JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

Cite This: J. Agric. Food Chem. XXXX, XXX, XXX−XXX

pubs.acs.org/JAFC

Characterization of Dialkyldithiophosphates as Slow Hydrogen Sulfide Releasing Chemicals and Their Effect on the Growth of Maize

Justin M. Carter,[†] Eric M. Brown,[†] Erin E. Irish,[‡] and Ned B. Bowden*[,†]

[†]Department of Chemistry, University of Iowa, Iowa City, Iowa 52242, United States

[‡]Department of Biology, University of Iowa, Iowa City, Iowa 52242, United States

Supporting Information

ABSTRACT: Hydrogen sulfide is a key gasotransmitter for plants and has been shown to greatly increase their growth and survival in the presence of environmental stressors. Current methods for slowly releasing hydrogen sulfide use chemicals, such as GYY-4137, but these result in the release of chemicals not found in the environment, and chemicals used may lack structures that can be readily tuned to affect the rate of release of hydrogen sulfide. In this article, we describe the synthesis and slow release of hydrogen sulfide from dialkyldithiophosphates, which are a new set of hydrogen sulfide releasing chemicals that can be used in agriculture. The rates of hydrolysis of dibutyldithiophosphate and GYY-4137 were measured in water at 85 °C and compared with each other to investigate their differences. GYY-4137 is widely used as a chemical that slowly releases H_2S, but its rate of release was not previously quantified. The release of hydrogen sulfide in water at room temperature was measured for a series of dialkyldithiophosphates using a hydrogen sulfide electrode. It was shown that the structure of the dialkyldithiophosphate affected the amount of hydrogen sulfide released. The final degradation products of dibutyldithiophosphate were shown to be phosphoric acid and butanol, which are chemicals found in the environment. This result was notable because it demonstrated that dialkyldithiophosphates degrade to safe, natural chemicals that will not pollute the environment. To demonstrate that dialkyldithiophosphates have potential applications in agriculture, maize was grown for 4.5 weeks after exposure to 1−200 mg of dibutyldithiophosphate, and the weight of corn plants increased by up to 39% at low loadings of dibutyldithiophosphate.

KEYWORDS: *dialkyldithiophosphates, maize, harvest yield, hydrogen sulfide, GYY-4137, kinetics*

Downloaded via UNIV OF IOWA on October 29, 2019 at 23:58:47 (UTC).
See https://pubs.acs.org/sharingguidelines for options on how to legitimately share published articles.

INTRODUCTION

Hydrogen sulfide (H_2S) has recently been recognized as a key gasotransmitter in numerous plants. H_2S can increase plant growth, survival in the presence of environmental stressors, and harvest yields.[1−4] In 2004 and 2005, several enzymes that produce H_2S from cysteine were discovered, complementing the discovery in 1987 of an H_2S producing enzyme found in chloroplasts and mitochondria.[5−7] Since 2005, scientists have repeatedly demonstrated that H_2S delivered at optimal concentrations to plants has dramatic effects: more than doubling the sizes of roots, protecting plants from drought and heat, inhibiting freezing stress in leaves, protecting plants from high concentrations of salt, and prolonging the shelf-lives of harvested fruit.[8−13] Prior to 2007, most research showed that H_2S had a negative effect on plants (i.e., inhibited their growth or killed the plants), but since then, it has been shown to have positive effects when delivered at optimal doses on lettuce, strawberries, wheat, corn, soybeans, sweet potatoes, cucumbers, rice, peas, spinach, tomatoes, broccoli, lettuce, sugar beets, radishes, alfalfa, and kiwi.[14−39] The identity of the "optimal dose" of H_2S delivered to plants differs widely from paper to paper because of the challenges of delivering specified amounts of a low boiling point gas that rapidly evaporates after delivery (the boiling point of H_2S is −60 °C).

Many of the early and current reports of H_2S in agriculture used aqueous H_2S that was made by dissolving solid NaSH or Na_2S in water. In these studies, seeds or plants were watered once or twice daily with aqueous H_2S at concentrations from micromolar to several millimolar. Low concentrations of aqueous H_2S led to positive effects, such as those already mentioned as well as increased root length; increased number of roots; increased rate of germination; increased mass of the plant; and increased survival in the face of environmental stressors such as excessive heat, drought, low iron, high concentrations of heavy metals, and more.[14−39] At higher loadings of several millimolar H_2S, plants suffered and did poorly compared with the plants grown without exogenous H_2S. Although informative, in these experiments most (likely >95%) of the H_2S evaporated, and only a small, unknown amount was adsorbed by the plants. In addition, the administration of aqueous H_2S to plants also resulted in the release of H_2S into the atmosphere, which was a problem because it was toxic at loadings as low as several parts per million and possessed a strong, unpleasant odor of rotten eggs.[40] Prior work with aqueous H_2S did not identify the amount of H_2S needed to have a positive effect on plants, but these papers described the importance of controlling the amount of H_2S delivered to plants to have a beneficial effect and avoid negative effects. In this paper we describe a new set of chemicals never used in agriculture that slowly degrade to release H_2S, can be used to control the amount of H_2S

Received: July 15, 2019
Revised: September 30, 2019
Accepted: October 9, 2019
Published: October 9, 2019

ACS Publications © XXXX American Chemical Society

DOI: 10.1021/acs.jafc.9b04398
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

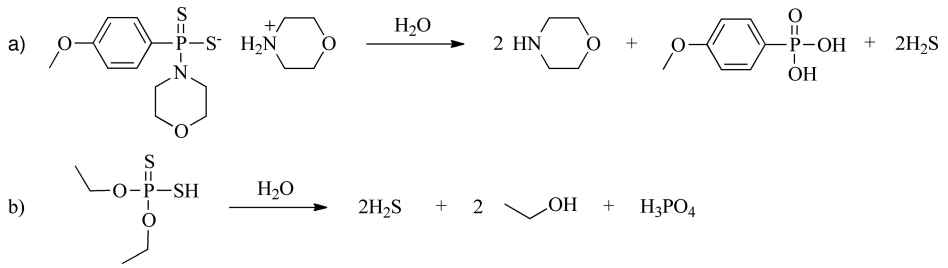

Figure 1. Degradation products of (a) GYY-4137 and (b) diethyldithiophosphate. GYY-4137 releases 2 equiv of H_2S but also 2 equiv of morpholine and a non-natural phosphate. Diethyldithiophosphate releases 2 equiv of H_2S; 2 equiv of ethanol; and phosphoric acid, which is commonly used in fertilizer.

delivered to plants, and have degradation products that are natural, safe chemicals in the environment.

To address the challenges of working with aqueous H_2S, scientists have used small chemicals that slowly release H_2S, such as GYY-4137 (Figure 1).[3,41−44] In our prior work, the administration of 1−250 mg quantities of GYY-4137 per seed was shown to double the weight of radish plants and nearly double the weight of a head of lettuce.[3] The doubling of the weight of radishes happened at loadings of only 10 mg of GYY-4137; at this loading of GYY-4137, the radish plants were only exposed to a maximum of 1.8 mg of H_2S over 4.5 weeks.

Although GYY-4137 has been widely used by us and others in agricultural and medicinal studies, its rate of hydrolysis and release of H_2S are poorly understood.[3,42,44,45] We and others reported that only a few percent of GYY-4137 hydrolyzed in water after 30 days but that the rate of hydrolysis was much faster in organic solvents such as chloroform or DMSO with residual water present.[3,42,44] In many agricultural studies, the use of GYY-4137 to deliver H_2S was an improvement from the use of aqueous H_2S because it did not lead to toxic levels of H_2S in the atmosphere above the plants, and the location of release of H_2S could be controlled by the careful addition of GYY-4137 on a plant. GYY-4137 has two limitations that hinder its use and future applications. First, upon hydrolysis, GYY-4137 releases an amine and a phosphate that are not naturally found in the environment and have unknown effects on plants. Second, the structure of GYY-4137 does not readily lend itself to changes that could alter its rate of release of H_2S. A recent paper reported the synthesis of derivatives of GYY-4137 by replacing morpholine with other amines, but the kinetics of the rates of release of H_2S were not measured.[46] Unfortunately for applications in agriculture, these H_2S-releasing chemicals hydrolyzed to release H_2S and chemicals not naturally found in the environment.

In this article, we report the one-step synthesis of dialkyldithiophosphates and their rates of release of H_2S (Figure 1b). Dialkyldithiophosphates are commonly used as ligands for zinc to yield lubricants, but their applications in agriculture have not been investigated before.[47−52] Their rates of hydrolysis and release of H_2S were initially reported in 1962 and 1984.[53,54] A report in 1962 described the half-life of hydrolysis of diethyldithiophosphate at room temperature to be 250 h in 1 M HCl and 4.8 h in 10 M HCl. The rate of hydrolysis decreased by orders of magnitude when the size of the alkyl groups was increased, according to a report in 1984.[53] In this report, the half-life of hydrolysis of di(2-ethylhexyl)-dithiophosphate at room temperature was estimated to be over 2 years in 1 M HCl on the basis of an observation that only 10% hydrolyzed after 5 months. This prior work demonstrated that hydrolysis and the release of H_2S from dialkyldithiophosphates were sensitive to the sizes of the alkyl groups used in their synthesis and could provide a method to control the rate of release of H_2S. We believe dialkyldithiophosphates are an improvement on GYY-4137 because in addition to having controlled rates of hydrolysis, they degrade to release fatty alcohols and phosphoric acid, which are already present in the environment. Their degradation products are safe and do not present a hazard to their use.

