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Safener Benoxacor Induces Indirect Photolysis of Herbicides Metolachlor Under Sunlight

Leandra Marie Caywood

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Safener Benoxacor Induces Indirect Photolysis of Herbicide Metolachlor Under Sunlight

by

Leandra Marie Caywood

An Honors Capstone

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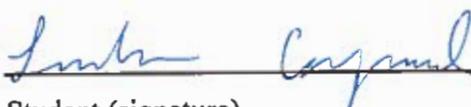
for the Honors Diploma

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of

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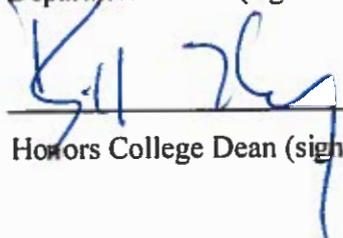
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Dedication: To my mom, who still proofreads all of my papers.

Abstract

Benoxacor, a safener, is available in a formulation with herbicide *S*-metolachlor that is commonly applied pre-emergence to crops such as corn. Benoxacor protects the crop from the active ingredient of the herbicide (metolachlor) while still allowing the herbicide to kill weeds. In this work, the decay of metolachlor and benoxacor in simulated sunlight was studied, both separately under direct photolysis and in mixtures under indirect photolysis. Experiments on a quartz surface and in water were performed. Metolachlor was shown to be stable under sunlight, as suggested by its molar absorptivity in the solar spectrum, while benoxacor was shown to decay rapidly under direct photolysis both in water and on the surface. In water samples, benoxacor exhibited a comparable sensitizing strength to nitrate. On the surface, metolachlor decay was 18 times faster when loaded with benoxacor by pipette in a 1:0.5 ratio ($k = 0.11$ h^{-1}) than when metolachlor was loaded individually ($k = 0.006$ h^{-1}).The enhancement of metolachlor decay by the presence of benoxacor increased with increasing benoxacor concentration ($k = 0.14$ h^{-1} and $k = 0.13$ h^{-1} for a 1:1 ratio of metolachlor to benoxacor when loaded by pipette and surfactant, respectively). These results show that benoxacor has the potential to induce indirect photolysis on otherwise stable metolachlor when applied to the soil. Future studies should address the extent of such impact and mechanistic pathways in order to assess the environmental fate of these chemicals.

Introduction

  S-metolachlor is a type of chloroacetamide herbicide with a long-standing history of use in control of grassy weeds and is most commonly applied to corn and soybeans. Commercially, metolachlor is available in conjunction with its safener, benoxacor, in a formula called Dual II Magnum. Benoxacor is a dichloroacetamide safener that plays an important role in protecting crops from its paired herbicide S-metolachlor, allowing the herbicide to kill weeds without inflicting harm on the crop itself. Benoxacor has been shown to do this in corn by inducing glutathione-S-transferase (GST) activity in the plant.[1-3] The GST enzyme can then bond to the metolachlor substrate and create a conjugate compound that is much less toxic to the crop.[4] This is effective because crops metabolize herbicides very quickly, catalyzed by GST, while weeds undergo much slower detoxification.[5] Dual II Magnum is applied pre-emergence (i.e., before the seed is germinated) directly onto the soil surface where it sits under direct sunlight during the day.

  Because these compounds see significant sunlight after they are applied to the field, the chemical fate process of photodegradation becomes important. Metolachlor absorbs very little light above 290 nm, where the sunlight spectrum begins, and therefore is stable in sunlight and does not readily undergo direct photolysis. Benoxacor, however, has ample light absorbance overlapping with the sunlight spectrum, and can undergo direct photolysis, where a compound directly absorbs light energy and then undergoes a chemical reaction to form a photoproduct.[6] Additionally, because benoxacor can absorb sunlight, it has the potential to act as a sensitizer to metolachlor, increasing the decay of metolachlor when applied together in a formulation. In this scenario, metolachlor undergoes indirect photolysis. Benoxacor becomes electronically excited from the light and either reacts with metolachlor as a sensitizer to form a photoproduct or

transforms into another reactive intermediate which then reacts with metolachlor to form a photoproduct.[7] The potential reactive intermediate formed via indirect photolysis is typically a hydroxyl or carbonate radical, or a singlet oxygen which facilitates the reaction, although the specific pathways of indirect photolysis of metolachlor as induced by benoxacor remain unknown.[8-10]

