observable from week one through week three at concentrations 0.025 and 0.1 mM DBDTP, plants exposed to these treatments exhibited better resilience in comparison to the control group and those at 0.4 mM DBDTP concentration (Figure 2.6C-E)

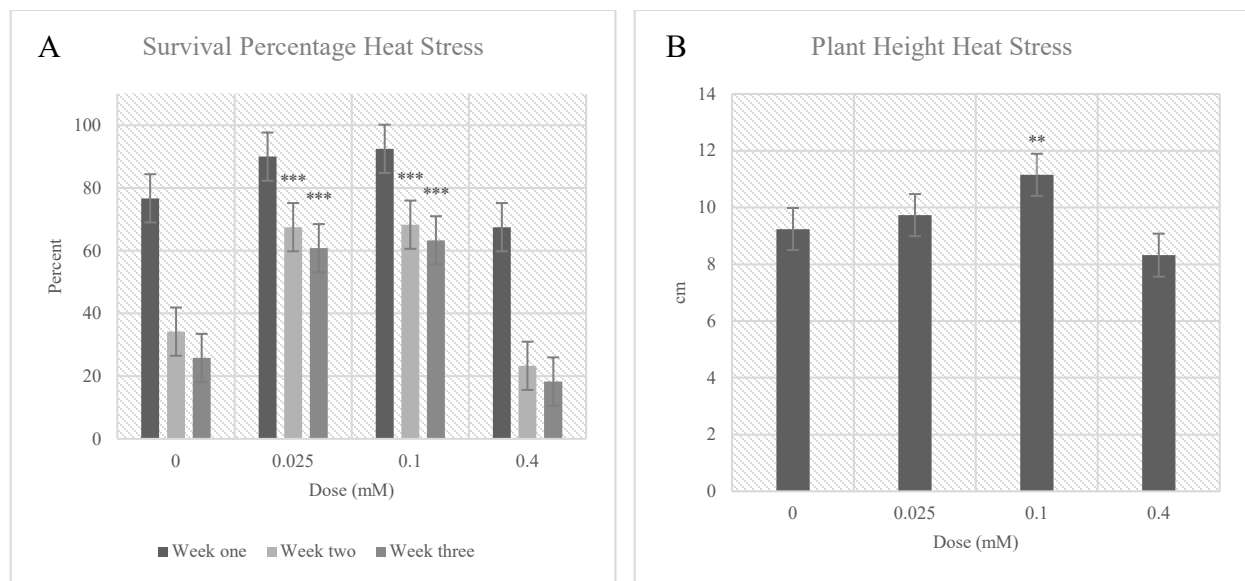


Figure 2.6 Continued

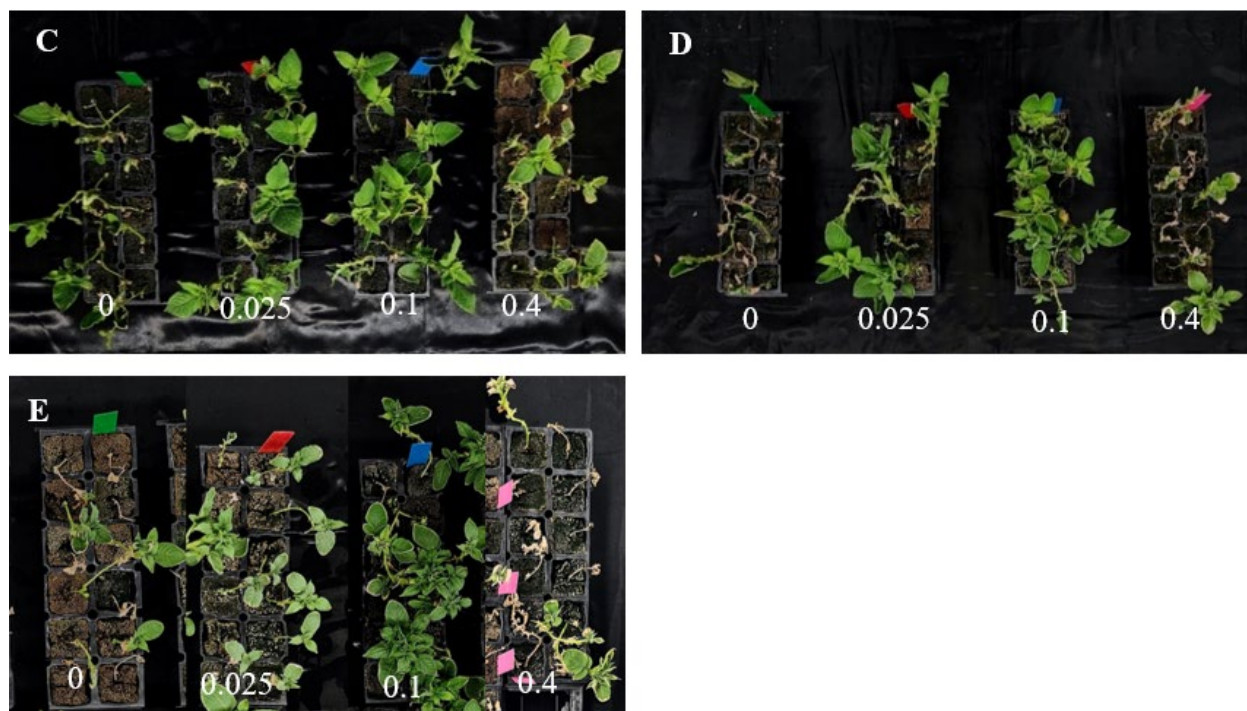


Figure 2.6. Effect of daily DBDTP applications in a solution of 200 ppm 20-20-20 NPK on potato plantlets exposed to heat stress for three weeks. (A) Potato survival percentage by week for four DBDTP treatments, (B) Potato height average from one week, two weeks, and three weeks after heat stress across four DBDTP treatments, (C) Potato plantlets one week after heat stress DBDTP treatment 0, 0.025, 0.1, and 0.4 mM left to right, (D) Potato plantlets two weeks after heat stress, DBDTP treatment 0, 0.025, 0.1, and 0.4 mM left to right, (E) Potato plantlets three weeks after heat stress, DBDTP treatment 0, 0.025, 0.1, and 0.4 mM left to right. Each data point in the figure represents the estimated mean ($\pm$ SE) from one trial. Error bars represent the standard error (SE) of the estimated marginal means. A single asterisk (*) indicates a significant difference from the control (0 mM DBDTP) at $P < 0.05$, double asterisks (**) indicate strong significance at $P < 0.01$, and triple asterisks (***) indicate highly significant differences at $P < 0.001$, based on post hoc analysis.

Discussion

Research on hydrogen sulfide (H_2S) has shown its importance in various fields such as medicine, plant science, and animal science when it is used at the right concentrations. In plants, it has been reported that treatment with H_2S increased seed vigor, root activity, the

contents of photosynthetic pigments, plant growth and development, and stress tolerance of plants (Dai et al. 2024; Li, Min, & Zhou, 2016; Ali et al., 2013).

H₂S plays a vital role in promoting the growth of primary roots as well as the development of lateral and adventitious roots. Furthermore, exogenous applications of H₂S have been shown to lead to the accumulation of auxins, which are critical for root growth (Mukherjee & Corpas, 2020; Wu et al., 2021). In tissue culture experiments summarized in this study; we confirmed that the application of DBDTP induced root growth by significantly increasing root length for all tested concentrations in contrast to the control. Specifically, at low concentrations, results indicated that 0.025 mM increased root length by 53.29%, 0.05 mM by 52.88%, and 0.1 mM by 55.96%. Even at the highest concentration of 0.2 mM, root length was increased by 39.5% (Figure 2.1A). Previous studies demonstrated that H₂S promotes the formation of adventitious roots in cucumber plants by inducing the expression of genes related to haem oxygenase-1 (*HO-1*), which is part of a signaling pathway that enhances root development (Lin et al., 2012). H₂S also has shown an effect in increasing the number of lateral roots, total root length, and the total root surface area (Jia et al., 2015; Wu et al., 2021). Larger root systems are crucial for plants to acquire water and maximize nutrient uptake from the soil, while also providing support and stress tolerance (Epstein, 1977). Despite the increase in root length, our findings suggest that DBDTP can have a significant, negative effect on shoot height and fresh weight when applied at high levels, such as 0.2 mM. At this concentration, shoot height was reduced by 20.37% and fresh weight by 22.22% (Figure 2.1A and B). Alternatively, a positive trend was observed at 0.05 mM DBDTP for height (2.70%) and fresh weight (11.11%). In the

case of dry weight, there was a non-significant negative effect at 0.2 mM and a non-significant positive effect of 18.18% at 0.025 mM.

Many authors have reported the effects of H₂S on lateral root development and the formation of adventitious roots. It will be essential to evaluate these parameters alongside root dry weight and the expression of genes in the roots in response to DBDTP treatment. Wu et al. (2021) demonstrated that in peaches exogenous H₂S led to a 44.50% increase in the concentration of endogenous auxin. In another study under in vitro conditions in potatoes, Kolachevskaya et al. (2014) created transgenic lines harboring the *Agrobacterium* auxin biosynthesis gene *tms1*. Expression of *tms1* was detected mostly in tubers rather than in shoots, and a positive correlation was observed between *tms1* expression, Indoleacetic acid (IAA) content in tubers, and stimulation of tuber formation, implying an important role of auxins in potato tuberization. Auxins are known for being key regulators in root development. Results from tissue culture demonstrated that the DBDTP H₂S donor added to the growth media increased root length. This enhancement may be due to the ability of H₂S to improve the concentration of endogenous auxins, which could have a direct impact on yield due to the role of auxins in potato tuberization.

A positive trend in plant height and vigor was observed for potato plants treated with DBDTP in the 2023 field (Figure 2.2A, B). Treatment with 0.5 mM DBDTP increased height by 15.86% and vigor by 17.24% compared to the control in the first month. Additionally, the 2 mM DBDTP treatment led to a 13.8% increase in height and an 11.19% increase in vigor when compared to the control at the second month time-point. In 2024 we observed again a small non-significant effect on height and vigor due to DBDTP treatments; one month after transplanting

treatment 1 mM increased height by 3.1% and treatment 0.5 mM increased vigor by 2.6% compared to the control, meanwhile, two months after transplanting height was increased by 2.0% and vigor 7.8% (Figure 2.4A) when 0.5 mM was applied. Even when these results were not significant, they support information reported by other authors on the positive effects of H₂S on plant growth.

A significant negative effect on height and vigor was observed in 2023 when the highest concentration of DBDTP (10 mM) was used (Figure 2.2A, B). Plant height in the first and second months was reduced by 19.04% and 21.00% respectively, compared to the control treatment (0 mM). However, in 2024 the only significant negative effect was observed for vigor in the first month at 10 mM DBDTP, with a reduction of 10.5% (Figure 2.4A). In many studies, it has been reported that the use of high concentrations of H₂S can have a negative impact on plant growth. Thompson and Kats (1978) found that the use of 3000 ppm H₂S in greenhouses caused leaf lesions, defoliation, and reduced growth. High levels of the H₂S donor NaHS inhibit auxin transport and lead to changes in root system development. This inhibition occurs by repressing primary root growth through the activation of a signal transduction pathway involving the accumulation of reactive oxygen species (ROS), activation of *Mitogen-Activated Protein Kinase 6 (MPK6)*, and production of nitric oxide (NO) (Jia, Hu, Fan, & Li, 2015; Zhang, Luo, Wang, & Xu, 2017). Furthermore, seeds pre-soaked in 8.0 mM NaHS delayed germination (Dai et al., 2024).

In 2023, the potato harvest was carried out early at two months after transplanting due to the unexpected infection by Potato virus Y (PVY). In 2024 the harvest was carried out at the standard three months after transplanting. Data on aboveground biomass per plant, tubers per

plant, and tuber weight per plant were collected immediately after harvest. The highest treatment (10 mM DBDTP) exhibited a significant negative effect on biomass and reduced fresh weight by 45.9% per plant compared to the control in 2023. However, the use of 0.5 mM and 2 mM concentrations showed a positive non-significant effect and increased fresh weight by 17.29% and 14.87% respectively (Figure 2.2C). While not significant, plants treated with DBDTP in 2024 trended in the same direction as in 2023. Fresh weight was reduced by 21.8% when treated with 10 mM DBDTP, while treatment at 0.5 mM increased fresh weight by 5.3% compared to 0 mM (Figure 2.4B).

In previous studies, it was shown that using dibutyldithiophosphate significantly increased biomass of corn plants by 39% when only 1 mg per plant of the dibutyldithiophosphate was used. However, higher concentrations (100 mg and 200 mg) had a negative impact on biomass and height of the plants (Carter, Brown, Irish, & Bowden, 2019). Additionally, in a study from 2018, it was found that using H₂S with the donor GYY-4137 significantly boosted the growth of radishes, peas, and lettuce. But once again, higher concentrations had a negative effect on plant growth (Carter et al., 2018). Both studies revealed a similar pattern where low concentrations increased growth, some middle concentrations had a similar effect as the control, and higher concentrations ultimately led to reduced growth. Our findings align with those of these authors, indicating that the highest concentrations resulted in negative effects on plant growth, while low concentrations had a positive impact.

Concerning the number of tubers per plant, the difference for the treatments approached statistical significance (p-value= 0.054) in 2023 but not in 2024 (p-value= 0.54). In 2023 0.5 mM and 2 mM increased the number of tubers by 31.02% and 19.05% compared to the control

treatment (Figure 2.2D), while in 2024 the same concentrations increased tubers by 5.29% and 9.21%, suggesting once more the role of DBDTP in promoting plant developmental processes.

Tuber weight trended in the same direction as number of tubers. In 2023 we obtained a significant effect on tuber weight per plant, where 0.5 mM significantly increased weight by 72.83% per plant and 2 mM treatment increased tuber weight by 52.83% compared to the control treatment (Figure 2.3A). In 2024, the results did not show a significant effect, however, once again 0.5 mM showed trended 5.32% higher 5.32% than the control (Figure 2.3B). Furthermore, the 10 mM concentration reduced tuber weight per plant by 29.79% in 2023 and 20.09% in 2024 compared to 0 mM even though.

An interesting pattern was observed in our data from 2023 for all the response variables. The 0.5 mM treatment increased the responses and 1 mM treatment showed results similar to the control. Subsequently, the 2 mM treatment again enhanced the responses, while the 10 mM treatment negatively affected them. Similar trends have been reported by other authors. Carter et al. (2018, 2019) showed comparable patterns in their studies on corn plant height and mass, radish root weight, pea shoot height and fresh weight, and lettuce biomass. In these cases, the lowest concentration had a positive effect, an intermediate concentration resembled the control, other intermediate concentrations enhanced plant growth, and the highest concentrations negatively impacted the plants. In 2024 our data only showed this pattern for the response variable tubers per plant, height, and vigor one month after transplanting, but not for the other variables.

These results emphasize the necessity of defining an appropriate concentration of DBDTP for its field application and that this will likely vary depending on crop species. As

indicated by all response variables, using concentrations that are too high has toxic effects on plants, leading to reduced height, vigor, and yield. This observation confirms the findings of many authors who have noted that while exogenous H_2S can have positive effects on plants at low concentrations, it can be phytotoxic at higher levels, negatively impacting germination, root growth, and other aspects of plant growth and development (Gupta et al., 2021; Li, Gong, & Liu, 2012; Zhang, Luo, Wang, & Xu, 2017).

Our findings on phytotoxicity indicate that the foliar application of DBDTP needs to be carried out with caution, as high concentrations cause significant foliage damage (Figure 2.7A-C, Figure 2.8A-E). In Figure 2.5A and Figure 2.5B, we observed that concentrations of 1, 2, and 10 mM can lead to significant damage when applied at one day, eight days, fifteen days, and one month after transplanting compared to the control. However, 0.5 mM concentration exhibited a similar response to the control group for eight, fifteen, and one month after transplanting; however, it caused significant phytotoxicity damage at one day after transplanting in comparison with 0 mM. This highlights the importance of establishing timing and concentration recommendations for foliar applications. Furthermore, our results indicate that the 1 mM concentration generally caused 38%, 23.2%, and 20% foliage damage one day, eight days, and fifteen days post-transplanting, respectively. At one month after transplanting in the April repetition, the damage was approximately 21.9%, whereas, in June, it was just 8.25% compared to the control but not significant. The variation in damage observed between April and June may be attributed to human error in visual assessment or mistakes in the collection of data. For the 2 mM concentration, the damage was 52.75% 1DAT, 32.9% 8DAT, 36.75% 15DAT, and 36.5% and 37.5% in 1MAT for the April and June rounds. In the case of the 10 mM concentration,

damage increased to 90.5% 1DAT, 74.5% 8DAT, 81.5% 15DAT, and reached 91.76% and 99% in April and June (1MAT), respectively. The 0.5 mM concentration generally did not exhibit significant phytotoxicity. It only caused significant damage (22%) at 1DAT compared to the control plants, but the damage remained under 10% for subsequent application times. These results indicate that 0.5 mM might be suitable for application in foliar trials one week after transplanting.

We observed an exponential increase in phytotoxicity with higher doses. However, when considering the time variable, we noticed that for both the 2 mM and 10 mM concentrations, less damage appeared at eight days compared to fifteen days. This was surprising, as the general trend for lower doses indicated that older plants showed less or similar phytotoxicity. It is important to note that while this pattern was observed for the two higher doses, it was not statistically significant. Again, this discrepancy may be explained by the use of a visual assessment scale of 1-5 and the non-standardization in size for all plants used in the trial at the moment of transplanting (Figure 2.7, 2.8).



Figure 2.7. Phytotoxicity damage due to foliar application of five DBDP treatments at one, eight, fifteen, and one month after transplanting on potato plants grown in plugs. (A) Phytotoxicity at one day after transplanting with doses 0.5, 0, 10, 1, and 2 mM (left to right), (B) Phytotoxicity eight days after transplanting with doses 0, 10, 1, 2, and 0.5 mM (left to right), (C) Phytotoxicity fifteen days after transplanting with doses 0, 1, 2, 0.5, and 10 mM (left to right).