In this article, we report the synthesis and release of H_2S from a series of dialkyldithiophosphates synthesized from fatty alcohols with different lengths and compare their rates of hydrolysis with the rate of hydrolysis of GYY-4137, which was measured for the first time. To demonstrate the potential applications of dialkyldithiophosphates in agriculture, we also describe their effects on the growth of corn 4 weeks after exposure to milligram loadings of dibutyldithiophosphate. We believe that dialkyldithiophosphates are interesting new H_2S releasing chemicals for applications in agriculture because they have slow and controlled release of H_2S, their rate of H_2S release can be varied by changing their structures, and they ultimately degrade to release natural and safe chemicals.

EXPERIMENTAL PROCEDURES

Materials and Methods. The corn seeds used were Golden × Bantam (SVCOR113-qb5) from Eden Brother's in Arden, NC. Potting mixes were obtained from Beautiful Land Products in West Branch, IA. Potting mix #4 was peat/bark based general purpose growing mix. Pots were 6″ TEKU VCC 15 US 0600 (1.48 L) purchased from Hummert International.

All chemicals were obtained from Sigma-Aldrich. The dialkyldithiophosphates and GYY-4137 were synthesized by variation of published methods, and their NMR spectra were compared to literature values.[44] The NMR spectra were obtained using a Bruker Avance-300 at 300 MHz, a Bruker DRX-400 at 400 MHz, and a Bruker DPX-500 at 500 MHz. An amperometric H_2S microsensor for real time H_2S monitoring was purchased from Analysenmesstechnik GmBH.

Synthesis of Dialkyldithiophosphates. *Dibutyldithiophosphate Ammonium Salt.* *n*-Butanol (30.9 mL, 338 mmol) was added slowly over 2 min to a mixture of P_4S_{10} (18.66 g, 42.0 mmol) and toluene (75 mL). The contents were stirred at 85 °C for 16 h. Toluene was removed under reduced pressure, the crude dibutyldithiophosphate was cooled in an ice bath, and a 28% ammonium hydroxide solution in water (23.0 mL, 161.7 mmol) was added slowly over 2 min. Water was removed under reduced pressure yielding the dibutyldithiophosphate ammonium salt, which was recrystallized twice from hot toluene to give a white solid (79% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.97 (b, 4H), 4.00 (q, 4H), 1.67 (p, 4H), 1.38 (m, 4H), 0.94 (t, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 66.92, 32.60, 19.23, 13.97; ^{31}P NMR (300 MHz, $CDCl_3$) δ

DOI: 10.1021/acs.jafc.9b04398
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

109.89. The other ammonium salts were synthesized by the same procedure unless otherwise noted.

Dibutyldithiophosphate. The protonated acid of dibutyldithiophosphate was obtained by dissolving dibutyldithiophosphate ammonium salt (0.76 g, 2.95 mmol) in a cold solution of H_2SO_4 (0.62 mL, 11.63 mmol) in water and washing three times with 10 mL of $CHCl_3$. The organic layers were combined and washed with Na_2SO_4. The $CHCl_3$ was removed under reduced pressure, giving a tan, viscous liquid (98% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.15 (q, 4H), 2.82 (b, 1H), 1.72 (p, 4H), 1.43 (m, 4H), 0.95 (t, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 68.26, 32.11, 18.92, 13.73; ^{31}P NMR (300 MHz, $CDCl_3$) δ 86.15.

Diethyldithiophosphate Ammonium Salt. Ethanol (4.7 mL, 81.0 mmol), P_4S_{10} (4.38 g, 9.85 mmol), toluene (40 mL), 28% ammonium hydroxide in water (4.4 mL, 30.1 mmol). Recrystallized twice from hot ethanol to give a pink solid (31% yield). ^{1}H NMR (300 MHz, D_2O) δ 4.06 (q, 4H), 1.30 (t, 6H); ^{13}C NMR (75 MHz, D_2O) δ 65.58, 18.07; ^{31}P NMR (300 MHz, D_2O) δ 111.60.

Dihexyldithiophosphate Ammonium Salt. *n*-Hexanol (2.5 mL, 19.5 mmol), P_4S_{10} (1.08 g, 2.43 mmol), toluene (20 mL), 28% ammonium hydroxide in water (0.7 mL, 5.2 mmol). White solid (75% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.09 (b, 4H), 3.96 (q, 4H), 1.69 (p, 4H), 1.32 (m, 12H), 0.89 (t, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 67.34, 31.70, 30.49, 25.69, 22.78, 14.19; ^{31}P NMR (300 MHz, $CDCl_3$) δ 109.69.

Dioctyldithiophosphate Ammonium Salt. *n*-Octanol (15.0 mL 94.4 mmol), P_4S_{10} (5.07 g, 11.4 mmol), toluene (60 mL), 28% ammonium hydroxide in water (5.9 mL, 40.1 mmol). White solid (73% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.96 (b, 4H), 3.95 (q, 4H), 1.68 (p, 4H), 1.27 (m, 20H), 0.88 (t, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 67.24, 32.03, 30.57, 29.50, 29.36, 26.07, 22.83, 14.25; ^{31}P NMR (300 MHz, $CDCl_3$) δ 109.67.

Didecyldithiophosphate Ammonium Salt. *n*-Decanol (5.80 mL, 30.4 mmol), P_4S_{10} (1.69 g, 3.80 mmol), toluene (25 mL), 28% ammonium hydroxide in water. White solid (74% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.65 (b, 4H), 3.97 (q, 4H), 1.68 (p, 4H), 1.26 (m, 28H), 0.88 (t, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 67.34, 32.10, 30.59, 29.89, 29.83, 29.64, 29.55, 26.09, 22.85, 14.26; ^{31}P NMR (300 MHz, $CDCl_3$) δ 109.57.

Didodecyldithiophosphate Ammonium Salt. *n*-Dodecanol (16.7 mL, 73.5 mmol), P_4S_{10} (4.10 g, 9.22 mmol), toluene (30 mL), 28% ammonium hydroxide in water (4.20 mL, 29.5 mmol). White solid (82% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.13 (b, 4H), 3.95 (q, 4H), 1.68 (p, 4H), 1.26 (m, 28H), 0.88 (t, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 67.24, 32.10, 30.59, 29.92, 29.67, 29.56, 29.64, 26.10, 22.85, 14.25; ^{31}P NMR (300 MHz, $CDCl_3$) δ 109.58.

Stability of Dibutyldithiophosphate and Dibutyldithiophosphate Ammonium Salt. Dibutyldithiophosphate (viscous liquid, 122.8 mg, 0.50 mmol) and dibutyldithiophosphate ammonium salt (white solid, 133.5 mg, 0.51 mmol) were added to separate vials and capped. The chemicals were stored at room temperature. ^{31}P NMR spectra (300 MHz, D_2O) were taken on days 0 and 41. The hydrolysis percents were measured by integrating peaks in the ^{31}P NMR spectra. The dibutyldithiophosphate was 35% hydrolyzed after 41 days, and the dibutyldithiophosphate ammonium salt showed no change in the ^{31}P NMR spectrum and did not hydrolyze.

Investigation of the Degradation of Dibutyldithiophosphate by ^{31}P NMR Spectroscopy. Dibutyldithiophosphate (77.0 mg, 0.318 mmol) was dissolved in 1.60 mL of 90% H_2O/D_2O buffered with Bis-Tris (1 M) to pH 7, yielding a 0.20 M solution. The solution was added to an NMR tube and placed in an 85 °C oil bath. ^{31}P NMR spectra (300 MHz) were taken periodically to track degradation.

The hydrolysis of dibutyldithiophosphate was monitored in buffered 90% H_2O/D_2O at pH 7 at room temperature by ^{31}P NMR spectroscopy. Two studies were completed at 0.20 and 0.52 M. Dibutyldithiophosphate (77.0 mg, 0.318 mmol) was dissolved in 1.60 mL of 90% H_2O/D_2O buffered with Bis-Tris (1 M) at pH 7, yielding a 0.20 M solution. Dibutyldithiophosphate (99.0 mg, 0.409 mmol) was dissolved in 0.79 mL of 90% H_2O/D_2O buffered with Bis-Tris (1 M) at pH 7, yielding a 0.52 M solution. The ^{31}P NMR spectra (300 MHz, 90% H_2O/D_2O) of both samples were collected at day 0 and day 35.

The hydrolysis of dibutyldithiophosphate was also measured in $CDCl_3$ at room temperature by ^{31}P NMR spectroscopy. Dibutyldithiophosphate (67.2 mg, 0.277 mmol) was dissolved in 1.30 mL of $CDCl_3$, yielding a 0.21 M solution. The ^{31}P NMR spectra (300 MHz, $CDCl_3$) were collected at day 0 and day 29.