The suggestion that benoxacor plays a role in chemically affecting the herbicide it is paired with has direct environmental implications. The U.S. Federal Insecticide, Fungicide, and Rodenticide Act only covers chemicals that affect pests as active ingredients.[11] Because safeners only affect the crop, they are considered "inert" for regulatory purposes, although they clearly are not chemically inert. The U.S. Environmental Protection Agency defines the word "inert" as "not intended to imply nontoxicity; the ingredient may or may not be chemically active".[12] From an environmental standpoint, lack of regulation of chemically active and potentially toxic compounds is deeply concerning. In addition, because safeners are not regulated, their fate and transport has not been widely studied.

Metolachlor is used agriculturally in staggering amounts; more than 16 million kg year^{-1} was applied in 2012 in the United States alone.[13] While metolachlor is not always applied with a safener, upwards of 1.7 million kg year^{-1} of benoxacor or another dichloroacetamide safener was applied in the U.S.[14] However, the United States accounts for only 25% of total herbicide use, so worldwide annual application could be much higher.[15] Moreover, metolachlor and benoxacor have already been detected in soil,[16] ground water,[17,18] and surface waters[19,20] in the United States. In streams in Illinois and Iowa, part of the corn belt, metolachlor was detected in concentrations anywhere from 10 – 15,000 ng L^{-1}.[21] Benoxacor was detected about 30% as often as metolachlor in the streams, likely due to the difference in application rates of the two

compounds, and was seen in concentrations from 4 to 190 ng L^{-1}.[22] In a study by the U.S. Geological Survey, metolachlor and its photoproducts were the most detected herbicides in Iowa groundwater.[23] With this detection in ground and surface water, the toxicity of these compounds becomes a concern. Dual II Magnum was found to have a higher level of toxicity than either metolachlor or benoxacor individually.[24] Benoxacor has been shown to be acutely toxic to autotrophs, and moderately toxic to freshwater fish, although there is a lack of literature on chronic toxicity levels and on benoxacor toxicity in general.[25,26]

The objective of this work was to better understand the chemical fate and transport of metolachlor and benoxacor due to photolysis on the soil surface. In particular, the focus was to determine the role of benoxacor in inducing indirect photolysis of metolachlor and increasing the decay of this chemical. Because of the large quantities of these compounds applied annually, their detection in various components of the environment, and the uncertainty surrounding their degradation pathways and toxicity, gaining insight into their means of fate and transport is necessary. Experiments were designed to assess the sensitizing strength of benoxacor against other sensitizers commonly found in surface water such as nitrate, and to understand the impact of benoxacor on the decay of metolachlor in varying molar ratios both in water and on the surface. The photodegradation kinetics of benoxacor and metolachlor under both direct and indirect photolysis on the soil surface and in water was studied in simulated sunlight. The pseudo-first order rate constants were estimated and analyzed.

Procedure

Analytical Methods

UV-visible absorption spectra were recorded using an Agilent Cary 60 Spectrophotometer. Degradation was analyzed using high-performance liquid chromatography with a diode array detector (HPLC-DAD). Analyses were performed using an Agilent Technologies 1260 Infinity Quaternary HPLC with a C18 stationary phase column. Injection volumes of 15 µL were taken using an Agilent Standard Autosampler. The flow rate was 1 mL min^{-1} and the temperature was held at 30 $^{\circ}$C. Detection was done with an Agilent DAD VL+ Diode Array Detector at 230 nm for metolachlor and 291 nm for benoxacor.

Water Sample Preparation

Aqueous samples were prepared in test tubes. Desired volumes of herbicide and/or safener were added along with 1 mL of phosphate buffer (pH 7, 5 mM), and DI water to a total volume of 10 mL. For direct photolysis, 10 µM of metolachlor and 10 µM of benoxacor were irradiated separately. For indirect photolysis with sensitizers, 10 µM of metolachlor was always used, in 1:0.5, 1:1, 1:10, and 1:100 ratios with nitrate or benoxacor. Mixtures were prepared first and divided evenly between three test tubes or three dark controls. All aqueous samples were performed in triplicate, and at various time points 0.5 mL of solution would be removed from the test tube. At each time point, test tubes were removed from the sunlight simulator, samples were taken for HPLC analysis, and test tubes were placed back into the simulator. HPLC vials were kept in the dark.