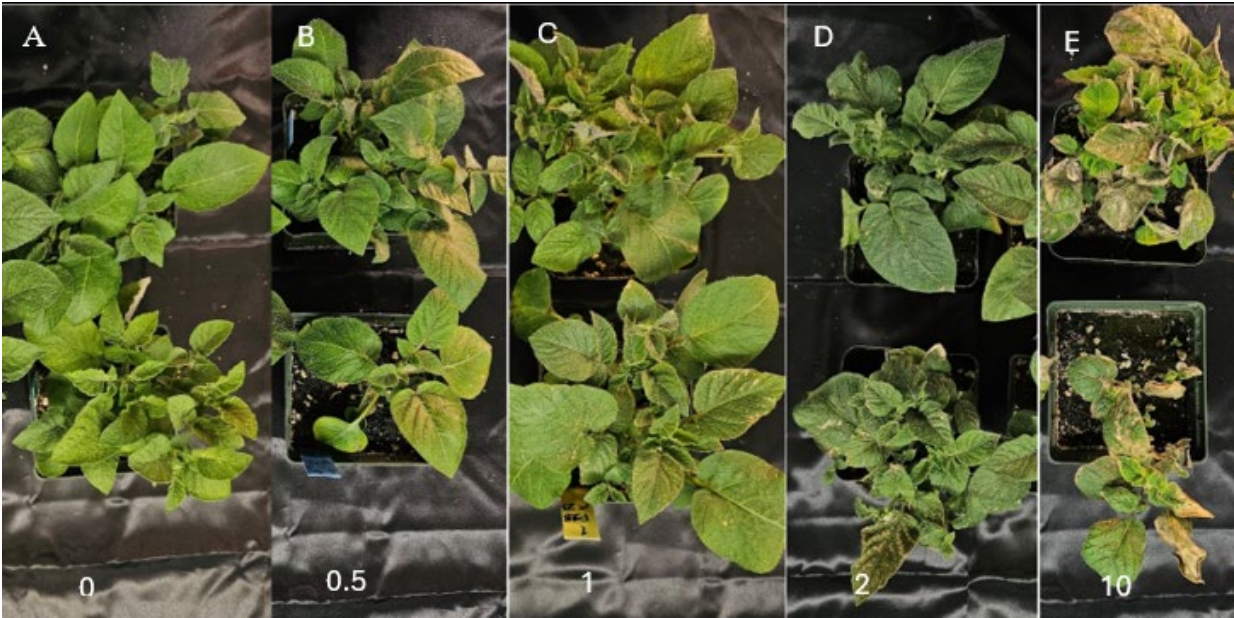


Figure 2.8. Phytotoxicity damage due to foliar applications of five DBDTP treatments one month after transplanting on potato plants, (A) 0 mM DBDTP treatment, (B) 0.5mM DBDTP treatment, (C) 1 mM DBDTP treatment, (D) 2 mM DBDTP treatment, (E) 10 mM DBDTP treatment (control)

Concerning shoot fresh weight, the data revealed that the 10 mM significantly reduced biomass by 47.56% at 1DAT compared to 0 mM, and a 24.32% for 15DAT, but for eight after transplanting a non-significant reduction of 11.83% was observed (Figure 2.5C). Additionally, after one month, similar trends were observed: biomass was reduced by 14.5% (non-significant) in the first trial (April) and 81.83% (significant) in the second trial (June) (Figure 2.5D) compared to the control. On the other hand, an interesting non-significant positive trend was noted for 0.5 mM DBSTP. In the data collected at one, eight, and fifteen days after transplanting, an increase of 1.21%, 5.02%, and 6.90% in biomass was observed respectively (Figure 2.5C). At 1MAT there was a non-significant 2% increase at 0.5 mM in April and a non-significant 6.5% increase at 1 mM in June (Figure 2.5D). These results align with field data, which also show a

positive effect on biomass at 0.5 mM. This experiment indicates that DBDTP may be used in foliar applications to boost plant growth, but more research regarding optimal dosage is needed.

Under heat stress conditions, as shown in Figure 2.6A, the concentration of 0.1 mM increased the survival percentage of potato plantlets from the first week onward. Specifically, 92.5% of the plantlets treated with 0.1 mM DBDTP survived in the first week, compared to only 76.7% survival in the control group (0 mM treatment). This showed an enhancement of more than 1.15X increase (non-significant) in survival rates for the 0.1 mM treatment compared to the control. Additionally, treatment 0.025 mM also displayed a positive non-significant effect, with a survival rate of 90% during the first week. During the second and third weeks of the experiment, a significant effect was observed at concentrations of 0.025 mM and 0.1 mM. The 0.025 mM treatment resulted in survival rates of 67.5% in week two and 60.5% in week three, which represents a 1.33X increase compared to the control group (34.2%) in week two and a 1.35X increase in week three (control 25.8%). For the 0.1 mM treatment, survival rates were 68.3% in week two and 63.3% in week three, translating to 1.34X and 1.38X, improvements respectively, when compared to the control group during those weeks. Interestingly, the highest treatment concentration of 0.4 mM did not demonstrate any significant effect compared to the control. Nevertheless, there was a noticeable negative impact on survival rates at this high concentration during all weeks of the study. This indicates one more time that high concentrations can lead to detrimental outcomes.

Previous studies have demonstrated that H₂S had a positive impact under heat stress conditions. Li et al., (2012) showed that H₂S donor NaHS significantly increased the survival rate of tobacco suspension-cultured cells at 43 °C and enhanced their regrowth ability after such

stress. Moreover, foliar applications of NaHS under heat stress (38 °C for 24 hours) resulted in significantly higher dry weights compared to controls in wheat seedlings (Yang et al., 2016). Additionally, aqueous solutions of NaHS and GYY4137 at various concentrations notably improved the survival percentage of maize seedlings subjected to heat stress at 48 °C (Li et al., 2013). Treatments with these donors also reduced electrolyte leakage, indicating better membrane integrity under stress (Li et al., 2013). Exogenous application of H₂S in wheat improved net photosynthetic rates and enhanced chlorophyll content, thereby stabilizing the photosynthetic apparatus under heat stress (Iqbal et al., 2021). Moreover, H₂S also contributed to lowering reactive oxygen species (ROS) levels, as evidenced by increased activities of antioxidative enzymes such as catalase (CAT), superoxide dismutase (SOD), and ascorbate peroxidase (APX). These enzymes help mitigate oxidative damage during heat stress and improve growth parameters, including increased leaf area and dry mass (Iqbal et al., 2021; Iqbal, Umar, et al., 2021). Experiments addressed at examining the expression of these genes and others in response to DBDTP treatment combined with varying environmental stress factors are needed to further understand and evaluate the usefulness of DBDTP as a potential growth stimulant to aid growers.

Plant height under heat stress conditions was significantly improved by the 0.1 mM treatment (Figure 2.6B), which resulted in a 20.5% increase compared to the control. In contrast, the 0.025 mM treatment only increased height by 5%, and the 0.4 mM treatment led to a 9.7% reduction in height, with both changes being statistically non-significant. In fact, Iqbal, Umar, et al. (2021) reported the enhancement of plant growth during heat stress conditions in wheat plants, the leaves presented significantly higher values of leaf area and dry mass when plants

were treated with 200 μM NaHS in foliar application at 15 days after sowing. Furthermore, Yang et al. (2016) reported that biomass (dry weight) was increased in 20-day pretreated wheat seedlings with NaHS at 0.15 mmol L^{-1} . Our findings under heat stress agree with reports from other authors and strengthen evidence indicating that H_2S treatment is beneficial to crops grown under heat stress.

Conclusion

The application of hydrogen sulfide donor DBDTP under different experimental conditions showed fascinating results, indicating its potential for further investigation. In tissue culture trials, DBDTP significantly improved root length across all tested concentrations (0.025, 0.05, 0.1, and 0.2 mM). While height and fresh weight did not show significant improvements, the 0.05 mM concentration demonstrated a slight enhancement in these traits. In field trials, the application of DBDTP enhanced plant vigor, height, biomass, tubers per plant, and yield under 0.5 mM concentration with an increase of 5% in key traits such as tuber weight per plant compared to the control. However, it should be mentioned that high concentrations, such as 10 mM , had a detrimental impact on plant performance, significantly affecting the evaluated traits.

The phytotoxicity trial results suggest that 0.5 mM is the most appropriate concentration for foliar applications out of tested concentrations, as it did not cause visible damage when applied from eight days after transplanting. However, additional research is needed to refine phytotoxicity assessments by incorporating more physiological parameters such as dry weight, height, and yield, alongside visual ratings. Future studies should also ensure uniformity in plant age, size, vigor, increased exposure periods, and mixed compatibility with products used in fertigation for more accurate evaluations.

Under heat stress conditions, continuous application of 0.025 and 0.1 mM DBDTP, in combination with 200 ppm fertilization significantly improved plant survival rates. Additionally, the 0.1 mM concentration enhanced plant height, suggesting its potential role in mitigating the effects of heat stress.

These findings reinforce DBDTP's potential to enhance plant growth and resilience to weather stress conditions. Further research is needed to optimize its application in agricultural systems such as fertigation in potato production to provide full benefits as a fertilizer additive and ensure appropriate doses and timings.

References

- Ali, S., Farooq, M. A., Hussain, S., Yasmeen, T., Abbasi, G. H., & Zhang, G. (2013). Alleviation of chromium toxicity by hydrogen sulfide in barley. *Environmental Toxicology and Chemistry*, 32(10), 2234–2239. <https://doi.org/10.1002/etc.2309>
- Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models Using lme4. *Journal of Statistical Software*, 67(1). <https://doi.org/10.18637/jss.v067.i01>
- Carter, J. M., Brown, E. M., Grace, J. P., Salem, A. K., Irish, E. E., & Bowden, N. B. (2018). Improved growth of pea, lettuce, and radish plants using the slow release of hydrogen sulfide from GYY-4137. *PLOS ONE*, 13(12), e0208732. <https://doi.org/10.1371/journal.pone.0208732>
- Carter, J. M., Brown, E. M., Irish, E. E., & Bowden, N. B. (2019). Characterization of Dialkyldithiophosphates as Slow Hydrogen Sulfide Releasing Chemicals and Their Effect on the Growth of Maize. *Journal of Agricultural and Food Chemistry*, 67(43), 11883–11892. <https://doi.org/10.1021/acs.jafc.9b04398>
- Dai, J., Wen, D., Li, H., Yang, J., Rao, X., Yang, Y., Yang, J., Yang, C., & Yu, J. (2024). Effect of hydrogen sulfide (H₂S) on the growth and development of tobacco seedlings in absence of stress. *BMC Plant Biology*, 24(1). <https://doi.org/10.1186/s12870-024-04819-w>
- Epstein, E. (1977). The Role of Roots in the Chemical Economy of Life on Earth. *BioSCIENCE*, 27(12), 783–787. <https://doi.org/10.2307/1297754>

- García Mata, C., & Lamattina, L. (2013). Gasotransmitters are emerging as new guard cell signaling molecules and regulators of leaf gas exchange. *Plant Science*, 201-202, 66–73. <https://doi.org/10.1016/j.plantsci.2012.11.007>
- Goodwin, L. R., Francom, D. M., Dieken, F. P., Taylor, J. D., Warenycia, M. W., Reiffenstein, R. J., & Dowling, G. P. (1989). Determination of Sulfide in Brain Tissue by Gas Dialysis/Ion Chromatography: Postmortem Studies and Two Case Reports. *Journal of Analytical Toxicology*, 13(2), 105–109. <https://doi.org/10.1093/jat/13.2.105>
- Gro Indoor. (2024). *IHORT Q-Plug 40/40, 1.25" x 1.25", case of 2100 – Gro Indoor*. Groindoor.com. https://groindoor.com/products/propagation-ihort-q-plug-40-40-1-25-x-1-25-case-of-2100?utm_source=google&utm_medium=cpc&campaignid=20569808052&adgroupid=&keyword=&device=c&gclid=Cj0KCQiAh8OtBhCQARIsAlkWb6_mDn5Eg49SqydpNhwjvwu0T8m5hFhQMXzmIOBjuqViKXnaUK8U7VMaAsqvEALw_wcB
- Gupta, S. K., Marwa, N., Pandey, A. K., Zhang, Y.-B., & Zhang, J.-L. (2021). Hydrogen sulfide homeostasis in plants: An overview. *Elsevier EBooks*, 341–363. <https://doi.org/10.1016/b978-0-323-85862-5.00017-8>
- Handayani, T., & Watanabe, K. N. (2021). Using in vitro technique for drought, heat, and combined drought-heat assay in potatoes. *IOP Conference Series: Earth and Environmental Science*, 762(1), 012061. <https://doi.org/10.1088/1755-1315/762/1/012061>
- Hosoki, R., Matsuki, N., & Kimura, H. (1997). The Possible Role of Hydrogen Sulfide as an Endogenous Smooth Muscle Relaxant in Synergy with Nitric Oxide. *Biochemical and Biophysical Research Communications*, 237(3), 527–531. <https://doi.org/10.1006/bbrc.1997.6878>
- Iqbal, N., Fatma, M., Gautam, H., Umar, S., Sofo, A., D'ippolito, I., & Khan, N. A. (2021). The Crosstalk of Melatonin and Hydrogen Sulfide Determines Photosynthetic Performance by Regulation of Carbohydrate Metabolism in Wheat under Heat Stress. *Plants*, 10(9), 1778. <https://doi.org/10.3390/plants10091778>
- Iqbal, N., Umar, S., Khan, N. A., & Corpas, F. J. (2021). Nitric Oxide and Hydrogen Sulfide Coordinately Reduce Glucose Sensitivity and Decrease Oxidative Stress via Ascorbate-Glutathione Cycle in Heat-Stressed Wheat (*Triticum aestivum* L.) Plants. *Antioxidants*, 10(1), 108–108. <https://doi.org/10.3390/antiox10010108>
- Jia, H., Hu, Y., Fan, T., & Li, J. (2015). Hydrogen sulfide modulates actin-dependent auxin transport via regulating ABPs results in changing of root development in Arabidopsis. *Scientific Reports*, 5(1). <https://doi.org/10.1038/srep08251>
- Kolachevskaya, O. O., Alekseeva, V. V., Sergeeva, L. I., Rukavtsova, E. B., Getman, I. A., Vreugdenhil, D., Buryanov, Y. I., & Romanov, G. A. (2014). Expression of auxin synthesis gene *tms1* under control of tuber-specific promoter enhances potato tuberization

- in vitro. Journal of Integrative Plant Biology*, 57(9), 734–744.
<https://doi.org/10.1111/jipb.12314>
- Kolupaev, Y. E., Karpets, Y. V., Shkliarevskiy, M. A., Yastreb, T. O., Plohovska, S. H., Yemets, A. I., & Blume, Y. B. (2022). Gasotransmitters in Plants: Mechanisms of Participation in Adaptive Responses. *the Open Agriculture Journal*, 16(1).
<https://doi.org/10.2174/18743315-v16-e2207050>
- Li, Z. G. (2013). Hydrogen sulfide: A multifunctional gaseous molecule in plants. *Russian Journal of Plant Physiology*, 60(6), 733–740.
<https://doi.org/10.1134/s1021443713060058>
- Li, Z.-G., Gong, M., & Liu, P. (2012). Hydrogen sulfide is a mediator in H₂O₂-induced seed germination in *Jatropha Curcas*. *Acta Physiologiae Plantarum*, 34(6), 2207–2213.
<https://doi.org/10.1007/s11738-012-1021-z>
- Li, Z.-G., Gong, M., Xie, H., Yang, L., & Li, J. (2012). Hydrogen sulfide donor sodium hydrosulfide-induced heat tolerance in tobacco (*Nicotiana tabacum* L) suspension cultured cells and involvement of Ca²⁺ and calmodulin. *Plant Science*, 185–186, 185–189. <https://doi.org/10.1016/j.plantsci.2011.10.006>
- Liu, R., & Lal, R. (2015). Effects of Low-Level Aqueous Hydrogen Sulfide and Other Sulfur Species on Lettuce (*Lactuca sativa*) Seed Germination. *Communications in Soil Science and Plant Analysis*, 46(5), 576–587. <https://doi.org/10.1080/00103624.2014.998341>
- Li, Z.-G., Min, X., & Zhou, Z.-H. (2016). Hydrogen Sulfide: A Signal Molecule in Plant Cross-Adaptation. *Frontiers in Plant Science*, 7. <https://doi.org/10.3389/fpls.2016.01621>
- Li, Z.-G., Yang, S.-Z., Long, W.-B., Yang, G.-X., & Shen, Z.-Z. (2013). Hydrogen sulphide may be a novel downstream signal molecule in nitric oxide-induced heat tolerance of maize (*Zea mays* L.) seedlings. *Plant, Cell & Environment*, 36(8), 1564–1572.
<https://doi.org/10.1111/pce.12092>
- Lin, Y.-T., Li, M.-Y., Cui, W.-T., Lu, W., & Shen, W.-B. (2012). Haem Oxygenase-1 is Involved in Hydrogen Sulfide-induced Cucumber Adventitious Root Formation. *Journal of Plant Growth Regulation*, 31(4), 519–528. <https://doi.org/10.1007/s00344-012-9262-z>
- Lisjak, M., Teklic, T., Wilson, I. D., Wood, M. E., Whiteman, M., & Hancock, J. T. (1988). Abstracts of Eleventh Annual Meeting, Japanese Society of Biorheology. *Biorheology*, 25(6), 893–915. <https://doi.org/10.3233/bir-1988-25610>
- Mukherjee, S., & Corpas, F. J. (2020). Crosstalk among hydrogen sulfide (H₂S), nitric oxide (NO) and carbon monoxide (CO) in root-system development and its rhizosphere interactions: A gaseous interactome. *Plant Physiology and Biochemistry*, 155, 800–814.
<https://doi.org/10.1016/j.plaphy.2020.08.020>