Investigation of the Degradation of GYY-4137 by ^{31}P NMR Spectroscopy. GYY-4137 (74.4 mg, 0.198 mmol) was dissolved in 1.51 mL of 90% H_2O/D_2O buffered with Bis-Tris to pH 7, yielding a 0.13 M solution. The solution was added to an NMR tube and placed in an 85 °C oil bath. ^{31}P NMR spectra (300 MHz, 90% H_2O/D_2O) were taken periodically to track degradation.

Investigation of the Degradation of Dialkyldithiophosphate Ammonium Salts Using an H_2S Electrode. To a glass jar, H_2O buffered with Bis-Tris (1 M) to pH 6.7 was added. A baseline of the concentration of H_2S was measured for an hour to confirm that it was zero. Next, dialkyldithiophosphates were added to the buffered water to yield different concentrations as described in this paper. The aqueous solution of H_2S was capped with a rubber stopper that had a hole cut into it for the H_2S electrode. Parafilm was wrapped tightly around the cap to ensure a closed system. The measurements were logged into a spreadsheet every two seconds.

GYY-4137 (13.79 g, 36.6 mmol) was added to 72.0 mL of buffered H_2O, yielding a 0.51 M solution.

GYY-4137 (3.21 g, 8.52 mmol) was added to 70.9 mL of buffered H_2O, yielding a 0.12 M solution.

GYY-4137 (1.52 g, 4.04 mmol) was added to 70.0 mL of buffered H_2O, yielding a 0.058 M solution.

Dibutyldithiophosphate (9.04 g, 34.9 mmol) was added to 70.0 mL of buffered H_2O, yielding a 0.50 M solution.

Dibutyldithiophosphate (4.42 g, 17.0 mmol) was added to 70.0 mL of buffered H_2O, yielding a 0.24 M solution.

Dibutyldithiophosphate (2.17 g, 8.38 mmol) was added to 70.9 mL of buffered H_2O, yielding a 0.19 M solution.

Diethyldithiophosphate (3.63 g, 17.9 mmol) was added to 71.0 mL of buffered H_2O, yielding a 0.25 M solution.

Dihexyldithiophosphate (5.57 g, 17.6 mmol) was added to 70.0 mL of buffered H_2O, yielding a 0.25 M solution.

Dioctyldithiophosphate (6.52 g, 17.5 mmol) was added to 70.3 mL of buffered H_2O, yielding a 0.25 M solution.

Didodecyldithiophosphate (8.46 g, 17.5 mmol) was added to 70.8 mL of buffered H_2O, yielding a 0.25 M solution.

Growth of Corn Exposed to Dibutyldithiophosphate. Corn was planted on September 5, 2017, in 6″ TEKU pots. The pots were packed finger tight with potting mix #4 from Beautiful Land Products. The corn seeds were planted approximately 1.5 in. deep. We planted 50 seeds of corn for every loading of dibutyldithiophosphate ammonium salt. After the seeds were added to the soil, the dibutyldithiophosphate ammonium salt was added as a fully dissolved aqueous solution. Tap water (500 mL) was added to a measured amount of the salt to yield the desired amount in 10 mL of the salt. After mixing well for 10 min, the aqueous solution was added to each plant via syringe (10 mL) immediately around and on the seed. After the salt was added, the plants were moved outside onto the balcony of a greenhouse in full sunlight until harvest. The corn plants were watered daily.

Corn was harvested on October 6, 2017. The height of a plant was taken by straightening the corn plant out by the leaves and measuring from the base of the shoot to the longest point on the leaf. The weight of the shoot and leaves was taken using a balance after the roots were removed.

Statistical Analysis. Statistical analysis was performed using IBM SPSS Statistics 25. A nonparametric Kruskal–Wallis test was performed to determine significance. Data represented are means ± standard error with two asterisks (**) indicating $\alpha < 0.05$ and one asterisk (*) indicating $\alpha < 0.1$.

DOI: 10.1021/acs.jafc.9b04398
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

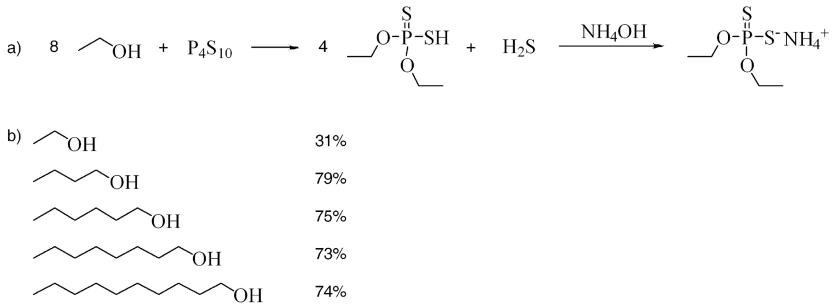

Figure 2. (a) Synthesis of dialkyldithiophosphates. The synthesis started with 8 equiv of an alcohol per 1 equiv of P_4S_{10}. The ammonium salt was isolated and could be converted into the protonated chemicals using dilute H_2SO_4. (b) Yields of the ammonium salts using different fatty alcohols.

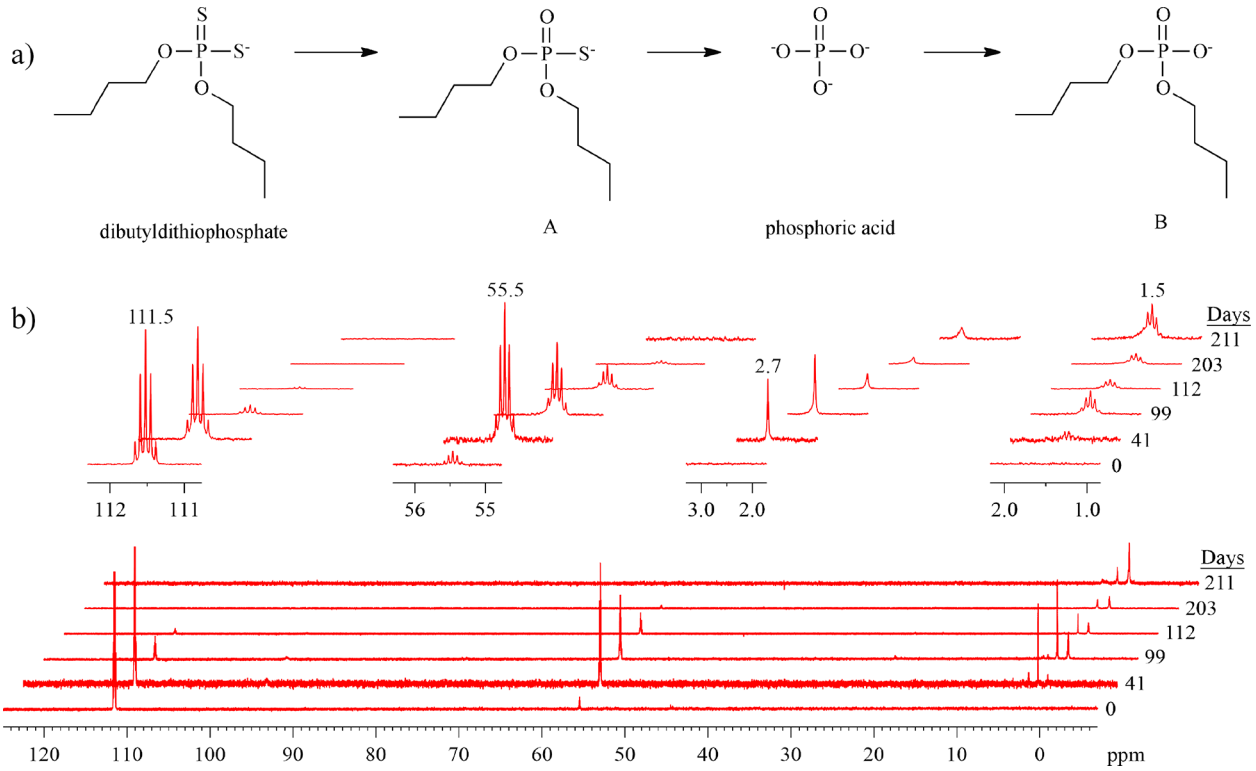

Figure 3. (a) Pathway for the hydrolysis of dibutyldithiophosphate in 90% H_2O/10% D_2O at 85 °C. (b) ^{31}P NMR spectra of the hydrolysis of dibutyldithiophosphate in 90% H_2O/10% D_2O at 85 °C on different days.

RESULTS AND DISCUSSION

Synthesis and Stability of Dialkyldithiophosphates. Dialkyldithiophosphates were readily synthesized from alcohols and commercially available P_4S_{10} using a method reported in the literature (Figure 2).[48,53,55] The dialkyldithiophosphates were not isolated; rather, they were converted to the ammonium salts and isolated by crystallization. This reaction sequence was used to synthesize dialkyldithiophosphate salts using ethanol, butanol, hexanol, octanol, decanol, and dodecanol, and the isolated yields of the salts are shown in Figure 2b.