Surface Sample Preparation

Calculations. Commercial Dual II Magnum Herbicide contains *S*-metolachlor (and *R*-enantiomer) as the active ingredient in a concentration of 915 g L^{-1}.[27] Recommended application rate for Dual II Magnum Herbicide in the environment as pre-emergence is 1.25 – 1.75 L ha^{-1}.[28] 4.02 x 10^{-8} mol cm^{-2} of S-metolachlor is applied in the environment. Using 1/8 of the recommended application rate on a quartz dish with a surface area of 20.26 cm^2, 0.102 µmol S-metolachlor should be applied to each quartz petri dish to mimic commercial application. Some deviations from this amount were used if the volume of safener was extreme or if the total volume would exceed that of the quartz dish.

Pipette Loading. Pipette loading was done using a micropipette in 10, 10 µL droplets equally spaced across the quartz dish. For direct photolysis on a quartz dish with a surface area of 20.26 cm^2, 0.100 µmol of S-metolachlor and 0.200 µmol of benoxacor were loaded per dish. For indirect photolysis, desired ratios of metolachlor and benoxacor were mixed first in a dark container and then pipetted onto the quartz dish. The amounts loaded for indirect photolysis of metolachlor induced by benoxacor are shown in Table 1.

Table 1. Amount of metolachlor and benoxacor loaded onto the surface of a 20.26 cm^2 quartz dish by pipette for indirect photolysis

Reaction Solution	Metolachlor (µmol)	Benoxacor (µmol)
1:0.5	0.080	0.040
1:1	0.067	0.067

Surfactant Loading. The surfactant used to encourage even spreading of the compounds across the dish was Tween 20, which was added in a 1:10 volumetric ratio to benoxacor. 1 mL of acetonitrile (ACN) was also added to each dish. For direct photolysis, 0.100 µmol of S-metolachlor and 0.100 µmol of benoxacor were loaded per dish, following the above

calculations. For indirect photolysis, the amounts of metolachlor and benoxacor loaded are

shown in Table 2.

Table 2. Amount of metolachlor and benoxacor loaded onto the surface of a 20.26 cm^2 quartz dish by surfactant for indirect photolysis

Reaction solution	Metolachlor (µmol)	Benoxacor (µmol)
1:1	0.05	0.05
1:10	0.05	0.50
1:100	0.05	5.00

Sample Recovery. After irradiation, 5 mL of ACN was added to each quartz dish, and the

samples were allowed to sit for 5 minutes in the dark. The solution was stirred 3 times with a

pipette, and samples were collected for HPLC analysis. HPLC vials were stored in the dark.

Percent sample recovery was calculated on the basis of 100% recovery in 5 mL ACN.

All surface experiments were performed in triplicate, meaning three individual quartz

dishes were prepared for each timepoint and ratio. Each sample was sacrificed after its

designated irradiation timepoint and new samples were prepared.

Irradiation in the Sunlight Simulator

Photolysis experiments were performed in a Q-Sun Xe-1 Xenon Test Chamber. Both

surface and water samples were placed in a water bath at 26 ± 3 $^{\circ}$C for the duration of irradiation.

A daylight-Q filter (X-7460) was used to ensure that samples only saw wavelengths within the

sunlight spectrum. The lamp was set to an intensity of 0.68 W m^{-2} at 340 nm. Test tubes for

water samples were inserted at a 45° from vertical to achieve maximum lamp exposure. Each

sample had an associated dark control that was not exposed to light for the duration of the

experiment and was sampled alongside the irradiated samples. Surface samples were placed in

quartz dishes, covered with quartz disks, and placed into the sunlight simulator so that they were

halfway submerged in the water bath. Dark controls for surface samples were also placed into the

sunlight simulator. All samples were then analyzed using HPLC to determine the concentration

of the compound of interest.

Data Analysis

All photolysis data was modeled assuming pseudo-first-order kinetics. These pseudo-

first-order rate constants were obtained by linear regression of the logarithmic concentration

values gathered by HPLC as a function of irradiation time. Standard deviations were calculated

for water samples where each sample was taken from the same reaction solution, but not from

surface samples due to the sacrificial nature of the sampling.