- Nelson, G. C., Valin, H., Sands, R. D., Havlík, P., Ahammad, H., Deryng, D., Elliott, J., Fujimori, S., Hasegawa, T., Heyhoe, E., Kyle, P., Von Lampe, M., Lotze-Campen, H., Mason d'Croz, D., van Meijl, H., van der Mensbrugghe, D., Müller, C., Popp, A., Robertson, R., & Robinson, S. (2013). Climate change effects on agriculture: Economic responses to biophysical shocks. *Proceedings of the National Academy of Sciences*, 111(9), 3274–3279. <https://doi.org/10.1073/pnas.1222465110>
- Ortiz, O., & Mares, V. (2017). The Historical, Social, and Economic Importance of the Potato Crop. *Compendium of Plant Genomes*, 1–10. https://doi.org/10.1007/978-3-319-66135-3_1
- Rensink, W. A., Iobst, S., Hart, A., Stegalkina, S., Liu, J., & Buell, C. R. (2005). Gene expression profiling of potato responses to cold, heat, and salt stress. *Functional & Integrative Genomics*, 5(4), 201–207. <https://doi.org/10.1007/s10142-005-0141-6>
- Sharma, S., Jaiswal, A. K., & Jaiswal, S. (2020). Potato. *Elsevier EBooks*, 339–347. <https://doi.org/10.1016/b978-0-12-812780-3.00021-0>
- Tang, R., Gupta, S. K., Niu, S., Li, X.-Q., Yang, Q., Chen, G., Zhu, W., & Haroon, M. (2020). Transcriptome analysis of heat stress response genes in potato leaves. *Molecular Biology Reports*, 47(6), 4311–4321. <https://doi.org/10.1007/s11033-020-05485-5>
- Thompson, C. R., & Kats, G. (1978). Effects of continuous hydrogen sulfide fumigation on crop and forest plants. *Environmental Science & Technology*, 12(5), 550–553. <https://doi.org/10.1021/es60141a001>
- US Department of Agriculture. (2024). *Plant Vigor Criteria*. Usda.gov. https://efotg.sc.egov.usda.gov/references/public/AZ/rtn_18.doc
- Wang, R. (2018). Overview of Gasotransmitters and the Related Signaling Network. In *Gasotransmitters* (pp. 1–28). The Royal Society of Chemistry. <https://doi.org/10.1039/9781788013000-00001>
- Wu, X., Du, A., Zhang, S., Wang, W., Liang, J., Peng, F., & Xiao, Y. (2021). Regulation of growth in peach roots by exogenous hydrogen sulfide based on RNA-Seq. *Plant Physiology and Biochemistry*, 159, 179–192. <https://doi.org/10.1016/j.plaphy.2020.12.018>
- Yang, M., Qin, B., Ma, X., Wang, P., Li, M., Chen, L., Chen, L., Sun, A., Wang, Z., & Yin, Y. (2016). Foliar application of sodium hydrosulfide (NaHS), a hydrogen sulfide (H₂S) donor, can protect seedlings against heat stress in wheat (*Triticum aestivum* L.). *Journal of Integrative Agriculture*, 15(12), 2745–2758. [https://doi.org/10.1016/s2095-3119\(16\)61358-8](https://doi.org/10.1016/s2095-3119(16)61358-8)

- Yang, Z., Wang, X., Feng, J., & Zhu, S. (2022). Biological Functions of Hydrogen Sulfide in Plants. *International Journal of Molecular Sciences*, 23(23), 15107. <https://doi.org/10.3390/ijms232315107>
- Zhang, G., Tang, R., Niu, S., Si, H., Yang, Q., Bizimungu, B., Regan, S., & Li, X.-Q. (2019). Effects of Earliness on Heat Stress Tolerance in Fifty Potato Cultivars. *American Journal of Potato Research*, 97(1), 23–32. <https://doi.org/10.1007/s12230-019-09740-9>
- Zhang, P., Luo, Q., Wang, R., & Xu, J. (2017). Hydrogen sulfide toxicity inhibits primary root growth through the ROS-NO pathway. *Scientific Reports*, 7(1). <https://doi.org/10.1038/s41598-017-01046-2>

CHAPTER THREE

THE EFFECTS OF TOXIC GAS HYDROGEN SULFIDE
RELEASED BY DIBUTYLDITHIOPHOSPHATES ON
RADISHES AND GREEN BEANS

Contribution of Authors and Co-Authors

Manuscript in Chapter 3

Author: Luis Esquivel-Cervantes

Contributions: conceptualization, methodology, validation, formal analysis, investigation, data curation, writing – original draft, visualization.

Co-Author: Nina Zidack

Contributions: conceptualization, supervision, writing – review and editing, funding acquisition.

Co-Author: Alanna Oiestad

Contributions: conceptualization, investigation, writing – review, and editing.

Co-Author: Jack Martin

Contributions: formal analysis, supervision, writing – review, and editing.

Co-Author: Ned Bowden

Contributions: writing – review and editing, funding acquisition.

Co-Author: Michael Giroux

Contributions: conceptualization, methodology, investigation, resources, supervision, project administration, writing – review and editing, funding acquisition.

Manuscript Information

Esquivel-Cervantes, L., Zidack N., Oiestad A., Bowden N., Martin J., & Giroux M. (2025). The Potential Effects of Toxic Gas Hydrogen Sulfide Released by Dithiophosphates on Radishes Under Nutrient Deficiency

Status of Manuscript:

- ☒ Prepared for submission to a peer-reviewed journal
- ☐ Officially submitted to a peer-reviewed journal
- ☐ Accepted by a peer-reviewed journal
- ☐ Published in a peer-reviewed journal

Abstract

Hydrogen Sulfide (H_2S) is a gasotransmitter that has been shown to promote growth and protect plants from environmental stressors. It is known for its unpleasant rotten egg odor and its toxic effects on multiple organisms at high concentrations. In this study, H_2S was slowly released via the breakdown of dibutyl dithiophosphate (DBDTP). The purpose of the present study was to investigate the potential roles of hydrogen sulfide on the growth of radish and bush beans. Experiments examined the application of DBDTP as a seed treatment, drench, and foliar spray to improve plant growth. The findings of the study indicated that in greenhouse conditions, the exposure of radishes to one application as seed treatment of 12.5 mM DBDTP plus three applications of 0.5 mM H_2S in drench led to a non-significant minimum increase of 4.4% in overall fresh weight and root fresh weight under 0 ppm and 100 ppm NPK fertilization. Under field conditions, slow release of hydrogen sulfide in a seed treatment could be useful to improve the growth of radishes. In this study, overall fresh weight was increased by 5.2% and 7.2% while root fresh weight was increased by 7.3% and 12.35% when DBDTP was applied at 12.5 mM and 25 mM as seed treatment concentrations respectively. A further experiment examining the phytotoxicity of DBDTP foliar sprays revealed that concentrations lower than 0.5 mM need to be tested to avoid foliage damage. Finally, the H_2S effect on green beans as a seed treatment in a field trial showed a non-significant positive effect on overall fresh weight and pod fresh weight when DBDTP was applied at 12.5 mM, 25 mM, and 50 mM concentrations. These results suggest that DBDTP may be used as an H_2S donor to improve plant growth in agricultural settings and also highlight the importance of continuing future testing under diverse conditions.

Introduction

In 2002 Wang classified hydrogen sulfide (H_2S) as a gasotransmitter in plants, along with nitric oxide (NO) and carbon monoxide (CO). Since then its role in plant science started to be further investigated. During the last few years, extensive research has shown the effects of exogenous applications of H_2S on plant physiology including resistance to abiotic and biotic stress conditions, delayed senescence, and improved seed germination (Li & Zhu, 2014; Bloem et al., 2004; Hancock & Whiteman, 2014).

Hydrogen sulfide can be delivered to plants using H_2S donors. Dithiophosphates (DTPs) are one of these donors. They were recently formulated for their use in agriculture to slowly release hydrogen sulfide in the presence of water, aid plant survival under environmental stressors, and increase plant growth (Carter et al., 2019). Opposed to other H_2S donors, dithiophosphates have varying hydrolysis rates, degrade to release safe compounds such as fatty alcohol and monopotassium phosphate, and are inexpensive to produce (Carter et al., 2019). This study focuses solely on the DTP dibutyl dithiophosphate (DBDTP).

The identification of low-cost fertilizer additives that can increase plant growth or aid vegetables under environmental stressors is crucial to addressing decisive challenges in agriculture. Therefore, testing such compounds in different application methods, environments, and crops becomes essential to ensure their proper functioning in agricultural settings.

Radishes (*Raphanus sativus*) are a widely grown vegetable worldwide and are cultivated for their roots, leaves, pods, and oil seeds, with distinct varieties in Eastern and Western cultures (Ahuja et al. 1987; Nishio, 2017). Radishes are consumed as fresh salad vegetables, garnishes, and boiled dishes, important for their low-calorie content and for being a good source of

antioxidants, electrolytes, minerals, dietary fiber, and vitamin C (Nishio, 2017; Quais et al., 2013).

Bush beans (*Phaseolus vulgaris* L.) are a very important legume worldwide. They are consumed as dry seeds or unripe pods, and occasionally the leaves are eaten as a vegetable. Additionally, the straw is used to feed livestock. The main forms of consumption of *P. vulgaris* are dry grain or green pods highlighting their significance in human nutrition due to their rich content in protein, calories, vitamins, minerals, and fiber (Broughton et al., 2003).

Both bush beans and radishes are fast-growing crops. Bush beans can be harvested around eight weeks after planting and radishes one month after planting, making them a practical option for testing compounds such as DBDTP in a short time frame. Besides that, it is important to diversify the testing of DBDTP in as many crops as possible to ensure that this compound can be broadly used across crop species.

To elucidate the potential effects of DBDTP on radishes and bush beans, DBDTP was administered as a seed treatment and plants were grown under normal field conditions. Additional greenhouse studies were carried out in radish. In the greenhouse, radishes were grown in two fertilizer treatments: 0 ppm NPK and 100 ppm NPK. This setup was planned to assess the impact of hydrogen sulfide on nutrient uptake, ensuring that any effects of H₂S would not be masked by normal or high fertility levels. The treatment with low and no fertilizer would provide a clearer view of this compound's effects on the response variables. In addition, five DBDTP treatments were tested as foliar applications in radishes to examine phytotoxicity.

Materials and Methods

The effect of DBDTP was evaluated on Rover radishes under greenhouse and field conditions in 2023 and 2024. Additionally, it was assessed on Provider bush beans in field conditions in the same years.

DBDTP Treatment Preparation

DBDTP was applied first as a seed treatment and subsequently as a weekly drench application for radishes. To prepare the seed treatment, 7% of the weight of seeds treated within each treatment group was calculated. This percentage was used as the volume (dd water + surfactant) needed to dissolve the DBDTP. A surfactant called SPRET was used to improve DBDTP adhesion, using 1.25 ml of SPRET in 1 liter of water.

The doses used in this experiment for seed treatment were 0, 12.5, 50, 100, and 200 millimolar (mM) DBDTP. Weekly drench application doses included 0, 0.5, 1, 2, and 10 mM DBDTP. This choice was based on our previous experiments in the field with potatoes where some of these concentrations showed good results, indicating the importance of evaluating DBDTP effects on other crops. A consistent water volume of 150 ml was used for drench applications, as the soil was saturated at this volume.

Greenhouse Experimental Design

The DBDTP treatment experiment was divided into two groups: one group of plants was treated using DBDTP as seed treatment and drenched weekly with a final application occurring one week before harvesting which resulted in a total of four drench applications. The second group only received the seed treatment. The seeds treated with 0 mM in the seed treatment corresponded to 0 mM in the drench application, 12.5 mM to 0.5 mM, 50 mM to 1 mM, 100 mM

to 2 mM, and 200 mM to 10 mM. In the case of the 0 mM in the drench application, only water was used.

Radishes were sown in 15 cm pots, with 10 seeds per pot using Profile® Greens Grade™ an inorganic soil amendment that resists compaction, eliminates localized dry spots, promotes drainage, and facilitates healthy root systems (Reinders, 2022). Seven days after planting, germination was recorded and pots were thinned to 5 seedlings.

Plants were grown under two different fertilizer conditions: a low fertilizer rate (100 ppm) using 20-20-20 NPK fertilizer, and no fertilizer (0 ppm). Pots were watered as needed. According to Sparks (2023), when radishes are cultivated using soilless media in a flood-and-drain hydroponic system, the best fertilizer concentration ranges from 200 to 300 ppm N. Radishes grown under 100 ppm N tend to be very small, which is why we used 100 ppm NPK as a low fertilizer treatment.

The trial was repeated in May 2024 and once again in September 2024. Overall fresh weight and root fresh weight were collected at harvest one month after planting, and a completely randomized design (CRD) with 3 replicates per treatment combination was used for this study.

Greenhouse Phytotoxicity Trial

In the greenhouse drench trial, the concentrations used in the drench application caused damage to the leaves upon contact. To investigate this further, a new experiment was carried out using foliar application concentrations 0, 0.5, 1, 2, and 10 mM DBDTP, as was used previously. Five replications per treatment combination in a Completely Randomized Design (CRD) with ten radish seeds were sown per 15 cm pot filled with a 3:1 peat-moss perlite substrate. After

germination pots were thinned to 5 plants. Radishes were subjected to two different application times: 8 days after germination (8DAG) and 15 days after germination (15DAG). The level of phytotoxicity was assessed using a visual scale 1 to 5, where 1 indicated no burns on the leaves; 2 represented small individual burns on some leaves; 3 indicated at least one leaf with spots that merged into a bigger spot but covering no more than $\frac{1}{4}$ of the leaf; 4 implied larger spots in at least one leaf covering more than $\frac{1}{3}$ of the leaves but without dead leaves; and 5 represented at least one true leaf dead and more leaves with scores 3 and 4 or dead plant (Figure 3.1). The trial was repeated twice. Radishes were harvested 8 days after the corresponding application time and overall fresh weight was measured.



Figure 3.1. Phytotoxicity scale, where 1 indicates no burns on the leaves; 2 represents small individual burns on some leaves; 3 indicates at least one leaf with spots that merged into a bigger spot but covering no more than $\frac{1}{4}$ of the leaf; 4 represents bigger spots in at least one leaf covering more than $\frac{1}{3}$ of the leaves but without dead leaves; and 5 indicates at least one true leaf dead and more leaves with scores 3 and 4 or dead plant

Radish and Bush Bean Field Experimental Design

In the field in 2023 and 2024, five DBDTP concentrations (0, 12.5, 25, 50, and 100 mM) were tested as a seed treatment on radishes and bush beans. For Provider bush beans, 3% of the weight of the seeds was used for the seed treatment volume, and for radishes 7%. The change in the percentage used was due to the size of the seeds. The trials were conducted in a completely

randomized design (CRD) with 15 replicates in 0.91m long rows in 2023 and with 20 replicates in 2024. For radishes, seeds were sown 2.5cm apart, with 30cm between rows, and 30 seeds per replicate. For bush beans, 34 seeds were planted in a 2-row plot with each seed spaced 5cm apart. In 2024, 30 seeds were used for both cases, but the distance between rows increased to 76.2cm due to more available space. Germination percentage was taken for both crops 10 days after planting. Radishes were harvested 1 month after planting, and the overall fresh weight and the root fresh weight were recorded. Bush beans were harvested two months after planting and above-ground fresh weight, pod fresh weight, and number of pods per plant were recorded.

Statistical analysis

The data were analyzed using analysis of variance (ANOVA) in R (R Foundation for Statistical Computing, Version 4.0.5, Vienna, Austria). The values are presented as the means of two independent experiments, analyzed either individually or collectively based on the specific objectives of each trial, comparing control (0 mM DBDTP) versus the DBDTP treatments. Differences with a p-value of less than 0.05 were considered significant. Figures were created using Microsoft Excel.

In the case of greenhouse experiments, due to the variation in the trend response and the interaction between DBDTP seed treatments, DBDTP drench applications, and the two rounds of the experiment (May and September) data were analyzed separately for each round using a linear model and log-transformation to meet normality and constant variance assumptions. In the Phytotoxicity experiment, data on the ratings from five plants were averaged and the two rounds of the experiment were analyzed in a single linear mixed model for response phytotoxicity, however, in the case of the total fresh weight although plants in the 8DAG and 15DAG trials

received the same DBDTP doses, they were harvested at different ages (eight days after the corresponded application). Therefore, direct comparisons between application times were not conducted. Instead, each application time was analyzed independently in a linear mixed model combining the data from the two rounds to assess the effect of DBDTP within the context of its specific growth duration. To meet assumptions data were log-transformed and subsequently estimated marginal means were back-transformed on the original scale for easier interpretation of fresh weight. The data from the field experiment were analyzed by combining the two trials 2023 and 2024, in a linear mixed-effect model for all the response variables.

For those variables where log transformation was needed; after performing ANOVA and post hoc analysis, the back transformation was used for the estimated marginal means on the original scale for easier interpretation of the response variable.

Results and Discussion

The effects of DBDTP were evaluated in 2023 and 2024 on radishes and bush beans under different experimental conditions. Greenhouse radish trials were carried out using low rates of fertilizer and no fertilizer, while field trials were carried out under normal conditions. For bush beans DBDTP effects were tested only under normal field conditions.

Our research aimed to evaluate how H₂S influences germination percentage, overall fresh weight, and root fresh weight under low fertility. To ensure no additional fertilizer was presented in the substrate, the radishes were grown only in Profile Greens. Profile® Greens Grade™ is an inorganic soil amendment that resists compaction, eliminates localized dry spots, promotes drainage, and facilitates healthy root systems (Reinders, 2022).

Effect of DBDTP on radishes under greenhouse conditions at 0 ppm NPK fertilization

Data were log-transformed before statistical analysis for 0 ppm NPK and 100 ppm NPK, and estimated marginal means were back-transformed for presentation to facilitate biological interpretation. Germination percentage was recorded seven days after planting. The combined data analysis from two independent trials indicated that DBDTP seed treatment did not have a significant effect on germination (Figure 2), although a 3% improvement was seen at 12.5 mM compared to the control, while germination rates trended down at 200 mM DBDTP.

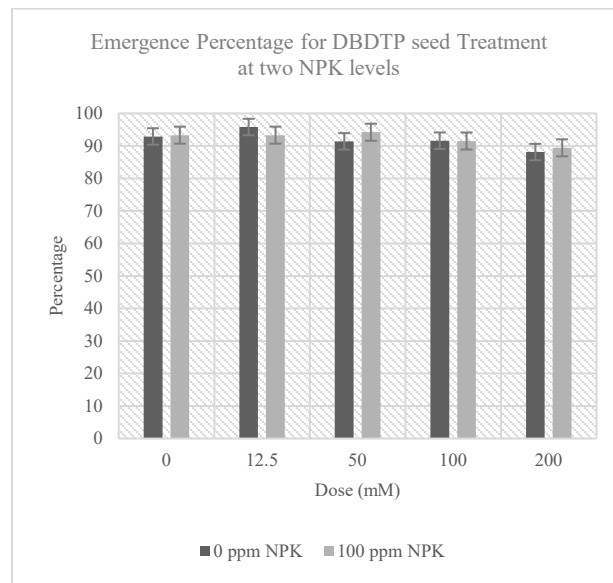


Figure 3.2. Emergence Percentage seven days after planting by five DBDTP seed treatments using 0 ppm NPK and 100 ppm NPK. Error bars represent the standard error (SE) of the estimated marginal means. Each data point in the figure represents the estimated mean ($\pm$ SE) from two trials. A single asterisk (*) indicates a significant difference from the control (0 mM DBDTP) at $P < 0.05$, double asterisks (**) indicate strong significance at $P < 0.01$, and triple asterisks (***) indicate highly significant differences at $P < 0.001$, based on post hoc analysis.