The salts were isolated rather than the protonated dialkyldithiophosphates for two reasons. First, the salts were easy to purify by crystallization. Second, the salts were stable at room temperature, but the protonated dialkyldithiophosphates were not. Samples of the ammonium salt of dibutyldithiophosphate and the protonated acid of this chemical were placed in separate vials without any solvent and stored at room temperature for 41 days. The salt was a solid, and the protonated dibutyldithiophosphate was a viscous liquid. These samples were characterized by ^{31}P NMR spectroscopy at days 0 and 41. The salt showed no degradation after 41 days, but the protonated dibutyldithiophosphate hydrolyzed by 35% over this time period (Figures S1 and S2).

Hydrolysis of Dibutyldithiophosphate Monitored by ^{31}P NMR Spectroscopy. There are numerous methods to monitor the release of H_2S, such as the use of gas chromatography, HPLC, H_2S electrodes, and fluorescent probes and the formation of tin sulfide.[56−62] Recent work has focused on the development of probes that strongly fluoresce after reacting with H_2S and that can work in cellular environments. We chose to investigate the hydrolysis of dibutyldithiophosphate by ^{31}P NMR spectroscopy and by use of a H_2S electrode. ^{31}P NMR spectroscopy was chosen because it provided the most quantitative data about the long-term hydrolysis of dibutyldithiophosphate in a range of solvents and at a range of temperatures. Furthermore, ^{31}P NMR spectroscopy allowed the rate constants for the degradation of

DOI: 10.1021/acs.jafc.9b04398
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

dibutyldithiophosphate to be extracted and its final degradation products to be identified. ^{31}P NMR spectroscopy did not directly measure the release of H_2S, because it did not measure the concentration of H_2S, but an H_2S electrode was used for those measurements. The H_2S electrode was chosen because it provided real time data about the concentration of H_2S for numerous hours and was simple to set up.

The degradation of dibutyldithiophosphate at room temperature was followed by ^{31}P NMR spectroscopy in water and in $CDCl_3$. The hydrolysis of dibutyldithiophosphate in 90% H_2O/10% D_2O at room temperature was slow, as determined by ^{31}P NMR spectroscopy. At concentrations of 0.20 and 0.52 M, less than 3% of dibutyldithiophosphate had hydrolyzed after 35 days, as shown by ^{31}P NMR spectroscopy. This result was similar to the slow hydrolysis of GYY-4137 at room temperature. In prior work by us and others, we demonstrated that less than 3% of GYY-4137 hydrolyzed after 35 days, but its hydrolysis was faster in organic solvents. In DMSO-d_6, 74% of GYY-4137 had hydrolyzed using residual water after 7 days, and in $CDCl_3$, GYY-4137 had degraded by 50% using residual water after 13 days.[42] To investigate whether the hydrolysis of dibutyldithiophosphate was strongly affected by its local environment, its hydrolysis was measured in $CHCl_3$ by ^{31}P NMR spectroscopy. After 29 days, 15% of dibutyldithiophosphate had hydrolyzed using residual water, which demonstrated that the local environment affects the rate of hydrolysis of dibutyldithiophosphate (Figure S3).

The hydrolysis of dibutyldithiophosphate was investigated in 90% H_2O/10% D_2O at 85 °C by ^{31}P NMR spectroscopy to measure its rate of hydrolysis (Figure 3). This temperature was chosen because the hydrolysis was very slow at room temperature, and it was the highest temperature we felt could be used accurately without evaporation of solvent from the NMR tube. Even at this elevated temperature, the reaction was followed for 211 days. As expected on the basis of literature precedent, dibutyldithiophosphate was a pentet in the ^{31}P NMR spectrum at 111.5 ppm. The hydrolysis was slow, and it took 47 days for 50% of dibutyldithiophosphate to hydrolyze to release 1 equiv of H_2S and chemical A, which was characterized in prior work. Dibutyldithiophosphate did not completely disappear from the ^{31}P NMR spectra until after 112 days, and chemical A did not disappear until after 211 days. The next peak that appeared in the ^{31}P NMR spectra had a shift at 2.7 ppm and was due to phosphoric acid. The identity of phosphoric acid as the peak at 2.7 ppm was confirmed by the addition of phosphoric acid at the completion of the experiment (Figure S4). This addition of phosphoric acid increased the intensity of the peak assigned to phosphoric acid and no new peaks were observed. Although the hydrolysis of chemical A to phosphoric acid required the release of two butanols and 1 equiv of H_2S, no other intermediates were observed.

As the reaction proceeded, a new peak at 1.5 ppm appeared and grew in intensity. This peak was a pentet, was labeled chemical B in Figure 3, and slowly grew in intensity at the expense of phosphoric acid. The structure of the chemical responsible for chemical B was hypothesized to be the two butanols bonded to the phosphorus because of the splitting pattern of the peak at 1.5 ppm. This hypothesis was tested by dissolving phosphoric acid in water and adding 20 equiv of butanol. After heating at 85 °C for 97 days, the peak due to chemical B appeared, which demonstrated that butanol could cleanly react with phosphoric acid to produce chemical B (Figure S5).

The rate constant for the disappearance of dibutyldithiophosphate was measured (Figure 4). The plot of the

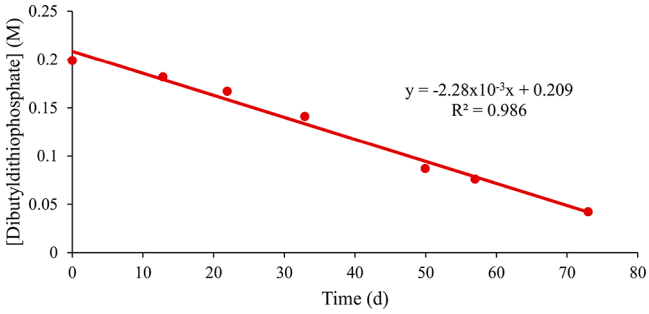

Figure 4. Hydrolysis of dibutyldithiophosphate following zero-order kinetics.

concentration of dibutyldithiophosphate versus time fit a straight line and followed zero-order kinetics because it was self-acid catalyzed. The rate constant for the disappearance of dibutyldithiophosphate was 2.28×10^{-3} M/h. We attempted to measure the rate constant for the disappearance of chemical A, but because it was an intermediate and its concentration was low, the data were too noisy to accurately measure it.

Hydrolysis of GYY-4137 Monitored by ^{31}P NMR Spectroscopy. Despite the widespread use of GYY-4137 in medicinal and agricultural studies, its rate of hydrolysis in water has not been reported because of its very slow kinetics of hydrolysis at room temperature. Here, we report the hydrolysis of GYY-4137 in 90% H_2O/10% D_2O at 85 °C and compare its rate of hydrolysis with that of dibutyldithiophosphate.

The ^{31}P NMR spectra of GYY-4137 were obtained periodically, and representative spectra are shown in Figure 5. The peak for GYY-4137 at 89.5 ppm was broad because of coupling with six hydrogens, and it disappeared within 2 days. A triplet at 74.5 ppm (chemical C in Figure 6b) grew at the expense of the GYY-4137 peak and slowly hydrolyzed over weeks. The identity of chemical C was confirmed by isolation of this chemical and its full characterization by ^{13}C, ^{31}P, and ^{1}H NMR spectroscopy as well as high resolution mass spectroscopy. The details of the full characterization of chemical C are in the Supporting Information (Figure S6). A small triplet at 47.2 ppm (chemical D) was observed as an intermediate to the final triplet peak at 12.4 ppm (chemical E). Chemical D was only a small peak in the ^{31}P NMR spectra and could not be isolated despite several attempts. The identity of chemical E was confirmed by comparison to reported spectra for this known chemical.

In our prior paper, the hydrolysis of GYY-4137 in DMSO-d_6 at room temperature was shown to follow the pathway in Figure 6a, but in H_2O/D_2O at 85 °C it followed the pathway shown in Figure 6b.[3] The biggest difference in mechanisms was that in water at 85 °C, the first hydrolysis event was loss of morpholine, but in DMSO-d_6, it was loss of a sulfur.

The kinetics for the disappearance of GYY-4137 followed pseudo-first-order kinetics with a rate constant of 9.72×10^{-2} h^{-1} (Figure 7a). The loss of morpholine was rapid, and as calculated, 50% of GYY-4137 had hydrolyzed to intermediate C in 5.2 h. The hydrolysis of intermediate C to release H_2S was slower and followed pseudo-first-order reaction kinetics with a rate constant of -2.59×10^{-3} h^{-1} (Figure 7b).