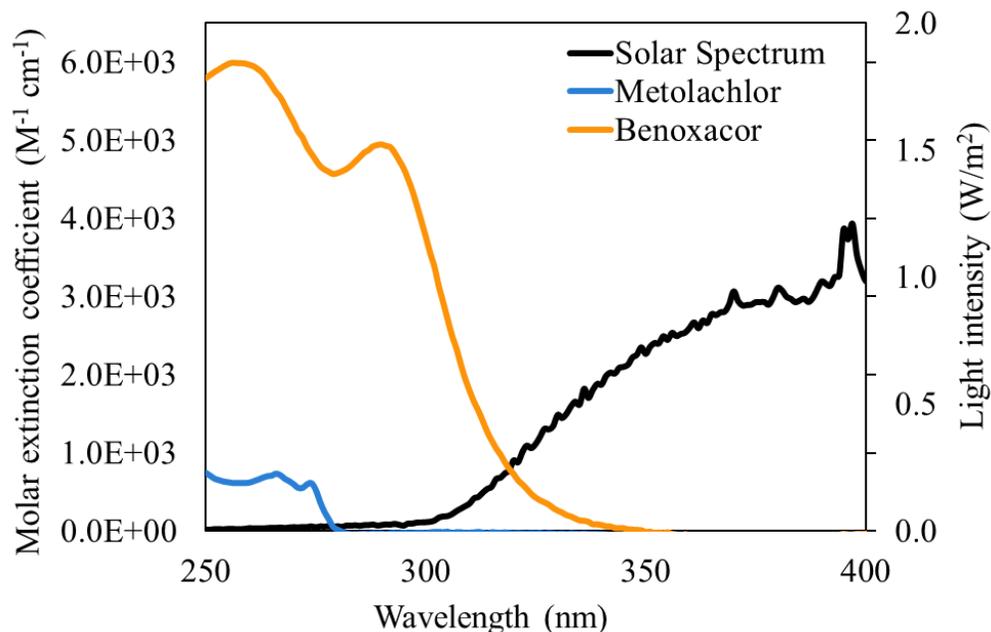## Results and Discussion

Light Absorption

Fig 1. Light absorbance of metolachlor and benoxacor as compared to the sunlight spectrum

The light intensity and molar extinction coefficient of the solar spectrum, the black line, from 250 to 400 nm is shown in Figure 1. This figure demonstrates that benoxacor, the orange line, has a much greater molar absorptivity in the solar spectrum than does metolachlor, the blue line, due to its greater area of overlap with the spectrum. This suggests that benoxacor can absorb sunlight and readily undergo direct photolysis, while metolachlor does not. This favors the hypothesis that benoxacor could be a good candidate to induce indirect photolysis in metolachlor, which otherwise does not significantly decay under sunlight, as indicated by the lack of significant overlap with the sunlight spectrum.
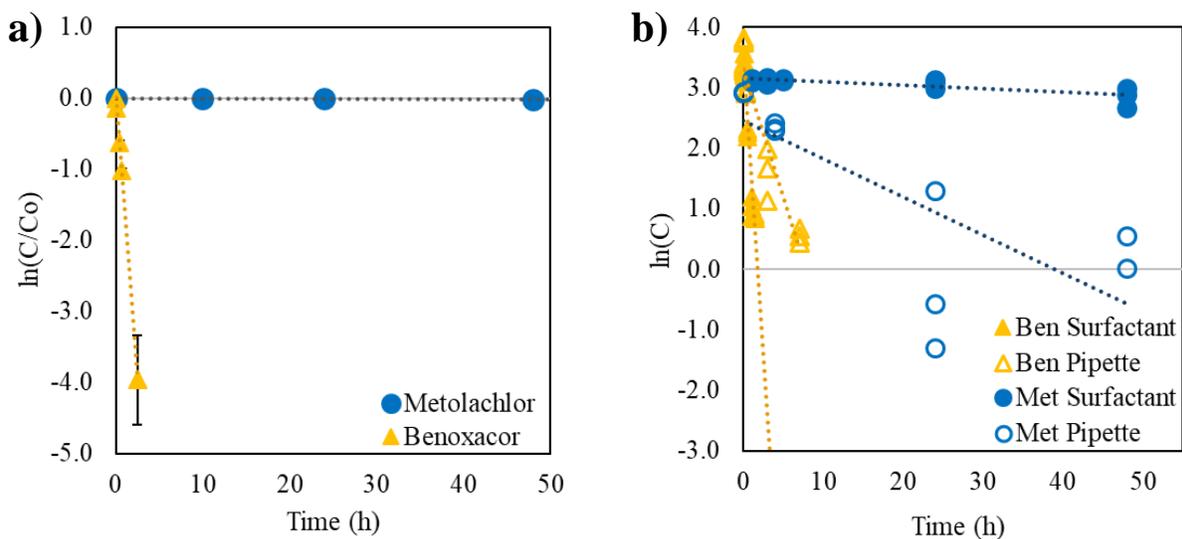
Direct Photolysis