It is important to mention that Figure 3.3 and Figure 3.4 display the doses corresponding to seed treatments on the x-axis, and also the doses corresponding to the subsequent drench

applications. As mentioned in the methods section, radishes were treated at first using DBDTP as a seed treatment, and three drench treatments were applied after emergence, resulting in a total of four applications before harvesting.

Overall fresh weight was recorded four weeks after planting. Data was analyzed for each experiment separately. The results from the first trial (May 2024) revealed an interaction between DBDTP treatments and the number of times that DBDTPs were applied, showing that the DBDTP 200 mM applied once plus three applications of 10 mM significantly reduced overall fresh weight and root fresh weight per plant compared to the control (Figure 3.3A-B). This significant reduction in weight in these response variables for 200 mM/10 mM treatment indicates that repeated high concentrations of H₂S are toxic for radishes, resulting in a detrimental effect on growth (Figure 3.3A-B).

In the second trial (September 2024), we observed no significant effect for DBDTP concentrations and the number of applications in overall fresh weight, but for root fresh weight, a significant effect was observed (p-value 0.031). This effect was not due to the difference between the control (0 mM) and the DBDTP concentrations (12.5/0.5, 50/1, 100/2, 200/10 mM) (Figure 3.3C-D), but rather among DBDTP concentrations themselves, for example, 50 mM one application and 200/10 mM with four applications). These comparisons are not the focus of this research, as our objective was to compare the effect of DBDTP relative to control.

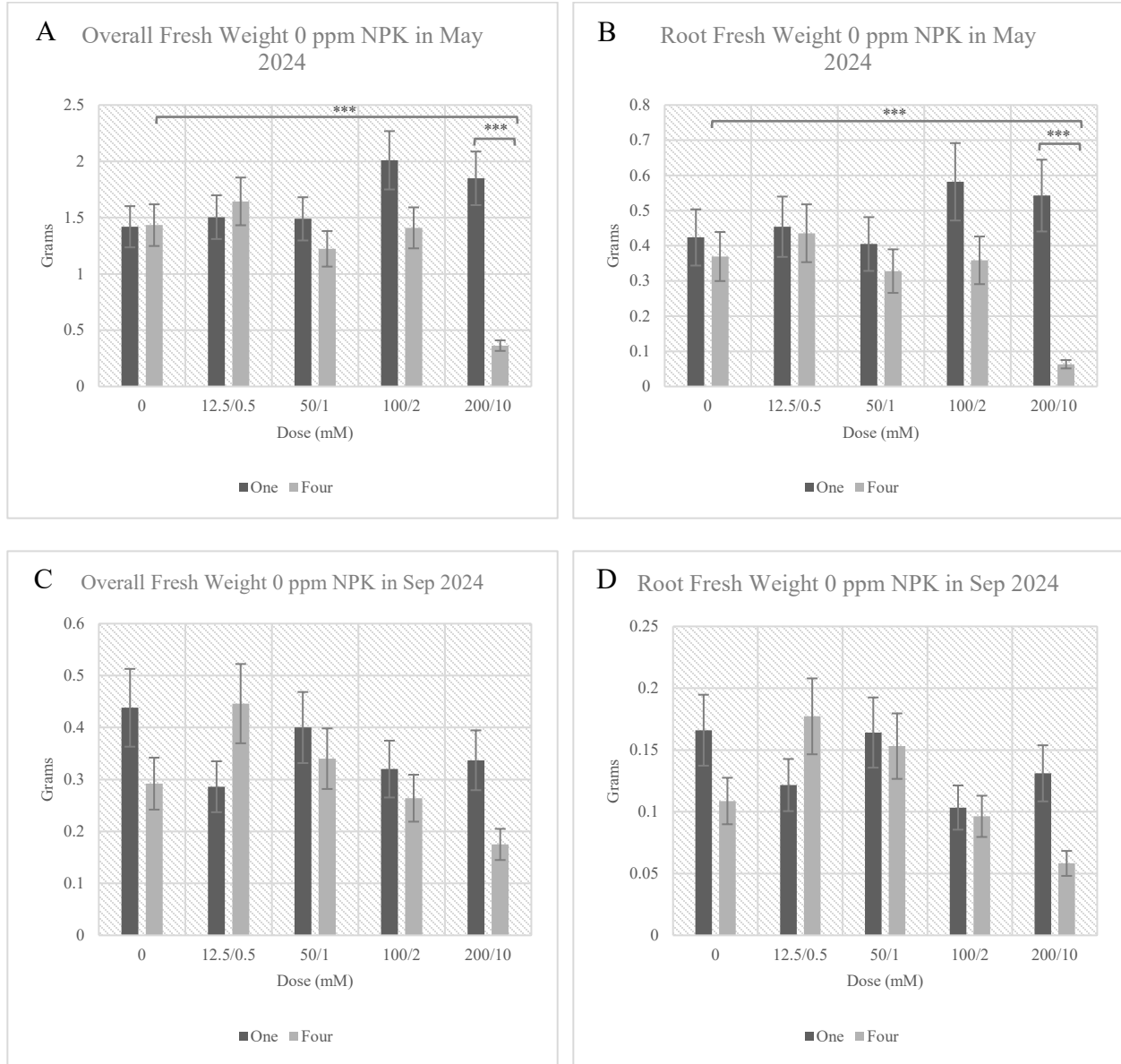


Figure 3.3. Radish Overall fresh weight and root fresh weight under greenhouse conditions for five DBDTP concentrations at 0 ppm 20-20-20 NPK fertilization for the first round of the experiment (May 2024) and the second round of the experiment (September 2024). The x-axis shows the doses used in seed treatments and the doses used in the drench applications. (A) Overall fresh weight in May 2024 by number of applications and DBDTP treatments. (B) Root fresh weight by number of applications and DBDTP treatments in May 2024. (C) Overall fresh weight by DBDTP treatments and number of applications in Sep 2024. (D) Root fresh weight in Sep 2024 by DBDTP treatments and the number of applications. Error bars represent the standard error (SE) of the estimated marginal means. Each data point in the figure represents the estimated mean ($\pm$ SE) from two trials analyzed individually. Statistical comparisons were made against the control in each group (0 mM DBDTP) and within each dose by comparing one

application vs. four applications. Comparisons were not made between different concentrations. A single asterisk (*) indicates a significant difference at $P < 0.05$, double asterisks (**) indicate strong significance at $P < 0.01$, and triple asterisks (***) indicate highly significant differences at $P < 0.001$, based on post hoc analysis

While no significant positive effects were detected in May 2024 and September 2024 with 0 ppm NPK combined with DBDTP treatments, interesting patterns emerged. In May, the 12.5/0.5 mM treatment led to an increase of 14% overall fresh weight when DDBTP was applied in total four times compared to the control, and an increase of 52% in September 2024 (Figure 3.3A and C). However, when comparing a single 12.5 mM application to four applications, the difference between the two was only about 9% in May 2024 and 55% in September 2024. Additionally, a single application of 100 mM and 200 mM increased overall fresh weight by 41% and 30 % respectively compared to the control in May 2024, but such effect was not seen in September 2024. In general, when these treatments were applied in total four times, they resulted in a decrease in fresh weight in both trials (Figure 3.3A-D). This may be due to sulfide toxicity. When sulfide is present in wetlands at high concentrations it leads to toxicity in aquatic organisms (Fort et al., 2017). Lamers et al. (2013) reviewed historical studies of sulfide toxicity, where a decrease in above-ground productivity has been observed at sulfide levels ranging from 30 to 8000 $\mu\text{mol L}^{-1}$, depending on the species. Sulfide concentrations that are too high can lead to poor nutrient uptake and utilization. This results in the potential inhibition of various oxidases, compromising metabolic capacity, inducing oxidative stress, and reducing gas exchange in the root systems (Armstrong & Armstrong, 2005; Allam, 1972). Previous studies have shown that higher and continuous exposure to H_2S can lead to negative effects such as necrotic lesions, limitation in plant growth, and affect plant functioning depending on the conditions of the environment and species. On sulfur-sufficient soils continuous exposure to H_2S -stimulated

growth of lettuce (*Lactuca sativa*), alfalfa (*Medicago sativa*), and sugar beet (*Beta vulgaris*) but negatively affected spinach cultivar, (Thompson & Kats, 1978; De Kok, 1990; De Kok et al., 2002).

In May, root fresh weight at 200 mM, and 100 mM-one-application showed similar patterns to overall fresh weight. There was a non-significant increase in root weight of 37% and 28% for 100 mM and 200 mM respectively compared to the control, but in September this effect was not present (Figure 3.3B and D). Additionally, the treatment combination 12.5/0.5 mM (total of four applications) led to a non-significant 17.8% increase in root weight in May and 63% in September compared to the control (Figure 3.3B and D).

Effect of DBDTP on radishes under greenhouse conditions at 100 ppm fertilization

Under low fertilization conditions (100 ppm 20-20-20 NPK), DBDTP treatments did not have a significant effect on germination percentage (Figure 3.2), all the treatments showed a very similar response, with positive effects below 1% compared to the control, suggesting that under low fertilization conditions, DBDTP does not improve germination. This may imply that the effects of DBDTP are more related to post-germination developmental processes. Nevertheless, in May and September, a significant interaction was detected between DBDTP treatments and the number of times DBDTP was applied for overall fresh weight and root fresh weight. Our results showed that the highest concentration (200/10 mM) reduced overall fresh weight and root fresh weight compared to 0 mM when DBDTPs were applied in total four times (Figure 3.4), demonstrating that extra application of 200/10 mM DBDTP is not beneficial. The patterns seen between each round varied slightly. In May, the treatment 12.5 mM increased overall fresh weight by 13.7% after one application and 5.7% after four applications compared to the control

(Figure 3.4A) (non-significant). In the same treatment in September, a non-significant 9.8% increase in weight was detected when DBDTP was applied in total four times, but no increase was observed with only one application compared to the control (Figure 3.4C). In May, 12.5/0.5 mM increased overall fresh weight similarly for both application frequencies (one vs four applications), however, in September when it was applied four times it resulted in a non-significant 12% weight increase compared to one time (Figure 3.4C). Furthermore, we noted that one application of 100 mM and 200 mM had a positive trend on overall weight by 21% and 15%, respectively in May, and 5.8% and 10% in September compared to controls (Figure 3.4A and C). In the case of root fresh weight, when treatments 12.5, 50, 100, and 200 mM were applied only once in May they yielded non-significant increases of 24%, 15%, 21%, and 16% compared to the control (Figure 3.4B). However, applying them four times led to a detrimental effect. In September, on the other hand, 50 mM showed a non-significant increase of 17% and 200 mM 4.4% when they were applied once. The 12.5/0.5 mM treatment increased root weight by 4.4% when it was applied in total four times (non-significant), but no improvement was observed when it was applied only once (Figure 3.4D).

Our results showed that the deficiency of fertilizer combined with repeated applications of high DBDTP concentrations negatively impacted the radish growth. Optimal levels of nitrogen (N), phosphorus (P), and potassium (K) are vital for proper plant growth, and their deficiency negatively affects plants (Khalofah et al., 2022; Kumar et al., 2021). Previous studies in cabbage have reported that, under normal nitrogen levels, low concentrations of exogenous H₂S can significantly increase biomass and crop quality. However, H₂S concentrations above 2.5 mM led to reductions in biomass and chlorophyll content (Montesinos-Pereira et al., 2016).

These findings reinforce the idea of testing DBDTP under normal fertilizer conditions, especially since our results exhibited positive trends at low concentrations under low NPK levels.

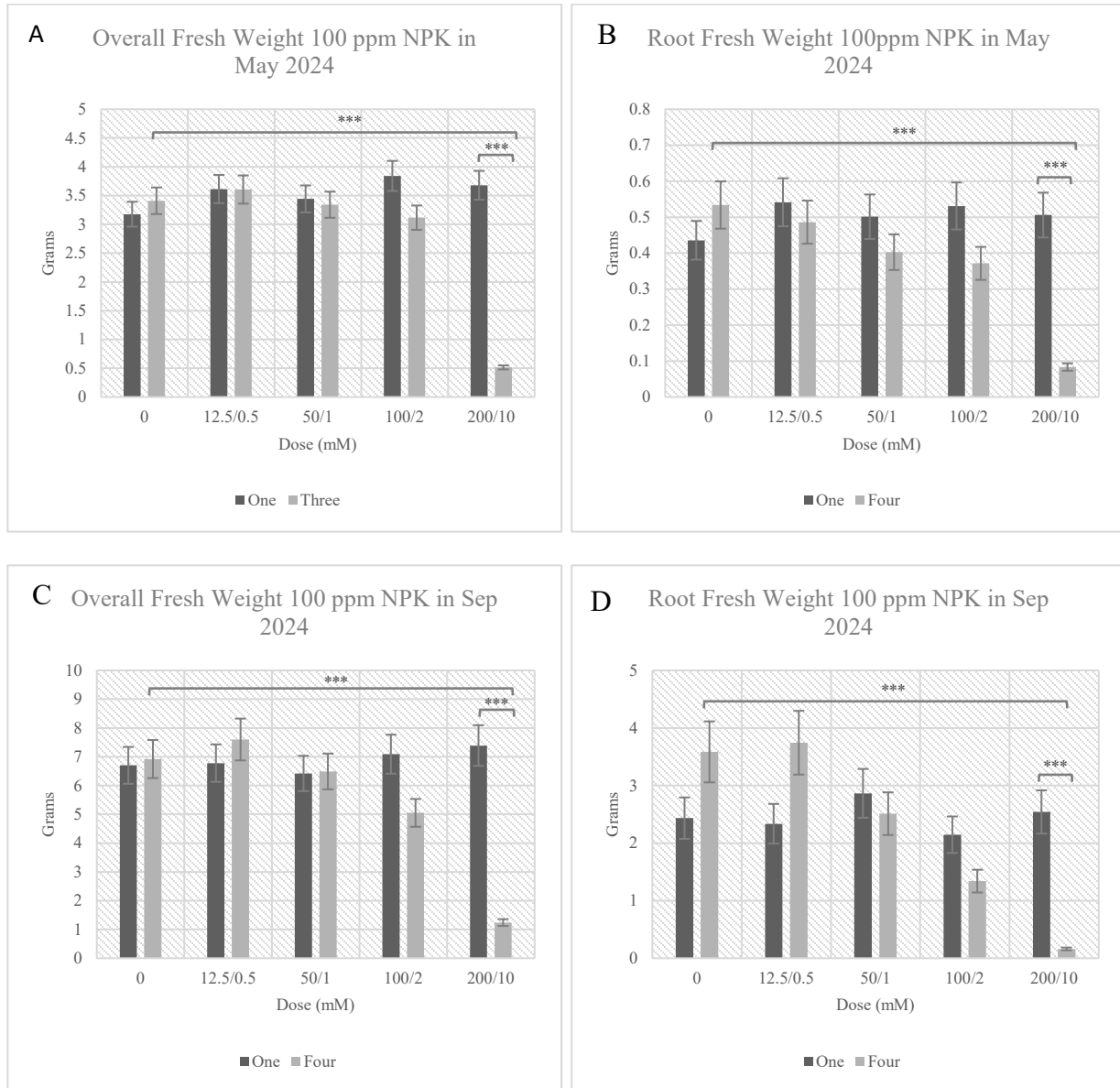


Figure 3.4. Radish Overall fresh weight and root fresh weight under greenhouse conditions for five DBDTP concentrations at 100 ppm 20-20-20 NPK fertilization for the first run of the experiment (May 2024) and the second run of the experiment (September 2024). The x-axis shows the doses used in seed treatments and the doses used in the drench applications. (A) Overall fresh weight in May 2024 by number of applications and DBDTP treatments. (B) Root fresh weight in May 2024 by the number of applications and DBDTP treatments. (C) Overall

fresh weight in Sep 2024 by number of applications and DBDTP treatments. (D) Root fresh weight in Sep 2024 by the number of applications and DBDTP treatments. Error bars represent the standard error (SE) of the estimated marginal means. Each data point in the figure represents the estimated mean ($\pm$ SE) from two trials analyzed individually. Statistical comparisons were made against the control in each group (0 mM DBDTP) and within each dose by comparing one application vs. four applications. Comparisons were not made between different concentrations. A single asterisk (*) indicates a significant difference at $P < 0.05$, double asterisks (**) indicate strong significance at $P < 0.01$, and triple asterisks (***) indicate highly significant differences at $P < 0.001$, based on post hoc analysis.

The data from both experiments (0 ppm and 100 ppm NPK) displayed different patterns for concentrations that exhibited a positive effect on overall or root fresh weight in the two application regimes. This could be explained by different water schedules between the two trials. Infrequent watering has shown a reduced yield in yams, affected flower quality, and decreased flowering in petunias (Flohr and Conover, 1994; Edi Santosa et al., 2004), therefore further research is needed to elucidate clearer the effects of DBDTP under no fertilizer and low fertilizer conditions in radishes. However, 12.5 mM under both fertilizer conditions and in the two DBDTP application regimes showed a similar behavior suggesting the potential of this dose for future experiments. It becomes complicated to obtain significance when looking at small changes in yield, however, small improvements in yield are important.

Phytotoxicity effect of DBDTP in foliar applications

Results show mean values combined over two independent trials. Foliar treatments were applied eight days after germination and fifteen days after germination using concentrations (0, 0.5, 1, 2, and 10 mM) we tested in our previous experiment. The data analysis showed no difference between the spraying times on phytotoxicity, suggesting that the effect of DBDTP is the same no matter when it is applied. On the other hand, DBDTP treatments showed a significant difference with all the treatments causing phytotoxicity compared to the control

(Figure 3.5A). Even the smallest concentration (0.5 mM) caused a 23.5% increase in phytotoxicity compared to the control. Our results showed that for radishes, none of these concentrations should be used in foliar applications. This suggests that further testing of smaller concentrations is needed. There is a large variation in H₂S susceptibility between species as well as between cultivars of the same species due to plant morphology (Ausma & De Kok, 2019), making it important to test different concentrations based on species.

In previous studies, very small concentrations of H₂S (50 µM NaHS) in foliar application improved fresh weight, shoot length, and physiology of soybean plants under cadmium (Cd) stress by the sulfur metabolism and expression of genes involved in glutathione synthesis (Oloumi et al., 2024), remarking again the need of testing DDBTP under different scenarios and low concentrations.