DOI: 10.1021/acs.jafc.9b04398
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

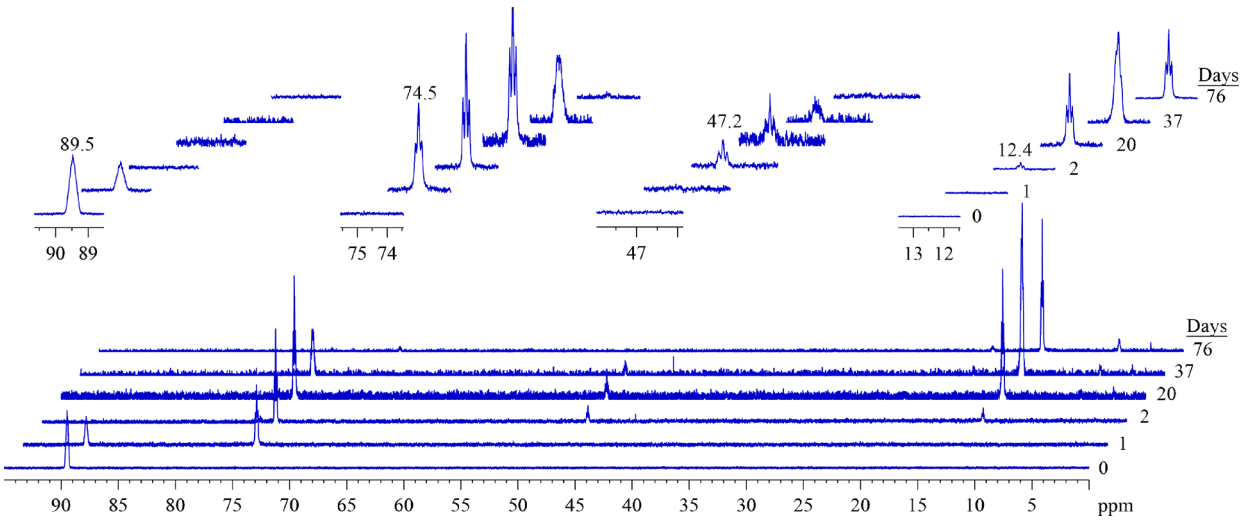

Figure 5. ^{31}P NMR spectra of the hydrolysis of GYY-4137 in water at 85 °C.

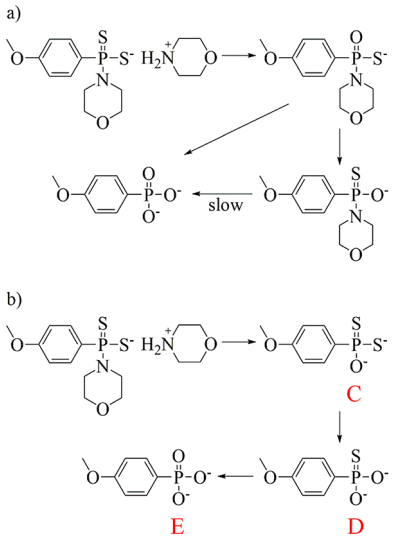

Figure 6. Hydrolysis of GYY-4137 (a) in DMSO-d_6 and (b) in water at 85 °C.

A direct comparison of the rates of release of H_2S by GYY-4137 and dibutyldithiophosphate is complicated by the differences in how they degrade. The kinetics of hydrolysis of dibutyldithiophosphate followed zero-order kinetics, and it took approximately 47 days for half of the dibutyldithiophosphate to release H_2S and form chemical A. It took approximately 13 days for GYY-4137 to release half of the first equivalent of H_2S. This demonstrated that the release of H_2S from GYY-4137 was faster than the release from dibutyldithiophosphate at 85 °C but that these rates were comparable.

Hydrolysis of Dialkyldithiophosphates Measured by a H_2S Electrode. The release of H_2S was monitored using a previously calibrated H_2S electrode that continuously reported the concentration of H_2S (Figure 8). In each of these experiments, the H_2S electrode was immersed in Bis-Tris buffer at a pH of 6.7 for 1 h, and then a dialkyldithiophosphate salt was added, as reported in Figure 8. The concentration of H_2S quickly rose after the addition of each dialkyldithiophosphate and then was mostly constant. The concentrations of H_2S were approximately 15, 6, and 1 μM for 0.25 M diethyldithiophosphate, dibutyldithiophosphate, and dihexyldithiophosphate, respectively; at these concentrations diethyldithiophosphate and dibutyldithiophosphate were fully dissolved, but dihexyldithiophosphate was only partially dissolved. This result demonstrated that the structures of the dialkyldithiophosphates can be used to control the rate of release of H_2S. An important result in these experiments was that the concentrations of H_2S were nearly 5 orders of magnitude lower than the concentrations of the dialkyldithiophosphates,

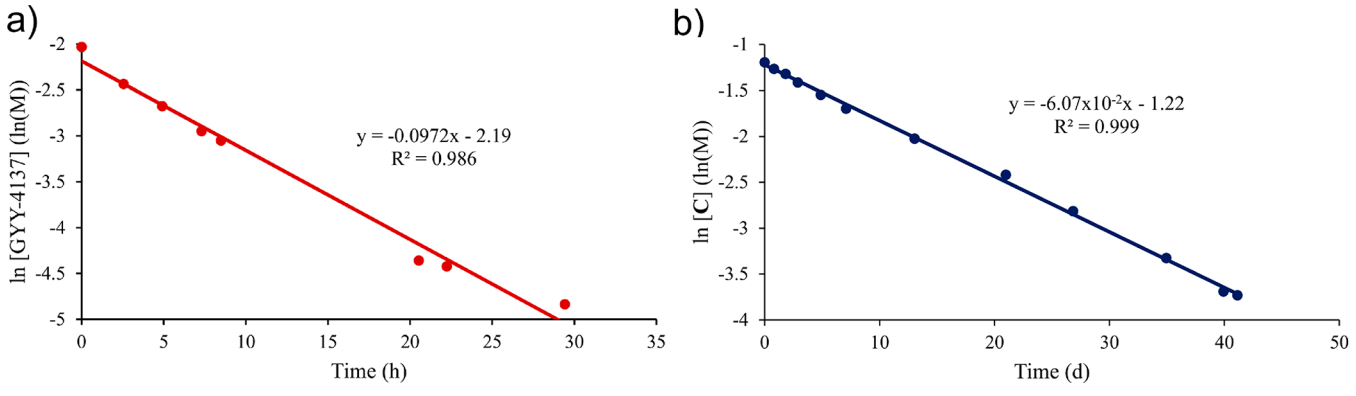

Figure 7. Kinetics of the disappearance of (a) GYY-4137 and (b) chemical C.

DOI: 10.1021/acs.jafc.9b04398
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

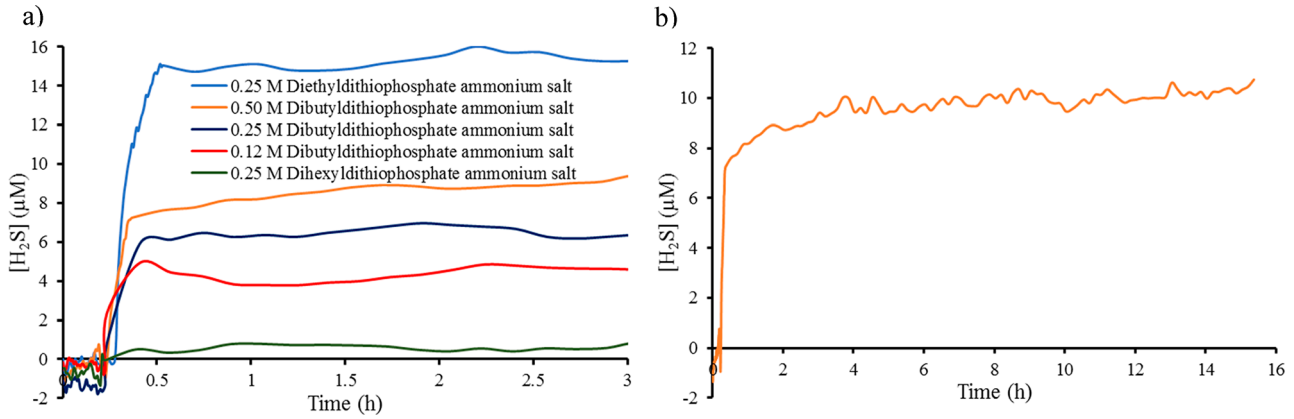

Figure 8. (a) Concentrations of H_2S from different concentrations of dialkyldithiophosphates. (b) Concentration of H_2S from 0.50 M dibutyldithiophosphate. The concentrations of H_2S were found using an H_2S electrode.

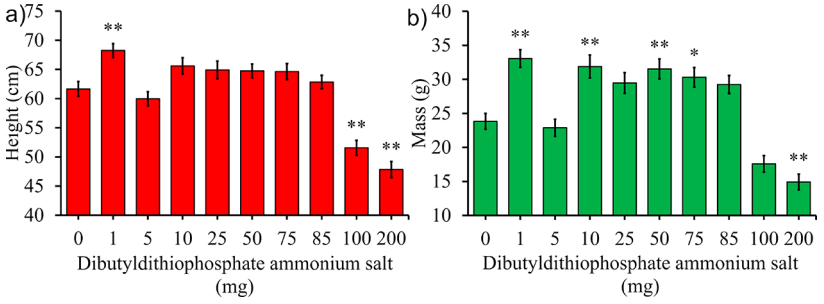

Figure 9. Average (a) heights and (b) masses of corn plants grown with different loadings of dibutyldithiophosphate ammonium salt added near the seeds and then watered with tap water for 4 weeks. Error bars are ±SE. Groups labeled with a unique number are statistically significant via the Tukey HSD test with $\alpha < 0.05$.

which was consistent with the ^{31}P NMR spectroscopy results that showed very slow hydrolysis of these chemicals at room temperature.