Fig 2. Direct photolysis of metolachlor (Met) and benoxacor (Ben) **a)** in water and **b)** on surface loaded by pipette and surfactant

The direct photolysis of metolachlor and benoxacor, separately, in water, is shown in Figure 2. As can be expected from Figure 1 and the molar extinction coefficients of the two compounds, metolachlor displayed very little decay after 50 hours of sunlight irradiation when tested in water. Benoxacor decayed very quickly in water under direct photolysis and fell below the detection limit of the HPLC after less than 5 hours, as shown in Figure 2 a). When direct photolysis was tested on the surface as shown in Figure 2 b), a similar trend was observed. Again, benoxacor was observed to decay much more quickly than metolachlor, which exhibited very little decay even after 50 hours of irradiation. There was no clear trend between rate of decay when the components were loaded by surfactant versus by pipette.
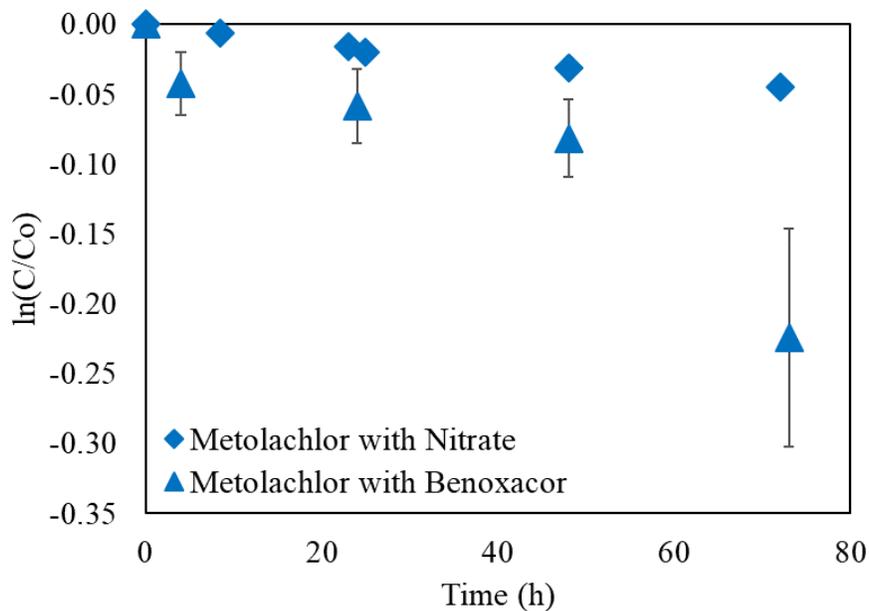
Indirect Photolysis

Water.

Fig 3. Indirect photolysis of 10 µM metolachlor paired with 100 µM nitrate or 100 µM benoxacor (1:10) in water
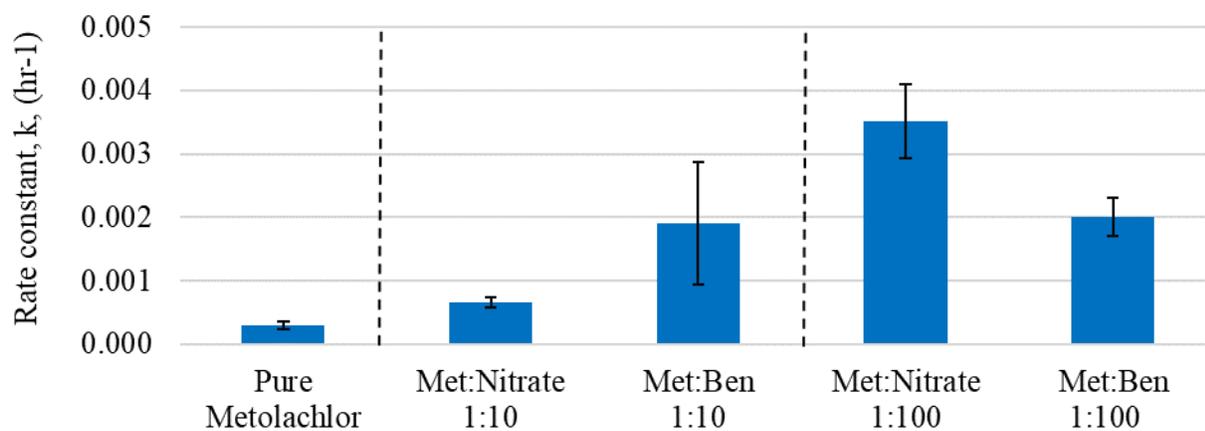