For overall fresh weight, data were analyzed for each application time individually by combining the data from both rounds of the experiment. This approach was necessary because plants for each application time were harvested one week following their respective application, making direct comparisons of weights between different application times impractical. Our analysis revealed that at 8DAG DBDTP concentrations were statistically significant, whereas at 15DAG, there was a significant interaction between the concentrations and the experimental repetitions. We noticed that fresh weight was significantly reduced in both application times when 10 mM was applied (Figure 3.5B and C). These results once again emphasize the necessity of testing doses below 0.5 mM in foliar applications on radishes to improve fresh weight. In nearly all treatments after the application of DBDTP, we observed a reduction in fresh weight. However, 8DAG application, we noted that 0.5 mM slightly increased weight by 4.33%, this

trend was not observed at 15DAG, suggesting that this effect is inconsistent. To avoid any reduction in fresh weight, it would be wise to test smaller concentrations.

Our data illustrates that the foliar application of very high concentrations (10 mM) of H_2S in radishes at 8DAG and 15DAG led to more than a 35% reduction in overall fresh weight compared to control. Prolonged exposures to H_2S in spinach have shown a reduction in photosynthesis reflected in decreased CO_2 fixation rates and disrupted chlorophyll fluorescence quenching provoking reduced growth (Maas et al., 1988). Photosynthesis is the cornerstone of plant growth and crop productivity, as it provides the primary source of energy for plants (Anbarasan & Ramesh, 2022; Gondim & Nolasco-Rondon, 2020), therefore, affecting the photosynthetic area in plants will affect their growth. Our results confirm that when too much damage is done to radish leaves due to H_2S exposure, a reduction in overall fresh weight results, suggesting that this effect may be due to decrease the photosynthetic area (Figure 3.6).

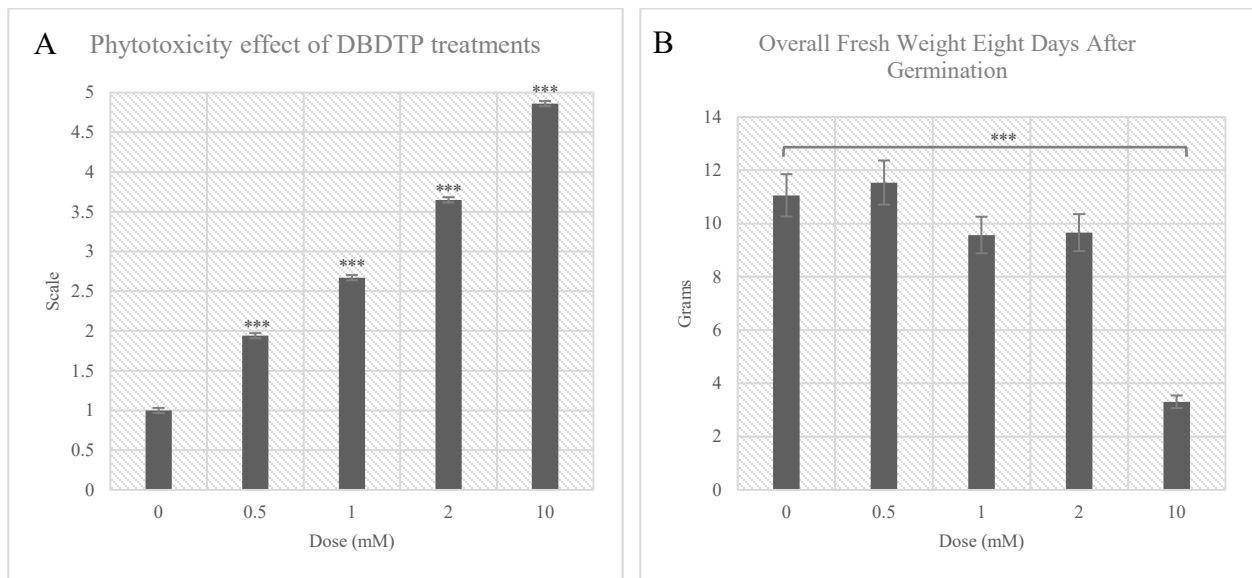


Figure 3.5 Continued

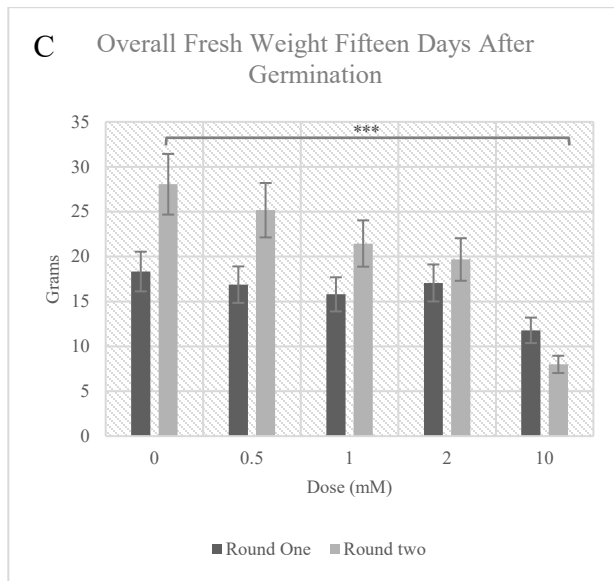


Figure 3.5. Phytotoxicity effect and fresh weight of DBDTP in foliar applications on radish. (A) Phytotoxicity effect of DBDTP foliar application on radishes. (B) Overall fresh weight by DBDTP treatments at 8DAG foliar application. (C) Overall fresh weight by DBDTP treatments at 15DAG foliar application. Error bars represent the standard error (SE) of the estimated marginal means. Each data point in the figure represents the estimated mean ($\pm$ SE) from two trials analyzed together. Statistical comparisons were made against control in each group (0 mM DBDTP). A single asterisk (*) indicates a significant difference at $P < 0.05$, double asterisks (**) indicate strong significance at $P < 0.01$, and triple asterisks (***) indicate highly significant differences at $P < 0.001$, based on post hoc analysis.



Figure 3.6. Phytotoxicity effect of DBDTP foliar applications on radish's foliage. (A) Phytotoxicity on radishes eight days after germination left to right 0 mM, 0.5 mM, 1 mM, 2 mM, and 10 mM. (B) Phytotoxicity on radishes fifteen days after germination left to right 0 mM, 0.5 mM, 1 mM, 2 mM, and 10 mM.

Effect of DBDTP on radishes under field conditions

For field trials, DBDTP was administered as a seed treatment and results correspond to the combined analysis of two independent trials carried out in 2023 and 2024. Comparisons were made between control versus DBDTP concentrations. Emergence percentage was collected ten days after planting; data analysis indicates that DBDTP had no significant effect on emergence (Figure 3.7A). Our results align with those reported by Liu and Lal (2015) where they found that H_2S did not significantly increase emergence of lettuce under laboratory conditions. However, they found that a treatment of 0.01 mM H_2S significantly increased shoot length by 40 percent and 0.1 mM by 24 percent. Regarding overall fresh weight and root fresh weight in this study, DBDTP seed treatments did not have a significant effect (Figure 3.7B). However, the use of low concentrations of DBDTP showed a positive trend for both overall fresh weight and root fresh weight with a 5.2% and 7.2% increase in overall fresh weight, and 7.3% and 12.35% root fresh

weight, for 12.5 mM and 25 mM treatments respectively. These results suggest that DBDTP can be used to improve fresh weight for doses within this range. In previous experiments, in which H_2S was used to boost early wheat growth, the overall mass of the plant, seeds, and roots, was significantly higher than the controls for all plants exposed to 1, 10, 100, and 500 μM H_2S (Dooley et al., 2013).

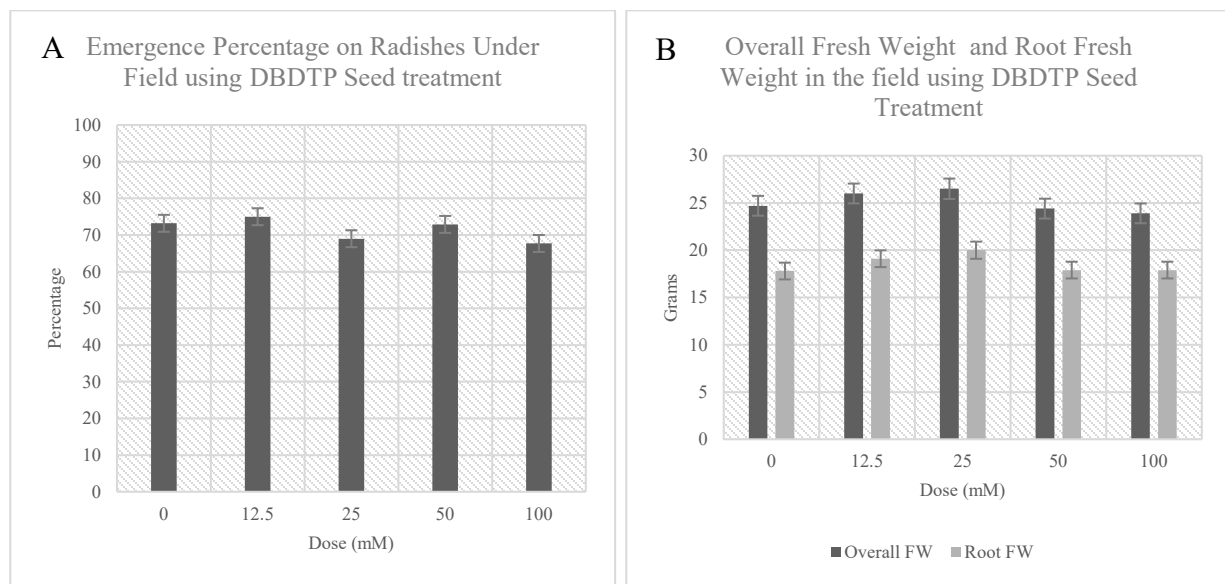


Figure 3.7. Radish emergence percentage, overall fresh weight, and root fresh weight for five DBDTP seed treatments in the field (data from 2023 and 2024). (A) Germination Percentage for radishes grown in the field using five DBDTP seed treatments. (B) Overall fresh weight and root fresh weight for radishes grown in the field using five DBDTP seed treatments. Error bars represent the standard error (SE) of the estimated marginal means. Each data point in the figure represents the estimated mean ($\pm$ SE) from two trials. Statistical comparisons were made against the control in each group (0 mM DBDTP). A single asterisk (*) indicates a significant difference at $P < 0.05$, double asterisks (**) indicate strong significance at $P < 0.01$, and triple asterisks (***) indicate highly significant differences at $P < 0.001$, based on post hoc analysis.

Effect of DBDTP on bush beans under field conditions

Under field conditions, the results presented mean values averaged over the 2023 and 2024 trials. The analysis of emergence percentage in the field showed an interaction effect

between year and DBDTP seed treatment concentration. In 2023 all DBDTP treatments showed a lower emergence percentage compared to the control, with a significant decrease observed in the 50 mM treatment group compared to 0 mM (Figure 3.8A). However, in 2024, this effect was not present (Figure 3.8A). In fact, in 2024 we observed a slight increase in emergence of 4.3% for 12.5 mM and 2.9% for 25 mM treatments compared to the control.

For overall fresh weight, pod fresh weight, and number of pods per plant no significant treatment effects were observed (Figure 3.8B and C). Nonetheless, treatments 12.5 mM, 25 mM, and 50 mM exhibited a positive impact on overall fresh weight by 5.50%, 6.42%, and 7.33% respectively. In addition, pod fresh weight trended 3.25%, 10.84%, and 4.33% higher for the same treatments. More research is needed to test DBDTP in bush beans.

The use of H₂S to mitigate stress has been tested on beans grown under salt stress, where foliar applications of 25 μ M H₂S increased shoot fresh mass by 11% (Ekinici et al., 2023). In another study, Dawood et al. (2022) reported that under normal growth conditions, H₂S increased shoot fresh weight by 8.57% at 50 μ M and 15.17% at 100 μ M. These concentrations also significantly boosted shoot fresh weight under salt stress conditions. Finally, Dooley et al. (2013) found a significant effect on fresh weight for bean seedlings treated with 5 μ M and 10 μ M H₂S. These results aligned with our findings, indicating that H₂S positively affects fresh weight. These studies also emphasize the importance of proceeding with further analysis of DBDTP under stress conditions.

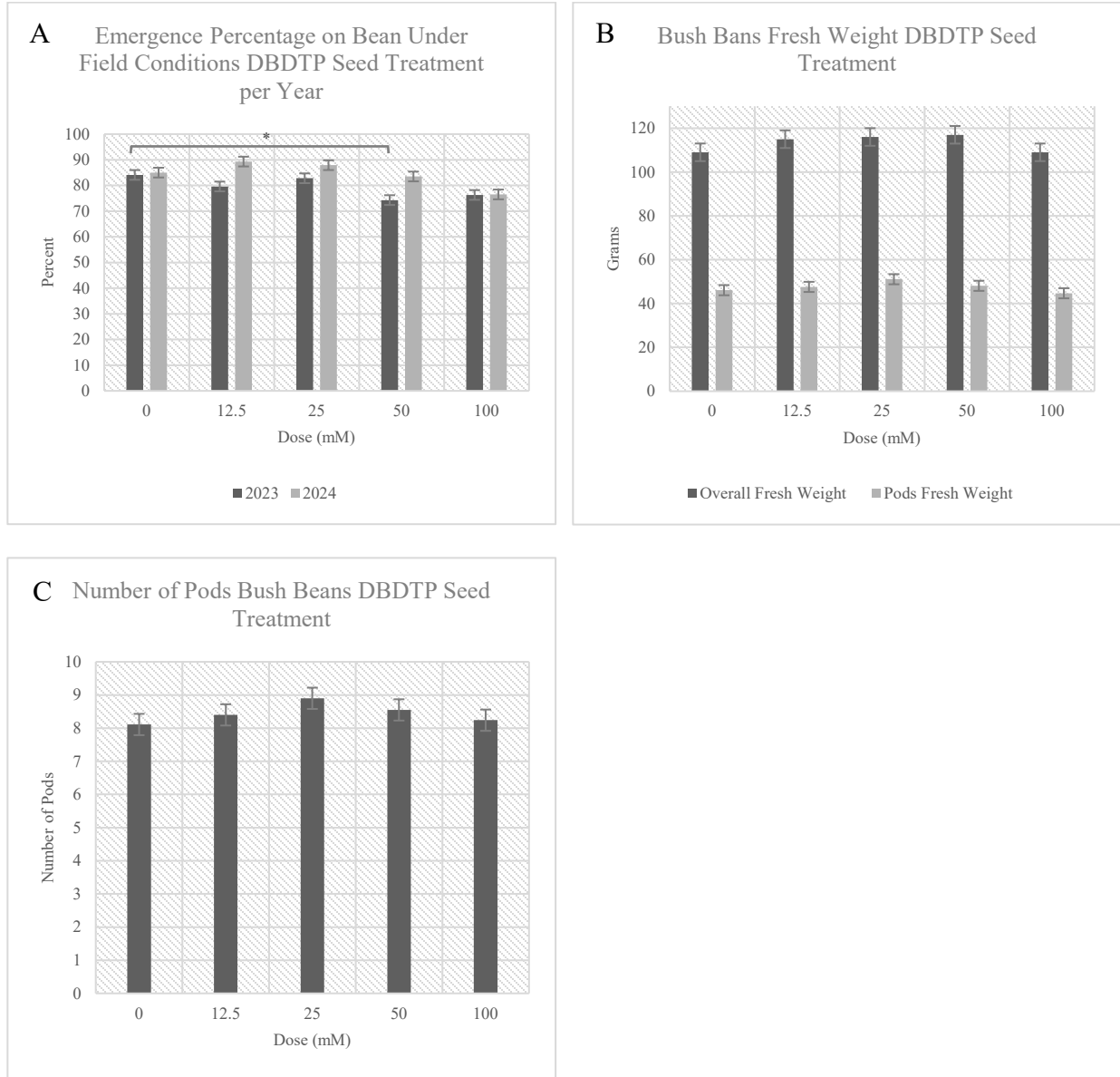


Figure 3.8. Emergence percentage, fresh weight, and number of pods in bush beans using DBDTP seed treatment in the field. (A) Germination percent of bush beans in the field in 2023 and 2024 using DBDTP seed treatments. (B) Overall fresh weight and pods fresh weight by DBDTP seed treatments in bush beans. (C) Number of pods in bush beans in the field using DBDTP seed treatments. Error bars represent the standard error (SE) of the estimated marginal means. Each data point in the figure represents the estimated mean ($\pm$ SE) from two trials. Statistical comparisons were made against the control (0 mM DBDTP). A single asterisk (*) indicates a significant difference at $P < 0.05$, double asterisks (**) indicate strong significance at $P < 0.01$, and triple asterisks (***) indicate highly significant differences at $P < 0.001$, based on post hoc analysis.

Conclusions

Further research is needed to better understand the effects of DBDTP treatments on radish growth when grown under no fertilizer and low fertilizer (20-20-20 NPK). However, the pattern observed for 12.5/0.5 mM seed treatment/drench application showing a positive trend on radish overall fresh and root weight under 0 ppm and 100 ppm NPK fertilizer when grown under replicated trials, suggests that the proper dose for radishes is within this range. Nevertheless, it is imperative to test DBDTP under normal fertilizer conditions. It would also be worth carrying out trials with a single application of DBDTP or a regime with less than four applications for high concentrations such as 1 mM, 2 mM, and 10 mM, since as it was demonstrated in this research that weekly applications of high concentrations of H₂S in radishes lead to a significant decrease in overall fresh weight and root fresh weight. Testing the effect of DBDTP under recommended fertilization doses in soilless culture in the greenhouse is essential due to the important interaction between macro-nutrients in uptake, transport, or assimilation of each other (Khalofah et al., 2022; Kumar et al., 2021). A study in chickpeas showed that the use of proper fertilization or even an increase of around 25% in combination with sulfur can improve yield, height, number of leaves, and number of branches (Mishra et al., 2023).

A dibutyl dithiophosphate (DBDTP) hydrogen sulfide donor seed treatment could be useful to improve the growth of radishes under field conditions. This study shows that increased trends of 5.2% and 7.2% in overall fresh weight and 7.3% and 12.35% root fresh weight were detected for 12.5 mM and 25 mM DBDTP seed treatment groups compared to 0 mM DBDTP.