Figure 8a also shows that the concentration of H_2S increased as the concentration of dibutyldithiophosphate increased from 0.12 to 0.50 M, but it was not a linear increase because the solubility limit of dibutyldithiophosphate was 0.28 M, so the sample at 0.50 M had solid dibutyldithiophosphate salt at the bottom of the apparatus. The hydrolysis of dibutyldithiophosphate was followed for 16 h to demonstrate the consistent release of H_2S (Figure 8b).

The amounts of H_2S released from dialkyldithiophosphates synthesized with fatty alcohols with six carbons or more were low because of their poor solubility in water. The solubility limit of dihexyldithiophosphate was 0.13 M, which is lower than the concentration of 0.25 M used in Figure 8a. No detectable amounts of H_2S were measured from aqueous solutions of dioctyldithiophosphate and didodecyldithiophosphate because these chemicals were mostly insoluble in water (Figures S7 and S8).

A comparison of the concentrations of H_2S measured by an H_2S electrode for GYY-4137 and dibutyldithiophosphate revealed that they had similar values for the steady state concentration of H_2S. In prior work, we reported that the concentration of H_2S was 3 μM at 0.12 M GYY-4137, which was similar to the value of 6 μM for 0.12 M dibutyldithiophosphate.[3]

Growth of Maize Using the Dibutyldithiophosphate Ammonium Salt. GYY-4137 has been used extensively in agriculture to investigate the effects of a slow H_2S releasing chemical on the germination of seeds, the first couple weeks of growth of plants, and the survival of plants in the presence of environmental stressors. We hypothesized that dialkyldithiophosphates could also be used in agricultural studies, and they would offer a method to control the rate of release of H_2S and degrade to release natural chemicals.

To investigate possible applications of dialkyldithiophosphates in agriculture, different loadings of dibutyldithiophosphate ammonium salt were used to grow corn. Corn seeds were planted in individual pots and different amounts of dibutyldithiophosphate ammonium salt were added to the soil adjacent to the seed at day 0. We planted 50 seeds for each of the concentrations of dibutyldithiophosphate ammonium salt shown in Figure 9 for a total of 500 corn seeds. The seeds were watered daily with tap water and grown for 4 weeks. The pH of the soil was not expected to change because complete hydrolysis of the potassium salt of dialkyldithiophosphate would yield the monopotassium salt of phosphoric acid, which is often used as a neutral pH buffer. The heights of the plants were measured by straightening the leaves and measuring from the base of each plant to the top of the longest leaf (Figure 9a). The plants were harvested by cutting at the base of the plant (i.e., removing the roots as shown in Figure S30), and each plant was weighed (Figure 9b).

The results in Figure 9 show that dibutyldithiophosphate has a strong effect on the growth of corn plants. The weights of the plants showed statistically significant improvements at 1, 10, 50, and 75 mg loadings of dibutyldithiophosphate, and there were improvements in their weights for all loadings except 100 and 200 mg. The weight of the corn plants increased by 39% when only 1 mg of the dibutyldithiophosphate salt was added to the soil adjacent to the seed compared with the weight of

DOI: 10.1021/acs.jafc.9b04398
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

the plants grown in the absence of the dibutyldithiophosphate salt. Importantly, 1 mg of this salt releases only 0.28 mg of H_2S. These results demonstrated that milligram amounts of H_2S delivered over 4 weeks could have a strongly positive effect on the weight of corn plants. Not surprisingly, few statistically significant differences were seen for the heights of the plants. The heights of the plants report one dimension of the overall sizes of the plants, but small differences in heights can lead to larger differences in weights.

The plants at 5 mg loadings of dibutyldithiophosphate salt had similar heights and weights to those of the control plants, and we are unsure of the reason for the small dips in the graphs. Prior work by others has shown that the response of plants to exogenous H_2S has a bell-shaped curve without a dip in the effect at intermediate loadings, but in our prior work, we observed dips in the responses of radish, pea, and lettuce plants at intermediate loadings of GYY-4137.[3] This work suggests that the response of plants to H_2S may be more complex than previously reported. Future work will investigate whether these dips are real or artifacts of the experiments.

These results are consistent with prior reports for how aqueous H_2S or GYY-4137 affected maize.[15,63−67] For instance, in a paper from 2014, exposure of 2.5 day old maize seedlings to 0, 100, 500, 1000, or 5000 μM GYY-4137 for 6 h followed by heat stress of 48 °C for 18 h resulted in low survival rates. The seedlings exposed to no GYY-4137 had a survival rate of only 31%, but those exposed to 100, 500, 1000, and 5000 μM GYY-4137 had survival rates of 69, 80, 57, and 39%, respectively.[63] This work and others demonstrated that the application of GYY-4137 or aqueous H_2S resulted in bell curve responses for maize and other plants. Too much GYY-4137 or aqueous H_2S was bad for plants, but intermediate loadings led to positive effects. These results were observed in our paper: at high loadings of 100 and 200 mg of dibutyldithiophosphate, the maize plants had lower weights and heights than plants not exposed to dibutyldithiophosphate. At intermediate loadings of 1 to 85 mg of dibutyldithiophosphate, the effect was positive, except for a dip in the mass of the maize plants at 5 mg loadings.

This paper describes the synthesis and characterization of a new series of chemicals that slowly release hydrogen sulfide. GYY-4137 is commonly used in agricultural studies, but its rate of hydrolysis cannot be easily varied by altering its structure, and it releases chemicals not found in the environment. Dialkyldithiophosphates are an advance in this field because they possess different rates of hydrolysis that depend on the alcohols used in their synthesis. The kinetics of the rates of hydrolysis of dibutyldithiophosphate were measured in water at 85 °C and compared with the kinetics for the rates of hydrolysis of GYY-4137. The rates were similar for both chemicals, and importantly, dibutyldithiophosphate degraded to release fatty alcohols and phosphoric acid, which are safe, natural chemicals. Although the dialkyldithiophosphates synthesized from octanol and longer alcohols were very poorly soluble in water, we believe they may be important because they may partition into hydrophobic subcellular parts of plant cells and complement the numerous hydrophilic chemicals already reported that release H_2S. These three characteristics of dialkyldithiophosphates, (1) the ability to tune their hydrophobicity, (2) the ability to tune their rates of release of H_2S, and (3) the biocompatibility of their degradation products, are important advances in the field of H_2S in agriculture.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.9b04398.

NMR spectra of the chemicals and measurement of the slow release of H_2S using an electrode (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: ned-bowden@uiowa.edu.

ORCID

Justin M. Carter: 0000-0002-2119-2166
Eric M. Brown: 0000-0001-7872-1658
Erin E. Irish: 0000-0003-4244-7938
Ned B. Bowden: 0000-0003-1628-8515

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors would like to acknowledge USDA NIFA-2018-67030-27352, NSF-PFI-1827336, and the Leopold Center for Sustainable Agriculture for funding.

■ REFERENCES

(1) Thompson, C. R.; Kats, G.; Lennox, R. W. Effects of fumigating crops with hydrogen sulfide or sulfur dioxide. *Calif. Agric.* **1979**, *33* (3), 9−10.

(2) Thompson, C. R.; Kats, G. Effects of continuous H_2S fumigation on crop and forest plants. *Environ. Sci. Technol.* **1978**, *12*, 550−553.

(3) Carter, J. M.; Brown, E. M.; Grace, J. P.; Salem, A. K.; Irish, E. E.; Bowden, N. B. Improved growth of pea, lettuce, and radish plants using the slow release of hydrogen sulfide from GYY-4137. *PLoS One* **2018**, *13*, No. e0208732.

(4) Cheng, T.; Shi, J.; Dong, Y.; Ma, Y.; Peng, Y.; Hu, X.; Chen, J. Hydrogen sulfide enhances poplar tolerance to high-temperature stress by increasing S-nitrosoglutathione reductase (GSNOR) activity and reducing reactive oxygen/nitrogen damage. *Plant Growth Regul.* **2018**, *84*, 11−23.

(5) Rennenberg, H.; Arabatzis, N.; Grundel, I. Cysteine desulphyactivity in higher plants: Evidence for the action of L- and D-cysteine specific enzymes. *Phytochemistry* **1987**, *26*, 1583−1589.

(6) Bloem, E.; Riemenschneider, A.; Voker, J.; Jutta, P.; Schmidt, A.; Salac, I.; Haneklaus, S.; Schnug, E. Sulphur supply and infection wiht Pyrenopeziza brassicae influence L-cystein desulphydrase activity in brassica napus L. *J. Exp. Bot.* **2004**, *55*, 2305−2312.

(7) Riemenschneider, A.; Wegele, R.; Schmidt, A.; Papenbrock, J. Isolation and characterization of a D-cysteine desulfydrase protein from arabidopsis thaliana. *FEBS J.* **2005**, *272*, 1291−1304.

(8) Guo, H.; Xiao, T.; Zhou, H.; Xie, Y.; Shen, W. Hydrogen sulfide: a versatile regulator of environmental stress in plants. *Acta Physiol. Plant.* **2016**, *38* (1), 16.

(9) Shi, H.; Ye, T.; Chan, Z. Exogenous application of hydrogen sulfide donor sodium hydrosulfide enhanced multiple abiotic stress tolerance in bermudagrass (Cynodon dactylon (L). Pers.). *Plant Physiol. Biochem.* **2013**, *71*, 226−234.

(10) Hu, L.-Y.; Hu, S.-L.; Wu, J.; Li, Y.-H.; Zheng, J.-L.; Wei, Z.-J.; Liu, J.; Wang, H.-L.; Liu, Y.-S.; Zhang, H. Hydrogen sulfide prolongs postharvest shelf-life of strawberry and plays an antioxidant role in fruits. *J. Agric. Food Chem.* **2012**, *60*, 8684−8693.

(11) Li, Z.-R.; Hu, K.-D.; Zhang, F.-Q.; Li, S.-P.; Hu, L.-Y.; Li, Y.-H.; Wang, S.-H.; Zhang, H. Hydrogen sulfide alleviates dark-promoted senescence in postharvest broccoli. *HortScience* **2015**, *50* (3), 416−420.

(12) Li, S.-P.; Hu, K.-D.; Hu, L.-Y.; Li, Y.-H.; Jiang, A.-M.; Xiao, F.; Han, Y.; Liu, Y.-S.; Zhang, H. Hydrogen sulfide alleviates postharvest

DOI: 10.1021/acs.jafc.9b04398
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

senescence of broccoli by modulating antioxidant defense and senescence-related gene expression. *J. Agric. Food Chem.* **2014**, *62*, 1119−1129.
(13) Christou, A.; Manganaris, G. A.; Papadopoulos, I.; Fotopoulos, V. Hydrogen sulfide induces systemic tolerance to salinity and non-ionic osmotic stress in strawberry plants through modification of reactive species biosynthesis and transcriptional regulation of multiple defence pathways. *J. Exp. Bot.* **2013**, *64* (7), 1953−1966.
(14) Duan, B.; Ma, Y.; Jiang, M.; Yang, Y.; Ni, L.; Lu, W. Improvement of photosynthesis in rice (Oryza sativa L.) as a result of an increase in stomatal aperture and density of exogenous hydrogen sulfide treatment. *Plant Growth Regul.* **2015**, *75*, 33−44.
(15) Li, Z.-G.; Ding, X.-J.; Du, P.-F. Hydrogen sulfide donor sodium hydrosulfide-improved heat tolerance in maize and involvement of proline. *J. Plant Physiol.* **2013**, *170*, 741−747.
(16) Li, Z. G. Hydrogen sulfide: a multifunctional gaseous molecule in plants. *Russ. J. Plant Physiol.* **2013**, *60*, 733−740.
(17) Alvarez, C.; Calo, L.; Romero, L. C.; Garcia, I.; Gotor, C. An O-acetylserine(thiol)lyase homolog with L-cysteine desulfhydrase activity regulates cysteine homeostasis in Arabidopsis. *Plant Physiol.* **2010**, *152* (2), 656−669.
(18) Bloem, E.; Rubekin, K.; Haneklaus, S.; Banfalvi, Z.; Hesse, H.; Schnug, E. H_2S and COS gas exchange of transgenic potato lines with modified expression levels of enzymes involved in sulphur metabolism. *J. Agron. Crop Sci.* **2011**, *197* (4), 311−321.
(19) Chen, J.; Wu, F.-H.; Wang, W.-H.; Zheng, C.-J.; Lin, G.-H.; Dong, X.-J.; He, J.-X.; Pei, Z.-M.; Zheng, H.-L. Hydrogen sulphide enhances photosynthesis through promoting chloroplast biogenesis, photosynthetic enzyme expression, and thiol redox modification in Spinacia oleracea seedlings. *J. Exp. Bot.* **2011**, *62* (13), 4481−4493.
(20) Christou, A.; Manganaris, G. A.; Papadopoulos, I.; Fotopoulos, V. Hydrogen sulfide induces systemic tolerance to salinity and non-ionic osmotic stress in strawberry plants through modification of reactive species biosynthesis and transcriptional regulation of multiple defence pathways. *J. Exp. Bot.* **2013**, *64* (7), 1953−1966.
(21) Fang, T.; Cao, Z.; Li, J.; Shen, W.; Huang, L. Auxin-induced hydrogen sulfide generation is involved in lateral root formation in tomato. *Plant Physiol. Biochem.* **2014**, *76*, 44−51.
(22) Fu, P.; Wang, W.; Hou, L.; Liu, X. Hydrogen sulfide is involved in the chilling stress response in Vitis vinfera L. *Acta Soc. Bot. Polym.* **2013**, *82* (4), 295−302.
(23) Gao, S.-P.; Hu, K.-D.; Hu, L.-Y.; Li, Y.-H.; Han, Y.; Wang, H.-L.; Lv, K.; Liu, Y.-S.; Zhang, H. Hydrogen sulfide delays postharvest senescence and plays an antioxidative role in fresh-cut kiwifruit. *HortScience* **2013**, *48* (11), 1385−1392.
(24) Garcia-Mata, C.; Lamattina, L. Hydrogen sulphide, a novel gasotransmitter involved in guard cell signalling. *New Phytol.* **2010**, *188* (4), 977−984.
(25) Hou, Z.; Wang, L.; Liu, J.; Hou, L.; Liu, X. Hydrogen sulfide regulates ethylene-induced stomatal closure in Arabidopsis thaliana. *J. Integr. Plant Biol.* **2013**, *55* (3), 277−289.
(26) Hu, L.-Y.; Hu, S.-L.; Wu, J.; Li, Y.-H.; Zheng, J.-L.; Wei, Z.-J.; Liu, J.; Wang, H.-L.; Liu, Y.-S.; Zhang, H. Hydrogen sulfide prolongs postharvest shelf life of strawberry and plays an antioxidative role in fruits. *J. Agric. Food Chem.* **2012**, *60* (35), 8684−8693.
(27) Krasensky, J.; Jonak, C. Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. *J. Exp. Bot.* **2012**, *63* (4), 1593−1608.
(28) Li, S.-P.; Hu, K.-D.; Hu, L.-Y.; Li, Y.-H.; Jiang, A.-M.; Xiao, F.; Han, Y.; Liu, Y.-S.; Zhang, H. Hydrogen sulfide alleviates postharvest senescence of broccoli by modulating antioxidant defense and senescence-related gene expression. *J. Agric. Food Chem.* **2014**, *62* (5), 1119−1129.
(29) Li, Z.-G.; Gong, M.; Liu, P. Hydrogen sulfide is a mediator in H_2O_2-induced seed germination in Jatropha Curcas. *Acta Physiol. Plant.* **2012**, *34* (6), 2207−2213.
(30) Lin, Y.-T.; Li, M.-Y.; Cui, W.-T.; Lu, W.; Shen, W.-B. Haem oxygenase-1 is involved in hydrogen sulfide-induced cucumber adventitious root formation. *J. Plant Growth Regul.* **2012**, *31* (4), 519−528.
(31) Liu, J.; Hou, L. X.; Liu, G. H.; Liu, X.; Wang, X. C. Hydrogen sulfide induced by nitric oxide mediates ethylene-induced stomatal closure of Arabidopsis thaliana. *Chin. Sci. Bull.* **2011**, *56* (33), 3547−3553.
(32) Papenbrock, J.; Riemenschneider, A.; Kamp, A.; Schulz-Vogt, H. N.; Schmidt, A. Characterization of cysteine-degrading and H_2S-releasing enzymes of higher plants - from the field to the test tube and back. *Plant Biol.* **2007**, *9* (5), 582−588.
(33) Shi, H.; Ye, T.; Chan, Z. Nitric oxide-activated hydrogen sulfide is essential for cadmium stress response in bermudagrass (Cynodon dactylon (L). Pers.). *Plant Physiol. Biochem.* **2014**, *74*, 99−107.
(34) Sun, Y.; Luo, W. Effects of exogenous hydrogen sulphide on the seed germination of pumpkin under NaCl stress. *J. Food, Agric. Environ.* **2013**, *11* (3), 1097−1100.
(35) Wang, B.-L.; Shi, L.; Li, Y.-X.; Zhang, W.-H. Boron toxicity is alleviated by hydrogen sulfide in cucumber (Cucumis sativus L.) seedlings. *Planta* **2010**, *231* (6), 1301−1309.
(36) Yadav, S. K. Heavy metals toxicity in plants: an overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. *S. Afr. J. Bot.* **2010**, *76* (2), 167−179.
(37) Zhang, H.; Hu, S.-L.; Zhang, Z.-J.; Hu, L.-Y.; Jiang, C.-X.; Wei, Z.-J.; Liu, J.; Wang, H.-L.; Jiang, S.-T. Hydrogen sulfide acts as a regulator of flower senescence in plants. *Postharvest Biol. Technol.* **2011**, *60* (3), 251−257.
(38) Zhang, H.; Tang, J.; Liu, X.-P.; Wang, Y.; Yu, W.; Peng, W.-Y.; Fang, F.; Ma, D.-F.; Wei, Z.-J.; Hu, L.-Y. Hydrogen sulfide promotes roots organogenesis in Ipomoea batatas, Salix matsudana and Glycine max. *J. Integr. Plant Biol.* **2009**, *51* (12), 1086−1094.
(39) Zhang, H.; Ye, Y.-K.; Wang, S.-H.; Luo, J.-P.; Tang, J.; Ma, D.-F. Hydrogen sulfide counteracts chlorophyll loss in sweet potato seedling leaves and alleviates oxidative damage against osmotic stress. *Plant Growth Regul.* **2009**, *58* (3), 243−250.
(40) Hydrogen Sulfide. *Occupational Safety and Health Administration, U.S. Department of Labor*. https://www.osha.gov/SLTC/hydrogensulfide/hazards.html (accessed Oct 16, 2019).
(41) Kloesch, B.; Steiner, G.; Mayer, B.; Schmidt, K. Hydrogen sulfide inhibits endothelial nitric oxide formation and receptor ligand-mediated Ca^{2+} release in endothelial and smooth muscle cells. *Pharmacol. Rep.* **2016**, *68* (1), 37−43.
(42) Alexander, B. E.; Coles, S. J.; Fox, B. C.; Khan, T. F.; Maliszewski, J.; Perry, A.; Pitak, M. B.; Whiteman, M.; Wood, M. E. Investigating the generation of hydrogen sulfide from the phosphonamidodithioate slow-release donor GYY4137. *MedChemComm* **2015**, *6*, 1649−1655.
(43) Lisjak, M.; Teklic, T.; Wilson, I. D.; Wood, M. E.; Whiteman, M.; Hancock, J. T. Hydrogen sulfide effects on stomatal apertures. *Plant Signaling Behav.* **2011**, *6* (10), 1444−1446.
(44) Li, L.; Whiteman, M.; Guan, Y. Y.; Neo, K. L.; Cheng, Y.; Lee, S. W.; Zhao, Y.; Baskar, R.; Tan, C. H.; Moore, P. K. Characterization of a novel, water-soluble hydrogen sulfide-releasing molecule (GYY4137): new insights into the biology of hydrogen sulfide. *Circulation* **2008**, *117*, 2351−2360.
(45) Lee, Z. W.; Zhou, J.; Chen, C. S.; Zhao, Y.; Tan, C. H.; Li, L.; Moore, P. K.; Deng, L. W. The slow-releasing hydrogen sulfide donor, GYY4137, exhibits novel anti-cancer effects *in vitro* and *in vivo*. *PLoS One* **2011**, *6*, No. e21077.
(46) Feng, W.; Teo, X.-Y.; Novera, W.; Ramanujulu, P. M.; Liang, D.; Huang, D.; Moore, P. K.; Deng, L.-W.; Dymock, B. W. Discovery of new H2S releasing phosphordithioates and 2,3-dihydro-2-phenyl-2sulfanylenebenzo[d][1,3,2]oxazaphospholes with improved antiproliferative activity. *J. Med. Chem.* **2015**, *58*, 6456−6480.
(47) Burn, A. J.; Dewan, S. K.; Gosney, I.; Tan, P. S. G. Phosphorus-31 nuclear magnetic resonance studey of the mechanism and kinetics of the hydrolysis of zinc(II) O,O-diethyl dithiophoshate and some related compounds. *J. Chem. Soc., Perkin Trans. 2* **1990**, 753−758.
(48) Qu, J.; Barnhill, W. C.; Luo, H.; Meyer, H. M., III; Leonard, D. N.; Landauer, A. K.; Kheireddin, B.; Gao, H.; Papke, B. L.; Dai, S.