The phytotoxicity results showed very important information. The foliar application of the treatments 0.5 mM, 1 mM, 2 mM, and 10 mM produces phytotoxicity no matter if they are applied

eight or fifteen days after germination, pointing out that lower concentrations of foliar treatments need to be tested.

The effect of DBDTP seed treatments on bush under field conditions showed that 12.5 mM, 25 mM, and 50 mM treatment levels led to positive trends in overall fresh weight, increasing 5.50%, 6.42%, and 7.33% respectively, and pod fresh weight by 3.25%, 10.84%, and 4.33% compared to the control. These results suggest the potential of DBDTP for future testing under diverse conditions such as when abiotic stress is present.

The importance of DBDTPs lies not only in their positive effect on improving plant growth but also in their ability to provide dual benefits. While much literature emphasizes the positive effects of H₂S under abiotic stresses, few mention its benefits under normal growth conditions. Therefore, having a compound such as DBDTP, that can improve plant growth, yet offer support in the presence of abiotic stress would be a valuable tool for agriculture. Although it is difficult to obtain significance for small changes in yield small improvements in yield become significantly important during large-scale production.

References

- Abe, K., & Kimura, H. (1996). The possible role of hydrogen sulfide as an endogenous neuromodulator. *The Journal of Neuroscience*, 16(3), 1066–1071. <https://doi.org/10.1523/jneurosci.16-03-01066.1996>
- Ahmed, M., Fahad, S., Ali, M. A., Hussain, S., Tariq, M., Ilyas, F., Ahmad, S., Saud, S., Hammad, H. M., Nasim, W., Wu, C., & Liu, H. (2021). Hydrogen Sulfide: A Novel Gaseous Molecule for Plant Adaptation to Stress. *Journal of Plant Growth Regulation*, 40(6), 2485–2501. <https://doi.org/10.1007/s00344-020-10284-0>
- Allam, A. I. (1972). Sulfide Inhibition of Oxidases in Rice Roots. *Phytopathology*, 62(6), 634–634. <https://doi.org/10.1094/phyto-62-634>

- Anbarasan, S., & Ramesh, S. (2022). Photosynthesis Efficiency: Advances and Challenges in Improving Crop Yield. *Plant Science Archives*, 7(3), 19–21.
<https://doi.org/10.51470/psa.2022.7.3.19>
- Armstrong, J., & Armstrong, W. (2005). Rice: Sulfide-induced Barriers to Root Radial Oxygen Loss, Fe²⁺ and Water Uptake, and Lateral Root Emergence. *Annals of Botany*, 96(4), 625–638. <https://doi.org/10.1093/aob/mci215>
- Ausma, T., & De Kok, L. J. (2019). Atmospheric H₂S: Impact on Plant Functioning. *Frontiers in Plant Science*, 10. <https://doi.org/10.3389/fpls.2019.00743>
- Bloem, E., Riemenschneider, A., Volker, J., Papenbrock, J., Schmidt, A., Salac, I., Haneklaus, S., & Schnug, E. (2004). Sulphur supply and infection with *Pyrenopeziza brassicae* influence L-cysteine desulphydrase activity in *Brassica napus* L. *Journal of Experimental Botany*, 55(406), 2305–2312. <https://doi.org/10.1093/jxb/erh236>
- Brown, E. M., Ranasinghe Arachchige, N. P. R., Paudel, A., & Bowden, N. B. (2021). Synthesis, Stability, and Kinetics of Hydrogen Sulfide Release of Dithiophosphates. *Journal of Agricultural and Food Chemistry*, 69(43), 12900–12908.
<https://doi.org/10.1021/acs.jafc.1c04655>
- Dawood, M. F. A., Sofy, M. R., Mohamed, H. I., Sofy, A. R., & Abdel-Kader, H. A. A. (2022). Hydrogen Sulfide Modulates Salinity Stress in Common Bean Plants by Maintaining Osmolytes and Regulating Nitric Oxide Levels and Antioxidant Enzyme Expression. *Journal of Soil Science and Plant Nutrition*, 22(3), 3708–3726.
<https://doi.org/10.1007/s42729-022-00921-w>
- De Kok, L. J. (1990). Sulfur metabolism in plants exposed to atmospheric sulfur. In H. Rennenberg, C. Brunold, L. J. De Kok, & I. Stulen (Eds.), *Sulfur Nutrition and Sulfur Assimilation in Higher Plants: Fundamental, Environmental and Agricultural Aspects* (pp. 111-130). Backhuys Publishers.
- De Kok, L. J., Stuiver, E. E., Westerman, S., & Stulen, I. (2002). Elevated Levels of Hydrogen Sulfide in the Plant Environment: Nutrient or Toxin. *Springer EBooks*, 201–219.
https://doi.org/10.1007/978-4-431-68388-9_10
- Dooley, F. D., Nair, S. P., & Ward, P. D. (2013). Increased Growth and Germination Success in Plants following Hydrogen Sulfide Administration. *PLoS ONE*, 8(4).
<https://doi.org/10.1371/journal.pone.0062048>
- Edi Santosa, Sugiyama, N., Eko Sulistyono, & Diddy Sopandie. (2004). Effects of Watering Frequency on the Growth of Elephant Foot Yams. *Nettai Nogyo/Nettai Nougyou*, 48(4), 235–239. <https://doi.org/10.11248/jsta1957.48.235>

- Ekinci, M., Turan, M., Ors, S., Dursun, A., & Yildirim, E. (2023). Improving salt tolerance of bean (*Phaseolus vulgaris* L.) with hydrogen sulfide. *Photosynthetica*, 61 (1). <https://doi.org/10.32615/ps.2023.005>
- Flohr, R. C., & Conover, C. A. (1994). Production and Postproduction Watering Schedule Effects on Plant Growth, Quality and Blooming of Pansies and Petunias. *Proc. Fla. State Hort Soc*, 107, 414–416. <https://journals.flvc.org/fshs/article/download/92449/88641>
- Fort, D. J., Todhunter, K., Fort, T. D., Mathis, M. B., Walker, R., Hansel, M., Hall, S., Richards, R., & Anderson, K. (2017). Toxicity of sulfide to early life stages of wild rice (*Zizania palustris*). *Environmental Toxicology and Chemistry*, 36(8), 2217–2226. <https://doi.org/10.1002/etc.3759>
- Gondim, E., & Nolasco-Rondon, J. (2020). The Crucial Role of Photosynthesis In Sustaining Plant Life and Its Impact on the Global Ecosystem, Climate, And Human Survival. *Australian Herbal Insight*, 3(1), 1–5. <https://doi.org/10.25163/ahi.319925>
- Hancock, J. T., & Whiteman, M. (2014). Hydrogen sulfide and cell signaling: Team player or referee? *Plant Physiology and Biochemistry*, 78, 37–42. <https://doi.org/10.1016/j.plaphy.2014.02.012>
- Khalofah, A., Ghramh, H. A., Al-Qthanin, R. N., & L'taief, B. (2022). The impact of NPK fertilizer on growth and nutrient accumulation in juniper (*Juniperus procera*) trees grown on fire-damaged and intact soils. *PLOS ONE*, 17(1), e0262685. <https://doi.org/10.1371/journal.pone.0262685>
- Kumar, S., Kumar, S., & Mohapatra, T. (2021). Interaction Between Macro- and Micro-Nutrients in Plants. *Frontiers in Plant Science*, 12. <https://doi.org/10.3389/fpls.2021.665583>
- Lamers, L. P. M., Govers, L. L., Janssen, I. C. J. M., Geurts, J. J. M., Van der Welle, M. E. W., Van Katwijk, M. M., Van der Heide, T., Roelofs, J. G. M., & Smolders, A. J. P. (2013). Sulfide as a soil phytotoxin—a review. *Frontiers in Plant Science*, 4. <https://doi.org/10.3389/fpls.2013.00268>
- Li, Z. B., & Zhu, L. (2014). Hydrogen sulfide donor sodium hydrosulfide-induced accumulation of betaine is involved in the acquisition of heat tolerance in maize seedlings. 38(1), 31–38. <https://doi.org/10.1007/s40415-014-0106-x>
- Liu, R., & Lal, R. (2015). Effects of Low-Level Aqueous Hydrogen Sulfide and Other Sulfur Species on Lettuce (*Lactuca sativa*) Seed Germination. *Communications in Soil Science and Plant Analysis*, 46(5), 576–587. <https://doi.org/10.1080/00103624.2014.998341>
- Maas, F. M., Van Loo, E. N., & Van Hasselt, P. R. (1988). Effect of long-term H₂S fumigation on photosynthesis in spinach. Correlation between CO₂ fixation and chlorophyll *a* fluorescence. *Physiologia Plantarum*, 72(1), 77–83. <https://doi.org/10.1111/j.1399-3054.1988.tb06625.x>

- Mishra, A., Gupta, H., Mishra, U. S., Sirothia, P., & Darwai, P. (2023). Interaction Effect of Sulphur and NPK on Growth Parameters and Yield of Mustard. *International Journal of Plant & Soil Science*, 35(15), 138–144. <https://doi.org/10.9734/ijpss/2023/v35i153089>
- Montesinos-Pereira, D., Barrameda-Medina, Y., Baenas, N., Moreno, D. A., Sánchez-Rodríguez, E., & Ruiz, J. M. (2016). Evaluation of hydrogen sulfide supply to biostimulate the nutritive and phytochemical quality and the antioxidant capacity of Cabbage (*Brassica oleracea* L. “Bronco”). *Journal of Applied Botany and Food Quality*, 89, 290–298. <https://doi.org/10.5073/jabfq.2016.089.038>
- Nishio, T. (2017). Economic and Academic Importance of Radish. In *Compendium of Plant Genomes* (pp. 1–10). Springer International Publishing AG. https://doi.org/10.1007/978-3-319-59253-4_1
- Oloumi, H., Maleki, M., Habibipour, L., & Lotfi, S. (2024). Foliar application of NaHS alleviates Cd toxicity in soybean plants through regulation of Glutathione metabolism. *Plant Stress*, 11(100363), 100363. <https://doi.org/10.1016/j.stress.2024.100363>
- Reinders. (2022). *Profile Products - Profile Greens Grade - 50 LB BAG*. Reinders.com. <https://www.reinders.com/profile-products-profile-greens-grade-50-lbs-bag/>
- Sparks, B. (2023, September 19). *How Mineral Nutrition Impacts Indoor-Grown Radishes*. Greenhouse Grower. <https://www.greenhousegrower.com/crops/how-mineral-nutrition-impacts-indoor-grown-radishes/>
- Thompson, C. R., & Kats, G. (1978). Effects of continuous hydrogen sulfide fumigation on crop and forest plants. *Environmental Science & Technology*, 12(5), 550–553. <https://doi.org/10.1021/es60141a001>
- Wang, R. (2002). Two’s company, three’s a crowd: can H₂S be the third endogenous gaseous transmitter? *The FASEB Journal*, 16(13), 1792–1798. <https://doi.org/10.1096/fj.02-0211hyp>

CHAPTER FOUR

CONCLUSION AND FUTURE DIRECTIONS

Dibutyldithiophosphates (DBDTPs) were developed to positively affect plants' growth, and yield by the controlled slow release of H_2S in the presence of water. This research has demonstrated that DBDTPs positively affected potatoes, radishes, and bush beans under different conditions, highlighting their versatility for use in agriculture across diverse crops and application methods. Their stability, ease of handling, and promising results in many response variables make them a valuable fertilizer additive to agricultural practices.

The value of DBDTPs lies beyond their positive effect for improving plant growth, they can provide dual benefits. While much literature emphasizes the positive effects of H_2S under abiotic stresses, few mention its benefits under normal growth conditions. Therefore, having a compound such as DBTPs, that can improve plant growth but at the same time offer support if abiotic stress occurs, becomes a valuable tool for crop management strategies.

In our research, we demonstrated that the addition of 0.025, 0.05, 0.1, and 0.2 mM DBDTP in tissue culture significantly increased root length compared to the control group (0 mM). However, such positive effects were not detected in height, fresh weight, and dry weight, and instead, a negative result in fresh weight, dry weight, and shoot height was observed when 0.2 mM was used. This indicates two important considerations: first, to prevent a decrease in these traits, the use of 0.2 mM should be avoided. Second, if potato plants are growing too fast in tissue culture and a delay in height is necessary, while still planning to increase root length, the use of 0.2 mM may be considered. Based on our findings, no difference between 0.025, 0.05, and

0.1 was seen for any of the evaluated traits, however, the only treatment that showed a slight improvement in shoot height and fresh weight was 0.05 mM.

In our field studies, we concluded that even when no significant positive effects in height, vigor, and yield were detected in 2023 and 2024 in potatoes, a positive impact of over 5% increase for 0.5 mM was seen for these parameters, suggesting the importance of this concentration in further testing to ensure its efficient use in field applications. Similarly, this concentration did not show phytotoxicity when applied in foliar applications at eight days, fifteen days, and one month after transplanting, strengthening its use in diverse application methods. However, it is crucial to test the effect of this dose in prolonged exposure time and more response variables besides phytotoxicity, such as yield.

Notably, in our experiment under three weeks of heat stress, the positive effects of 0.025 and 0.1 mM DBDTP in potato seedlings were evident in survival rates for the first round of the experiment when daily DBDTP applications in a mix with 200 ppm 20-20-20 NPK were done. This indicates that these concentrations may be useful under these conditions, however, more testing is required, to ensure a better control of parameters such as humidity and light intensity in the growth chamber.

In the case of radishes under nutrient deficiency, more research is needed to better understand the effects of this compound, ensuring better control of watering frequency, amount of water, and fertilizer. Nonetheless, an interesting outcome was seen when radishes were treated at first as seed treatment with 12.5 mM and then drenched weekly with 0.5 mM. Under both low NPK and zero NPK levels of 20-20-20 fertilizer, this treatment combination increased overall fresh weight and root fresh weight by more than 4.4% in both scenarios. However, further

assessment is needed, exploring fertilizer rates slightly above and below normal recommendations. This will help to clarify the impact of hydrogen sulfide on nutrient uptake. Contrary to the results in potatoes, DBDTP caused phytotoxicity in radishes at all tested concentrations (0.5, 1, 2, and 10 mM), implying that these concentrations should not be used in radishes, rather different treatments need to be explored.

Lastly, for bush beans and radishes in the field, our research showed that the use of DBDTP as seed treatment had positive trends on fresh weight when 12.5 and 25 mM were applied, increasing fresh weight by more than 5%.

In conclusion, it is important to highlight the large variation in H₂S susceptibility between species as well as between cultivars of the same species due to plant morphology (Ausma & De Kok, 2019), making it imperative to test different concentrations based on species and continue working on DBDTP application methods, doses, and timing in more crops. This will aid in expanding DBDTP use in agriculture, making the best use of its effect on plant growth to positively improve crop production.

CUMULATIVE REFERENCES CITED

- Abe, K., & Kimura, H. (1996). The possible role of hydrogen sulfide as an endogenous neuromodulator. *The Journal of Neuroscience*, 16(3), 1066–1071. <https://doi.org/10.1523/jneurosci.16-03-01066.1996>
- Ahmed, M., Fahad, S., Ali, M. A., Hussain, S., Tariq, M., Ilyas, F., Ahmad, S., Saud, S., Hammad, H. M., Nasim, W., Wu, C., & Liu, H. (2021). Hydrogen Sulfide: A Novel Gaseous Molecule for Plant Adaptation to Stress. *Journal of Plant Growth Regulation*, 40(6), 2485–2501. <https://doi.org/10.1007/s00344-020-10284-0>
- Alhrout, H. H., Khalifeh, H., in, H. A., Haddad, M. A., & Al-Dalein, S. Y. (2016). The impact of organic and inorganic fertilizer on yield and yield components of common bean (*Phaseolus vulgaris*). *Advances in Environmental Biology*, 10(9), 8–13. https://www.researchgate.net/publication/310828606_The_impact_of_organic_and_inorganic_fertilizer_on_yield_and_yield_components_of_common_bean_Phaseolus_vulgaris
- Ali, S., Farooq, M. A., Hussain, S., Yasmeen, T., Abbasi, G. H., & Zhang, G. (2013). Alleviation of chromium toxicity by hydrogen sulfide in barley. *Environmental Toxicology and Chemistry*, 32(10), 2234–2239. <https://doi.org/10.1002/etc.2309>
- Allam, A. I. (1972). Sulfide Inhibition of Oxidases in Rice Roots. *Phytopathology*, 62(6), 634–634. <https://doi.org/10.1094/phyto-62-634>
- Anbarasan, S., & Ramesh, S. (2022). Photosynthesis Efficiency: Advances and Challenges in Improving Crop Yield. *Plant Science Archives*, 7(3), 19–21. <https://doi.org/10.51470/psa.2022.7.3.19>
- Andersen, C. (n.d.). Agriculture and Natural Resources Home Gardening Series Radishes Environment. University of Arkansas. Retrieved October 23, 2023, from <https://www.uaex.uada.edu/publications/PDF/FSA-6075.pdf>
- Armstrong, J., & Armstrong, W. (2005). Rice: Sulfide-induced Barriers to Root Radial Oxygen Loss, Fe²⁺ and Water Uptake, and Lateral Root Emergence. *Annals of Botany*, 96(4), 625–638. <https://doi.org/10.1093/aob/mci215>
- Ausma, T., & De Kok, L. J. (2019). Atmospheric H₂S: Impact on Plant Functioning. *Frontiers in Plant Science*, 10. <https://doi.org/10.3389/fpls.2019.00743>
- Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models Usinglme4. *Journal of Statistical Software*, 67(1). <https://doi.org/10.18637/jss.v067.i01>
- Bhardwaj, S., & Kapoor, D. (2021). General view on H₂S and abiotic stress tolerance in plants. *Elsevier EBooks*, 113–132. <https://doi.org/10.1016/b978-0-323-85862-5.00010-5>
- Bitocchi, E., Nanni, L., Bellucci, E., Rossi, M., Giardini, A., Zeuli, P. S., Logozzo, G., Stougaard, J., McClean, P., Attene, G., & Papa, R. (2012). Mesoamerican origin of the common bean

- (*Phaseolus vulgaris* L.) is revealed by sequence data. *Proceedings of the National Academy of Sciences*, 109(14), E788–E796. <https://doi.org/10.1073/pnas.1108973109>
- Bláha, L. (2019). Importance Of Root-Shoot Ratio For Crops Production. *Agronomy & Agricultural Science*, 2(2), 1–7. <https://doi.org/10.24966/aas-8292/100012>
- Bloem, E., Riemenschneider, A., Volker, J., Papenbrock, J., Schmidt, A., Salac, I., Haneklaus, S., & Schnug, E. (2004). Sulphur supply and infection with *Pyrenopeziza brassicae* influence L-cysteine desulphydrase activity in *Brassica napus* L. *Journal of Experimental Botany*, 55(406), 2305–2312. <https://doi.org/10.1093/jxb/erh236>
- Bohl, W. H., & Johnson, S. B. (2010). Commercial Potato Production in North America The Potato Association of America Handbook Second Revision of American Potato Journal Supplement Volume 57 and USDA Handbook 267 by the Extension Section of The Potato Association of America. https://potatoassociation.org/wp-content/uploads/2014/04/A_ProductionHandbook_Final_000.pdf
- Broughton, W. J., Hernández, G., Blair, M., Beebe, S., Gepts, P., & Vanderleyden, J. (2003). Beans (*Phaseolus* spp.) – model food legumes. *Plant and Soil*, 252(1), 55–128. <https://doi.org/10.1023/a:1024146710611>
- Brown, E. M., Ranasinghe Arachchige, N. P. R., Paudel, A., & Bowden, N. B. (2021). Synthesis, Stability, and Kinetics of Hydrogen Sulfide Release of Dithiophosphates. *Journal of Agricultural and Food Chemistry*, 69(43), 12900–12908. <https://doi.org/10.1021/acs.jafc.1c04655>
- Burn, A. J., Dewan, S. K., Gosney, I., & Tan, P. S. G. (1990). Phosphorus-31 nuclear magnetic resonance study of the mechanism and kinetics of the hydrolysis of zinc(II)O,O-diethyl dithiophosphate and some related compounds. *Journal of the Chemical Society, Perkin Transactions 2*, 5, 753–758. <https://doi.org/10.1039/P29900000753>
- CABI. (2022). *Raphanus sativus* (radish). CABI Compendium, CABI Compendium. <https://doi.org/10.1079/cabicompendium.46796>
- Carter, J. M., Brown, E. M., Grace, J. P., Salem, A. K., Irish, E. E., & Bowden, N. B. (2018). Improved growth of pea, lettuce, and radish plants using the slow release of hydrogen sulfide from GYY-4137. *PLOS ONE*, 13(12), e0208732. <https://doi.org/10.1371/journal.pone.0208732>
- Carter, J. M., Brown, E. M., Irish, E. E., & Bowden, N. B. (2019). Characterization of Dialkyl dithiophosphates as Slow Hydrogen Sulfide Releasing Chemicals and Their Effect on the Growth of Maize. *Journal of Agricultural and Food Chemistry*, 67(43), 11883–11892. <https://doi.org/10.1021/acs.jafc.9b04398>
- Chatzianastasiou, A., Bibli, S.-I., Andreadou, I., Efentakis, P., Kaludercic, N., Wood, M. A., Whiteman, M., Di Lisa, F., Daiber, A., Manolopoulos, V. G., Szabó, C., &

- Papapetropoulos, D. A. (2016). Cardioprotection by H₂S Donors: Nitric Oxide-Dependent and -Independent Mechanisms. *J Pharmacol Exp Ther*, 358(3), 431–440. <https://doi.org/10.1124/jpet.116.235119>
- Dai, J., Wen, D., Li, H., Yang, J., Rao, X., Yang, Y., Yang, J., Yang, C., & Yu, J. (2024). Effect of hydrogen sulfide (H₂S) on the growth and development of tobacco seedlings in absence of stress. *BMC Plant Biology*, 24(1). <https://doi.org/10.1186/s12870-024-04819-w>
- Daniels, C. (2013). Growing Radishes in Home Gardens Crop at a Glance Selecting Types to Plant. Washington State University. <https://s3.wp.wsu.edu/uploads/sites/2071/2014/04/Radishes-FS127E.pdf>
- Datiles, M. J., & Acevedo-Rodríguez, P. (2022). *Solanum tuberosum* (potato). CABI Compendium, CABI Compendium. <https://doi.org/10.1079/cabicompendium.50561>
- Dawood, M. F. A., Sofy, M. R., Mohamed, H. I., Sofy, A. R., & Abdel-Kader, H. A. A. (2022). Hydrogen Sulfide Modulates Salinity Stress in Common Bean Plants by Maintaining Osmolytes and Regulating Nitric Oxide Levels and Antioxidant Enzyme Expression. *Journal of Soil Science and Plant Nutrition*, 22(3), 3708–3726. <https://doi.org/10.1007/s42729-022-00921-w>
- De Haan, S., & Rodriguez, F. (2016). Potato Origin and Production. *Advances in Potato Chemistry and Technology*, 1–32. <https://doi.org/10.1016/b978-0-12-800002-1.00001-7>
- De Kok, L. J. (1990). Sulfur metabolism in plants exposed to atmospheric sulfur. In H. Rennenberg, C. Brunold, L. J. De Kok, & I. Stulen (Eds.), *Sulfur Nutrition and Sulfur Assimilation in Higher Plants: Fundamental, Environmental and Agricultural Aspects* (pp. 111-130). Backhuys Publishers.
- De Kok, L. J., Stuiver, E. E., Westerman, S., & Stulen, I. (2002). Elevated Levels of Hydrogen Sulfide in the Plant Environment: Nutrient or Toxin. Springer EBooks, 201–219. https://doi.org/10.1007/978-4-431-68388-9_10
- Dida, G., & Etisa, D. (2018). Common bean (*Phaseolus Vulgaris* L.): A Review. *International Journal of Nutritional Science and Food Technology*, 4(7), 54–62. https://www.researchgate.net/publication/332179103_Common_bean_Phaseolus_Vulgaris_L_A_Review?enrichId=rgreq-9354211c390d0586f004e136a0d65664-XXX&enrichSource=Y292ZXJQYWdlOzMzMjE3OTEwMztBUzoXMDI0MzQyMDUzOTA0Mzg1QDE2MjEyMzM1ODUyNjk%3D&el=1_x_2&_esc=publicationCoverPdf
- Dooley, F. D., Nair, S. P., & Ward, P. D. (2013). Increased Growth and Germination Success in Plants following Hydrogen Sulfide Administration. *PLoS ONE*, 8(4). <https://doi.org/10.1371/journal.pone.0062048>

- Edi Santosa, Sugiyama, N., Eko Sulistyono, & Diddy Sopandie. (2004). Effects of Watering Frequency on the Growth of Elephant Foot Yams. *Nettai Nogyo/Nettai Nougyou*, 48(4), 235–239. <https://doi.org/10.11248/jsta1957.48.235>
- Ekinci, M., Turan, M., Ors, S., Dursun, A., & Yildirim, E. (2023). Improving salt tolerance of bean (*Phaseolus vulgaris* L.) with hydrogen sulfide. *Photosynthetica*, 61 (1). <https://doi.org/10.32615/ps.2023.005>
- Epstein, E. (1977). The Role of Roots in the Chemical Economy of Life on Earth. *BioSCIENCE*, 27(12), 783–787. <https://doi.org/10.2307/1297754>
- Ernest, E. (2024, April 19). High Plant Populations Needed for Best Snap Bean Yields. University of Delaware. <https://sites.udel.edu/weeklycropupdate/?p=24181>
- Feitosa de Vasconcelos, A. C., & Garófalo Chaves, L. H. (2019). Biostimulants and Their Role in Improving Plant Growth under Abiotic Stresses. In *www.intechopen.com*. IntechOpen. <https://www.intechopen.com/chapters/69956>
- Filipovic, M. R., & Jovanović, V. M. (2017). More than just an intermediate: hydrogen sulfide signaling in plants. *Journal of Experimental Botany*, 68(17), 4733–4736. <https://doi.org/10.1093/jxb/erx352>
- Firman, D. M., & Allen, E. J. (2007). Agronomic Practices. In *Potato Biology and Biotechnology Advances and Perspectives* (pp. 719–738). Elsevier. <http://base.dnsgb.com.ua/files/book/Agriculture/Cultures/Potato-Biology-and-Biotechnology.pdf>
- Flohr, R. C., & Conover, C. A. (1994). Production and Postproduction Watering Schedule Effects on Plant Growth, Quality and Blooming of Pansies and Petunias. *Proc. Fla. State Hort Soc*, 107, 414–416. <https://journals.flvc.org/fshs/article/download/92449/88641>
- Fort, D. J., Todhunter, K., Fort, T. D., Mathis, M. B., Walker, R., Hansel, M., Hall, S., Richards, R., & Anderson, K. (2017). Toxicity of sulfide to early life stages of wild rice (*Zizania palustris*). *Environmental Toxicology and Chemistry*, 36(8), 2217–2226. <https://doi.org/10.1002/etc.3759>
- García Mata, C., & Lamattina, L. (2013). Gasotransmitters are emerging as new guard cell signaling molecules and regulators of leaf gas exchange. *Plant Science*, 201-202, 66–73. <https://doi.org/10.1016/j.plantsci.2012.11.007>
- Gondim, E., & Nolasco-Rondon, J. (2020). The Crucial Role of Photosynthesis In Sustaining Plant Life and Its Impact on the Global Ecosystem, Climate, And Human Survival. *Australian Herbal Insight*, 3(1), 1–5. <https://doi.org/10.25163/ahi.319925>
- Goodwin, L. R., Francom, D. M., Dieken, F. P., Taylor, J. D., Warenycia, M. W., Reiffenstein, R. J., & Dowling, G. P. (1989). Determination of Sulfide in Brain Tissue by Gas Dialysis/Ion

- Chromatography: Postmortem Studies and Two Case Reports. *Journal of Analytical Toxicology*, 13(2), 105–109. <https://doi.org/10.1093/jat/13.2.105>
- Gotor, C., García, I., Aroca, A., Laureano-Marín, A. M., Arenas-Alfonseca, L., Jurado-Flores, A., Masero Moreno, I., & Rico Romero, L. (2019). Signaling by hydrogen sulfide and cyanide through post-translational modification. *Journal of Experimental Botany*, 70(16), 4251–4265. <https://doi.org/10.1093/jxb/erz225>
- Gro Indoor. (2024). IHORT Q-Plug 40/40, 1.25" x 1.25", case of 2100 – Gro Indoor. Groindoor.com. https://groindoor.com/products/propagation-ihort-q-plug-40-40-1-25-x-1-25-case-of-2100?utm_source=google&utm_medium=cpc&campaignid=20569808052&adgroupid=&keyword=&device=c&gclid=Cj0KCQIAh8OtBhCQARIsAlkWb6_mDn5Eg49SqydpNhwjvwu0T8m5hFhQMXzmIOBjuqViKXnaUK8U7VMaAsqvEALw_wcB
- Gupta, S. K., Marwa, N., Pandey, A. K., Zhang, Y.-B., & Zhang, J.-L. (2021). Hydrogen sulfide homeostasis in plants: An overview. Elsevier EBooks, 341–363. <https://doi.org/10.1016/b978-0-323-85862-5.00017-8>
- Hancock, J. T., & Whiteman, M. (2014). Hydrogen sulfide and cell signaling: Team player or referee? *Plant Physiology and Biochemistry*, 78, 37–42. <https://doi.org/10.1016/j.plaphy.2014.02.012>
- Handayani, T., & Watanabe, K. N. (2021). Using in vitro technique for drought, heat, and combined drought-heat assay in potatoes. *IOP Conference Series: Earth and Environmental Science*, 762(1), 012061. <https://doi.org/10.1088/1755-1315/762/1/012061>
- Hosoki, R., Matsuki, N., & Kimura, H. (1997). The Possible Role of Hydrogen Sulfide as an Endogenous Smooth Muscle Relaxant in Synergy with Nitric Oxide. *Biochemical and Biophysical Research Communications*, 237(3), 527–531. <https://doi.org/10.1006/bbrc.1997.6878>
- Iqbal, N., Fatma, M., Gautam, H., Umar, S., Sofo, A., D'ippolito, I., & Khan, N. A. (2021). The Crosstalk of Melatonin and Hydrogen Sulfide Determines Photosynthetic Performance by Regulation of Carbohydrate Metabolism in Wheat under Heat Stress. *Plants*, 10(9), 1778. <https://doi.org/10.3390/plants10091778>
- Iqbal, N., Umar, S., Khan, N. A., & Corpas, F. J. (2021). Nitric Oxide and Hydrogen Sulfide Coordinately Reduce Glucose Sensitivity and Decrease Oxidative Stress via Ascorbate-Glutathione Cycle in Heat-Stressed Wheat (*Triticum aestivum* L.) Plants. *Antioxidants*, 10(1), 108–108. <https://doi.org/10.3390/antiox10010108>
- Jacobs, A. (2012a). Plant Guide OILSEED RADISH *Raphanus sativus* L. Plant Symbol = RASA2. https://plants.usda.gov/DocumentLibrary/plantguide/pdf/pg_rasa2.pdf

- Jacobs, A. (2012b). Plant Guide for oilseed radish (*Raphanus sativus* L.). USDA-Natural Resources Conservation Service.
https://plants.usda.gov/DocumentLibrary/plantguide/pdf/pg_rasa2.pdf
- Jia, H., Hu, Y., Fan, T., & Li, J. (2015). Hydrogen sulfide modulates actin-dependent auxin transport via regulating ABPs results in changing of root development in *Arabidopsis*. *Scientific Reports*, 5(1). <https://doi.org/10.1038/srep08251>
- Jin, Z., Wang, Z., Ma, Q., Sun, L., Zhang, L., Liu, Z., Liu, D., Hao, X., & Pei, Y. (2017). Hydrogen sulfide mediates ion fluxes inducing stomatal closure in response to drought stress in *Arabidopsis thaliana*. *Plant and Soil*, 419(1-2), 141–152.
<https://doi.org/10.1007/s11104-017-3335-5>
- Kabil, O., Vitvitsky, V., & Banerjee, R. (2014). Sulfur as a Signaling Nutrient Through Hydrogen Sulfide. *Annual Review of Nutrition*, 34(1), 171–205. <https://doi.org/10.1146/annurev-nutr-071813-105654>
- Kang, J., Neill, D. L., & Xian, M. (2017). Phosphonothioate-Based Hydrogen Sulfide Releasing Reagents: Chemistry and Biological Applications. *Frontiers in Pharmacology*, 8.
<https://doi.org/10.3389/fphar.2017.00457>
- Khalofah, A., Ghramh, H. A., Al-Qthanin, R. N., & L'taief, B. (2022). The impact of NPK fertilizer on growth and nutrient accumulation in juniper (*Juniperus procera*) trees grown on fire-damaged and intact soils. *PLOS ONE*, 17(1), e0262685.
<https://doi.org/10.1371/journal.pone.0262685>
- Kolachevskaya, O. O., Alekseeva, V. V., Sergeeva, L. I., Rukavtsova, E. B., Getman, I. A., Vreugdenhil, D., Buryanov, Y. I., & Romanov, G. A. (2014). Expression of auxin synthesis gene *tms1* under control of tuber-specific promoter enhances potato tuberization in vitro. *Journal of Integrative Plant Biology*, 57(9), 734–744.
<https://doi.org/10.1111/jipb.12314>
- Kolupaev, Y. E., Karpets, Y. V., Shkliarevskyi, M. A., Yastreb, T. O., Plohovska, S. H., Yemets, A. I., & Blume, Y. B. (2022). Gasotransmitters in Plants: Mechanisms of Participation in Adaptive Responses. *the Open Agriculture Journal*, 16(1).
<https://doi.org/10.2174/18743315-v16-e2207050>
- Kumar, S., Kumar, S., & Mohapatra, T. (2021). Interaction Between Macro- and Micro-Nutrients in Plants. *Frontiers in Plant Science*, 12. <https://doi.org/10.3389/fpls.2021.665583>
- Lamattina, L., & García-Mata, C. (2016). *Gasotransmitters in Plants*. Springer.
<https://doi.org/10.1007/978-3-319-40713-5>
- Lamers, L. P. M., Govers, L. L., Janssen, I. C. J. M., Geurts, J. J. M., Van der Welle, M. E. W., Van Katwijk, M. M., Van der Heide, T., Roelofs, J. G. M., & Smolders, A. J. P. (2013).