DOI: 10.1021/acs.jafc.9b04398
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

Synergistic Effects Between Phosphonium-Alkylphosphate Ionic Liquids and Zinc Dialkyldithiophosphate (ZDDP) as Lubricant Additives. *Adv. Mater. (Weinheim, Ger.)* **2015**, *27* (32), 4767−4774.
(49) Gosvami, N. N.; Bares, J. A.; Mangolini, F.; Konicek, A. R.; Yablon, D. G.; Carpick, R. W. Mechanisms of antiwear tribofilm growth revealed in situ by single-asperity sliding contacts. *Science (Washington, DC, U. S.)* **2015**, *348* (6230), 102−106.
(50) Fujita, H.; Glovnea, R. P.; Spikes, H. A. Study of Zinc Dialkyldithiophosphate Antiwear Film Formation and Removal Processes, Part I: Experimental. *Tribol. Trans.* **2005**, *48* (4), 558−566.
(51) Chauhan, H. P. S. Chemistry of diorganodithiophosphate (and phosphinate) derivatives with arsenic, antimony and bismuth. *Coord. Chem. Rev.* **1998**, *173*, 1−30.
(52) Willermet, P. A.; Dailey, D. P.; Carter, R. O., III; Schmitz, P. J.; Zhu, W. Mechanism of formation of antiwear films from zinc dialkyldithiophosphates. *Tribol. Int.* **1995**, *28* (3), 177−87.
(53) Cote, G.; Bauer, D. Hydrolysis of the O,O-dialkyl phosphorodithoic acids used as extractants in liquid-liquid systems. *Anal. Chem.* **1984**, *56*, 2153−2157.
(54) Bode, H.; Arnswald, W. Bildung der metall-diathyldithiophosphate und ihre extraheirbarkeit aus mineralsauren losengen. *Fresenius' Z. Anal. Chem.* **1961**, *185*, 99−100.
(55) Ozturk, T.; Ertas, E.; Mert, O. A Berzelius reagent, phosphorus decasulfide (P4S10), in organic synthesis. *Chem. Rev.* **2010**, *110*, 3419−3478.
(56) Mittapelli, L. L.; Nawale, G. N.; Gholap, S. P.; Varghese, O. P.; Gore, K. R. A turn-on fluorescent GFP chromophore analog for highly selective and efficient detection of H2S in aqueous and in living cells. *Sens. Actuators, B* **2019**, *298*, 126875.
(57) Jia, X.; Li, W.; Guo, Z.; Guo, Z.; Li, Y.; Zhang, P.; Wei, C.; Li, X. An NBD-Based Mitochondrial Targeting Ratiometric Fluorescent Probe for Hydrogen Sulfide Detection. *ChemistrySelect* **2019**, *4* (29), 8671−8675.
(58) Lin, V. S.; Chen, W.; Xian, M.; Chang, C. J. Chemical probes for molecular imaging and detection of hydrogen sulfide and reactive sulfur species in biological systems. *Chem. Soc. Rev.* **2015**, *44* (14), 4596−4618.
(59) Montoya, L. A.; Pluth, M. D. Selective turn-on fluorescent probes for imaging hydrogen sulfide in living cells. *Chem. Commun. (Cambridge, U. K.)* **2012**, *48* (39), 4767−4769.
(60) Sasakura, K.; Hanaoka, K.; Shibuya, N.; Mikami, Y.; Kimura, Y.; Komatsu, T.; Ueno, T.; Terai, T.; Kimura, H.; Nagano, T. Development of a Highly Selective Fluorescence Probe for Hydrogen Sulfide. *J. Am. Chem. Soc.* **2011**, *133* (45), 18003−18005.
(61) Liu, C.; Pan, J.; Li, S.; Zhao, Y.; Wu, L. Y.; Berkman, C. E.; Whorton, A. R.; Xian, M. Capture and Visualization of Hydrogen Sulfide by a Fluorescent Probe. *Angew. Chem., Int. Ed.* **2011**, *50* (44), 10327−10329.
(62) Lippert, A. R.; New, E. J.; Chang, C. J. Reaction-Based Fluorescent Probes for Selective Imaging of Hydrogen Sulfide in Living Cells. *J. Am. Chem. Soc.* **2011**, *133* (26), 10078−10080.
(63) Li, Z.-G.; Yi, Z.-Y.; Li, Y.-T. Effect of pretreatment with hydrogen sulfide donor sodium hydrosulfide on heat tolerance in relation to antioxidant system in maize (Zea mays) seedlings. *Biologia* **2014**, *69*, 1001−1009.
(64) Li, Z.-G.; Yang, S.-Z.; Long, W.-B.; Yang, G.-X.; Shen, Z.-Z. Hydrogen sulfide may be a novel downstream signal molecule in nitric oxide-induded heat tolerance of maize (Zea mays L.) seedlings. *Plant, Cell Environ.* **2013**, *36*, 1564−1572.
(65) Dooley, F. D.; Nair, S. P.; Ward, P. D. Increased growth and germination success in plants following hydrogen sulfide administration. *PLoS One* **2013**, *8* (4), No. e62048.
(66) Peng, R.; Bian, Z.; Zhou, L.-G.; Cheng, W.; Hai, N.; Yang, C.; Yang, T.; Wang, X.; Wang, C. Hydrogen sulfide enhances nitric oxide-induced tolerance of hypoxia in maize (*Zea mays* L.). *Plant Cell Rep.* **2016**, *35*, 2325−2340.
(67) Chen, J.; Wu, F.-H.; Shang, Y.-T.; Wang, W.-H.; Hu, W.-J.; SImon, M.; Liu, X.; Shangguan, Z.-P.; Zheng, H.-L. Hydrogen sulfide improves adaptation of Zea mays seedlings to iron deficiency. *J. Exp. Bot.* **2015**, *66*, 6605−6622.

DOI: 10.1021/acs.jafc.9b04398
J. Agric. Food Chem. XXXX, XXX, XXX−XXX