- Sulfide as a soil phytotoxin—a review. *Frontiers in Plant Science*, 4. <https://doi.org/10.3389/fpls.2013.00268>
- Li, L., Rose, P., & Moore, P. K. (2011). Hydrogen Sulfide and Cell Signaling. *Annual Review of Pharmacology and Toxicology*, 51(1), 169–187. <https://doi.org/10.1146/annurev-pharmtox-010510-100505>
- Li, Z. B., & Zhu, L. (2014). Hydrogen sulfide donor sodium hydrosulfide-induced accumulation of betaine is involved in the acquisition of heat tolerance in maize seedlings. 38(1), 31–38. <https://doi.org/10.1007/s40415-014-0106-x>
- Li, Z. G. (2013). Hydrogen sulfide: A multifunctional gaseous molecule in plants. *Russian Journal of Plant Physiology*, 60(6), 733–740. <https://doi.org/10.1134/s1021443713060058>
- Li, Z.-G., Ding, X.-J., & Du, P.-F. (2013). Hydrogen sulfide donor sodium hydrosulfide-improved heat tolerance in maize and involvement of proline. *Journal of Plant Physiology*, 170(8), 741–747. <https://doi.org/10.1016/j.jplph.2012.12.018>
- Li, Z.-G., Gong, M., & Liu, P. (2012). Hydrogen sulfide is a mediator in H₂O₂-induced seed germination in *Jatropha Curcas*. *Acta Physiologiae Plantarum*, 34(6), 2207–2213. <https://doi.org/10.1007/s11738-012-1021-z>
- Li, Z.-G., Gong, M., Xie, H., Yang, L., & Li, J. (2012). Hydrogen sulfide donor sodium hydrosulfide-induced heat tolerance in tobacco (*Nicotiana tabacum* L) suspension cultured cells and involvement of Ca²⁺ and calmodulin. *Plant Science*, 185-186, 185–189. <https://doi.org/10.1016/j.plantsci.2011.10.006>
- Li, Z.-G., Min, X., & Zhou, Z.-H. (2016). Hydrogen Sulfide: A Signal Molecule in Plant Cross-Adaptation. *Frontiers in Plant Science*, 7. <https://doi.org/10.3389/fpls.2016.01621>
- Li, Z.-G., Yang, S.-Z., Long, W.-B., Yang, G.-X., & Shen, Z.-Z. (2013). Hydrogen sulphide may be a novel downstream signal molecule in nitric oxide-induced heat tolerance of maize (*Zea mays* L.) seedlings. *Plant, Cell & Environment*, 36(8), 1564–1572. <https://doi.org/10.1111/pce.12092>
- Lin, Y.-T., Li, M.-Y., Cui, W.-T., Lu, W., & Shen, W.-B. (2012). Haem Oxygenase-1 is Involved in Hydrogen Sulfide-induced Cucumber Adventitious Root Formation. *Journal of Plant Growth Regulation*, 31(4), 519–528. <https://doi.org/10.1007/s00344-012-9262-z>
- Lisjak, M., Špoljarević, M., Wood, M., Whiteman, M., Wilson, I., Hancock, J., & Teklić, T. (2015). H₂S and NO signalling in plants. *Semantic Scholar*. <https://www.semanticscholar.org/paper/H2S-and-NO-signalling-in-plants-Lisjak-%C5%A0poljarevi%C4%87/c8fd18c43bc24469bc9cb918bc248ac7a1b72642>

- Lisjak, M., Teklic, T., Wilson, I. D., Wood, M. E., Whiteman, M., & Hancock, J. T. (1988). Abstracts of Eleventh Annual Meeting, Japanese Society of Biorheology. *Biorheology*, 25(6), 893–915. <https://doi.org/10.3233/bir-1988-25610>
- Lisjak, M., Teklić, T., Wilson, I. D., Wood, M., Whiteman, M., & Hancock, J. T. (2011). Hydrogen sulfide effects on stomatal apertures. *Plant Signaling & Behavior*, 6(10), 1444–1446. <https://doi.org/10.4161/psb.6.10.17104>
- Liu, R., & Lal, R. (2015). Effects of Low-Level Aqueous Hydrogen Sulfide and Other Sulfur Species on Lettuce (*Lactuca sativa*) Seed Germination. *Communications in Soil Science and Plant Analysis*, 46(5), 576–587. <https://doi.org/10.1080/00103624.2014.998341>
- Maas, F. M., Van Loo, E. N., & Van Hasselt, P. R. (1988). Effect of long-term H₂S fumigation on photosynthesis in spinach. Correlation between CO₂ fixation and chlorophyll a fluorescence. *Physiologia Plantarum*, 72(1), 77–83. <https://doi.org/10.1111/j.1399-3054.1988.tb06625.x>
- Mishra, A., Gupta, H., Mishra, U. S., Sirothia, P., & Darwai, P. (2023). Interaction Effect of Sulphur and NPK on Growth Parameters and Yield of Mustard. *International Journal of Plant & Soil Science*, 35(15), 138–144. <https://doi.org/10.9734/ijpss/2023/v35i153089>
- Montesinos-Pereira, D., Barrameda-Medina, Y., Baenas, N., Moreno, D. A., Sánchez-Rodríguez, E., & Ruiz, J. M. (2016). Evaluation of hydrogen sulfide supply to biostimulate the nutritive and phytochemical quality and the antioxidant capacity of Cabbage (*Brassica oleracea* L. “Bronco”). *Journal of Applied Botany and Food Quality*, 89, 290–298. <https://doi.org/10.5073/jabfq.2016.089.038>
- Mukherjee, S., & Corpas, F. J. (2020). Crosstalk among hydrogen sulfide (H₂S), nitric oxide (NO) and carbon monoxide (CO) in root-system development and its rhizosphere interactions: A gaseous interactome. *Plant Physiology and Biochemistry*, 155, 800–814. <https://doi.org/10.1016/j.plaphy.2020.08.020>
- Natarajan, A., & G., J. W. (2023). Gasotransmitters: A Review. *International Journal of Basic & Clinical Pharmacology*, 12(4), 607–615. <https://doi.org/10.18203/2319-2003.ijbcp20231900>
- Navazio, J., Colley, M., & Dillon, M. (2007). Organic Seed Alliance Supporting the ethical development and stewardship of seed Principles and Practices of Organic Radish Seed Production in the Pacific Northwest This publication made possible through a grant from Western SARE. In Organic Seed Alliance. https://projects.sare.org/wp-content/uploads/1623radish_seed_manual.pdf
- Nelson, G. C., Valin, H., Sands, R. D., Havlík, P., Ahammad, H., Deryng, D., Elliott, J., Fujimori, S., Hasegawa, T., Heyhoe, E., Kyle, P., Von Lampe, M., Lotze-Campen, H., Mason d’Croz, D., van Meijl, H., van der Mensbrugghe, D., Müller, C., Popp, A., Robertson, R., & Robinson, S. (2013). Climate change effects on agriculture: Economic responses to

- biophysical shocks. *Proceedings of the National Academy of Sciences*, 111(9), 3274–3279. <https://doi.org/10.1073/pnas.1222465110>
- Nelson, G. C., Valin, H., Sands, R. D., Havlík, P., Ahammad, H., Deryng, D., Elliott, J., Fujimori, S., Hasegawa, T., Heyhoe, E., Kyle, P., Von Lampe, M., Lotze-Campen, H., Mason d’Croz, D., van Meijl, H., van der Mensbrugghe, D., Müller, C., Popp, A., Robertson, R., & Robinson, S. (2013). Climate change effects on agriculture: Economic responses to biophysical shocks. *Proceedings of the National Academy of Sciences*, 111(9), 3274–3279. <https://doi.org/10.1073/pnas.1222465110>
- Nishio, T. (2017). Economic and Academic Importance of Radish. In *Compendium of Plant Genomes* (pp. 1–10). Springer International Publishing AG. https://doi.org/10.1007/978-3-319-59253-4_1
- Occupational Safety and Health Administration, U.S. Department of Labor. (n.d.). Hydrogen Sulfide . [Www.osha.gov](http://www.osha.gov). Retrieved September 10, 2023, from <https://www.osha.gov/hydrogen-sulfide>
- OECD. (2016). *Safety Assessment of Transgenic Organisms in the Environment, Volume 6*. OECD Consensus Documents, Harmonisation of Regulatory Oversight in Biotechnology; OECD Publishing, Paris. https://www.oecd.org/en/publications/safety-assessment-of-transgenic-organisms-in-the-environment-volume-6_9789264253421-en.html
- Oloumi, H., Maleki, M., Habibipour, L., & Lotfi, S. (2024). Foliar application of NaHS alleviates Cd toxicity in soybean plants through regulation of Glutathione metabolism. *Plant Stress*, 11(100363), 100363. <https://doi.org/10.1016/j.stress.2024.100363>
- Oregon State University. (2010, July 21). Radish. College of Agricultural Sciences. <https://horticulture.oregonstate.edu/oregon-vegetables/radish-0>
- Ortiz, O., & Mares, V. (2017). The Historical, Social, and Economic Importance of the Potato Crop. *Compendium of Plant Genomes*, 1–10. https://doi.org/10.1007/978-3-319-66135-3_1
- Perdomo-Roldán, F., Mondragón-Pichardo, J., & Vibrans , H. (2009, July). *Raphanus sativus* - ficha informativa. [Www.conabio.gob.mx](http://www.conabio.gob.mx). <http://www.conabio.gob.mx/malezasdemexico/brassicaceae/raphanus-sativus/fichas/ficha.htm#3.%20Identificaci%C3%B3n%20y%20descripci%C3%B3n>
- Perdue, S., & Hamer, H. (2019). Specialty Crops Volume 2 • Subject Series • Part 8 United States Department of Agriculture. In USDA. https://www.nass.usda.gov/Publications/AgCensus/2017/Online_Resources/Specialty_Crops/SCROPS.pdf
- Petroff, R. (2002). Crop Profile for Potato in Montana. Montana State University. <https://ipmdata.ipmcenters.org/documents/cropprofiles/MTpotato.pdf>

- Powell, C. R., Dillon, K. M., & Matson, J. B. (2018). A review of hydrogen sulfide (H₂S) donors: Chemistry and potential therapeutic applications. *Biochemical Pharmacology*, 149, 110–123. <https://doi.org/10.1016/j.bcp.2017.11.014>
- PubChem. (2025). *Phaseolus vulgaris*. Nih.gov; PubChem. <https://pubchem.ncbi.nlm.nih.gov/taxonomy/3885>
- Ranasinghe Arachchige, N. P. R., Brown, E. M., & Bowden, N. B. (2022). Sustained Release of Hydrogen Sulfide from Di(t-butanol)dithiophosphate Phenethylamine Salt Encapsulated into Poly(lactic acid) Microparticles to Enhance the Growth of Radish Plants. *ACS Agricultural Science & Technology*, 2(5). <https://doi.org/10.1021/acsagscitech.2c00179>
- Reinders. (2022). Profile Products - Profile Greens Grade - 50 LB BAG. Reinders.com. <https://www.reinders.com/profile-products-profile-greens-grade-50-lbs-bag/>
- Rensink, W. A., Iobst, S., Hart, A., Stegalkina, S., Liu, J., & Buell, C. R. (2005). Gene expression profiling of potato responses to cold, heat, and salt stress. *Functional & Integrative Genomics*, 5(4), 201–207. <https://doi.org/10.1007/s10142-005-0141-6>
- Schellnhuber, H. J., & Cramer, W. P. (2006). Avoiding Dangerous Climate Change. In Google Books. Cambridge University Press. https://books.google.com/books?hl=es&lr=&id=7Ui8fBmNlm4C&oi=fnd&pg=PA10&dq=climate+change&ots=YAs5DrcMHO&sig=Xa24BEF1RuKQy_qZ6yMcvItamsk#v=onepage&q&f=false
- Sharma, P., Abrol, A., Qureshi, A., & Sharma, S. (2019). Role of biostimulants with special reference to Panchgavya and Jeevamrit in floriculture-a review. *AgricINTERNATIONAL*, 6(1). <https://www.semanticscholar.org/paper/Role-of-biostimulants-with-special-reference-to-and-Sharma-Abrol/65e765e38805b5f855e944ccb816f0c136ca63c4>
- Sharma, S., Jaiswal, A. K., & Jaiswal, S. (2020). Potato. Elsevier EBooks, 339–347. <https://doi.org/10.1016/b978-0-12-812780-3.00021-0>
- Shrestha, S., Dhungana, M., Sahani, S., & Bhattarai, B. (2021). Seed Quality Improvement to Approach Sustainable Yield Of Field Crops by Various Preparation Techniques: Seed Priming, Treatment and Inoculation_ A Review. *Plant Physiology and Soil Chemistry*, 1(1), 12–20. <https://doi.org/10.26480/ppsc.01.2021.12.20>
- Sparks, B. (2023, September 19). How Mineral Nutrition Impacts Indoor-Grown Radishes. *Greenhouse Grower*. <https://www.greenhousegrower.com/crops/how-mineral-nutrition-impacts-indoor-grown-radishes/>
- Spooner, D. M., & Knapp, S. (2013, November). *Solanum tuberosum* | Solanaceae Source. *Solanaceaesource.myspecies.info*. <https://solanaceaesource.myspecies.info/content/solanum-tuberosum>

- Struik, P. C. (2007). Above-Ground and Below-Ground Plant Development. In POTATO BIOLOGY AND BIOTECHNOLOGY ADVANCES AND PERSPECTIVES (pp. 219–236). Elsevier. <http://base.dnsgb.com.ua/files/book/Agriculture/Cultures/Potato-Biology-and-Biotechnology.pdf>
- Subbian, P., Lal, R., & Subramanian, K. S. (2000). Cropping Systems Effects on Soil Quality in Semi-Arid Tropics. *Journal of Sustainable Agriculture*, 16(3), 7–38. https://doi.org/10.1300/j064v16n03_03
- Tang, R., Gupta, S. K., Niu, S., Li, X.-Q., Yang, Q., Chen, G., Zhu, W., & Haroon, M. (2020). Transcriptome analysis of heat stress response genes in potato leaves. *Molecular Biology Reports*, 47(6), 4311–4321. <https://doi.org/10.1007/s11033-020-05485-5>
- Thompson, C. R., & Kats, G. (1978). Effects of continuous hydrogen sulfide fumigation on crop and forest plants. *Environmental Science & Technology*, 12(5), 550–553. <https://doi.org/10.1021/es60141a001>
- Thompson, C. R., & Kats, G. (1978). Effects of continuous hydrogen sulfide fumigation on crop and forest plants. *Environmental Science & Technology*, 12(5), 550–553. <https://doi.org/10.1021/es60141a001>
- US Department of Agriculture. (2024). Plant Vigor Criteria. Usda.gov. https://efotg.sc.egov.usda.gov/references/public/AZ/rtn_18.doc
- Wang, R. (2002). Two's company, three's a crowd: can H₂S be the third endogenous gaseous transmitter? *The FASEB Journal*, 16(13), 1792–1798. <https://doi.org/10.1096/fj.02-0211hyp>
- Wang, R. (2014). Gasotransmitters: growing pains and joys. *Trends in Biochemical Sciences*, 39(5), 227–232. <https://doi.org/10.1016/j.tibs.2014.03.003>
- Wang, R. (2018). Overview of Gasotransmitters and the Related Signaling Network. In *Gasotransmitters* (pp. 1–28). The Royal Society of Chemistry. <https://doi.org/10.1039/9781788013000-00001>
- Weil, R., White, C., & Lawley, Y. (2006). Forage Radish: New Multi-Purpose Cover Crop for the Mid-Atlantic. https://covercrop.org/wp-content/uploads/Weil_radish.pdf
- Welty, C., & Doohan, D. (2000, September). Crop Profile for Green Beans in Ohio. The Ohio State University Extension Program; The Ohio State University. <https://ipm.osu.edu/sites/ipm/files/imce/OHgreenbean.pdf>
- Willermet, P. A., Dailey, D. P., Carter, R. O., Schmitz, P. J., & Zhu, W. (1995). Mechanism of formation of antiwear films from zinc dialkyldithiophosphates. *Tribology International*, 28(3), 177–187. [https://doi.org/10.1016/0301-679x\(95\)98965-g](https://doi.org/10.1016/0301-679x(95)98965-g)

- Wu, X., Du, A., Zhang, S., Wang, W., Liang, J., Peng, F., & Xiao, Y. (2021). Regulation of growth in peach roots by exogenous hydrogen sulfide based on RNA-Seq. *Plant Physiology and Biochemistry*, 159, 179–192. <https://doi.org/10.1016/j.plaphy.2020.12.018>
- Xu, D., Cao, H., Fang, W., Pan, J., Chen, J., Zhang, J., & Shen, W. (2017). Linking hydrogen-enhanced rice aluminum tolerance with the reestablishment of GA/ABA balance and miRNA-modulated gene expression: A case study on germination. *Ecotoxicology and Environmental Safety*, 145, 303–312. <https://doi.org/10.1016/j.ecoenv.2017.07.055>
- Xuan, L., Li, J., Wang, X., & Wang, C. (2020). Crosstalk between Hydrogen Sulfide and Other Signal Molecules Regulates Plant Growth and Development. *International Journal of Molecular Sciences*, 21(13), 4593. <https://doi.org/10.3390/ijms21134593>
- Yang, M., Qin, B., Ma, X., Wang, P., Li, M., Chen, L., Chen, L., Sun, A., Wang, Z., & Yin, Y. (2016). Foliar application of sodium hydrosulfide (NaHS), a hydrogen sulfide (H₂S) donor, can protect seedlings against heat stress in wheat (*Triticum aestivum* L.). *Journal of Integrative Agriculture*, 15(12), 2745–2758. [https://doi.org/10.1016/s2095-3119\(16\)61358-8](https://doi.org/10.1016/s2095-3119(16)61358-8)
- Yang, Z., Wang, X., Feng, J., & Zhu, S. (2022). Biological Functions of Hydrogen Sulfide in Plants. *International Journal of Molecular Sciences*, 23(23), 15107. <https://doi.org/10.3390/ijms232315107>
- Yao, Y., Yang, Y., Li, C., Huang, D., Zhang, J., Wang, C., Li, W., Wang, N., Deng, Y., & Liao, W. (2019). Multiple environmental stressors can induce gasotransmitter production in plants. Abiotic stressors (drought, salt, heavy metal, temperature, light, and UV radiation) induced the generation of gasotransmitters (H₂, H₂S, NO, CO, and CH₄). In *Research Progress on the Functions of Gasotransmitters in Plant Responses to Abiotic Stresses*. <https://www.mdpi.com/2223-7747/8/12/605>
- Yao, Y., Yang, Y., Li, C., Huang, D., Zhang, J., Wang, C., Li, W., Wang, N., Deng, Y., & Liao, W. (2019). Research Progress on the Functions of Gasotransmitters in Plant Responses to Abiotic Stresses. *Plants*, 8(12), 605. <https://doi.org/10.3390/plants8120605>
- Zhang, G., Tang, R., Niu, S., Si, H., Yang, Q., Bizimungu, B., Regan, S., & Li, X.-Q. (2019). Effects of Earliness on Heat Stress Tolerance in Fifty Potato Cultivars. *American Journal of Potato Research*, 97(1), 23–32. <https://doi.org/10.1007/s12230-019-09740-9>
- Zhang, H. (2016). Hydrogen Sulfide in Plant Biology. In *Gasotransmitters in Plants*. Springer. <https://link.springer.com/book/10.1007/978-3-319-40713-5>
- Zhang, J., & Spikes, H. (2016). On the Mechanism of ZDDP Antiwear Film Formation. *Tribology Letters*, 63(2). <https://doi.org/10.1007/s11249-016-0706-7>

- Zhang, P., Luo, Q., Wang, R., & Xu, J. (2017). Hydrogen sulfide toxicity inhibits primary root growth through the ROS-NO pathway. *Scientific Reports*, 7(1). <https://doi.org/10.1038/s41598-017-01046-2>
- Zhou, Z., von Wantoch Rekowski, M., Coletta, C., Szabo, C., Bucci, M., Cirino, G., Topouzis, S., Papapetropoulos, A., & Giannis, A. (2012). Thioglycine and l-thiovaline: Biologically active H₂S-donors. *Bioorganic & Medicinal Chemistry*, 20(8), 2675–2678. <https://doi.org/10.1016/j.bmc.2012.02.028>
- Ziogas, V., Molassiotis, A., Fotopoulos, V., & Tanou, G. (2018). Hydrogen Sulfide: A Potent Tool in Postharvest Fruit Biology and Possible Mechanism of Action. *Frontiers in Plant Science*, 9(1375). <https://doi.org/10.3389/fpls.2018.01375>