Fig 4. Rate constants of metolachlor (Met) decay induced by benoxacor (Ben) or nitrate as compared to direct photolysis of metolachlor, in water

Indirect photolysis of metolachlor induced by nitrate or benoxacor was studied in various ratios. Nitrate is a common sensitizer found in surface water and would be expected to induce indirect photolysis in metolachlor. This is confirmed in Figure 4 which shows that the rate constants of metolachlor decay induced by nitrate are higher in both 1:10 and 1:100 ratios of metolachlor to nitrate than the rate constant of direct photolysis of metolachlor. Figure 3 shows that metolachlor undergoes decay more quickly when induced by benoxacor than by nitrate, a compound that is commonly regarded as a sensitizer. Figure 4 suggests that indirect photolysis of metolachlor is faster when paired with benoxacor in a 1:10 ratio, and faster when paired with nitrate in a 1:100 ratio, although all four variations are faster than the rate constant for direct photolysis of metolachlor. This suggests that benoxacor has a sensitizing strength that is worth studying, as it has a comparable affect to nitrate when present in the same molar ratio.
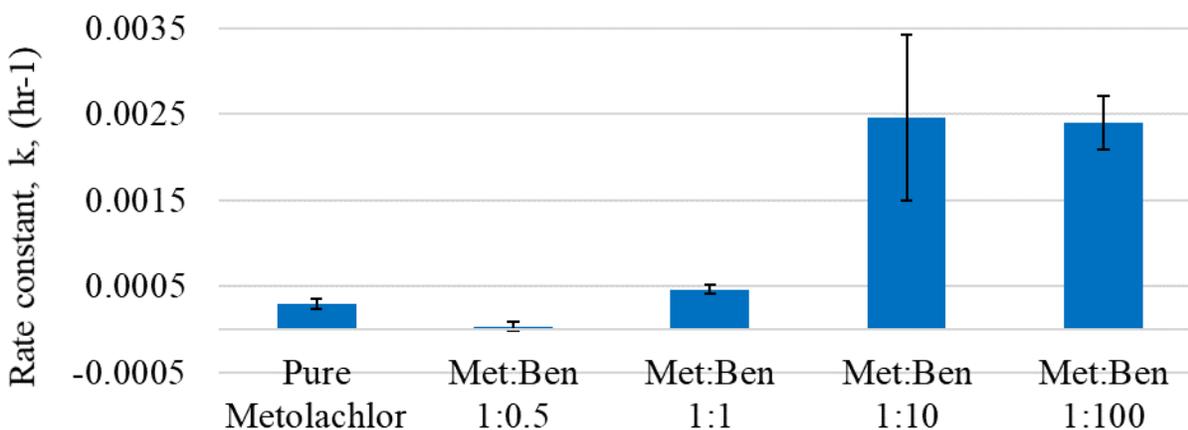

Fig 5. Rate constants of metolachlor (Met) decay induced by benoxacor (Ben) compared to direct photolysis of metolachlor, in water

Metolachlor undergoes indirect photolysis induced by benoxacor more quickly in 1:1, 1:10, and 1:100 ratios than it undergoes direct photolysis, in water, as shown in Figure 5. When paired with benoxacor in 1:10 ($k = 0.00245$ h^{-1} $\pm$ 0.00096) and 1:100 ($k = 0.00240$ h^{-1} $\pm$ 0.00031)

ratios, metolachlor decays over 8 times more quickly than it does when undergoing direct photolysis (k = 0.00030 h^{-1} ± 0.00006). In a 1:1 ratio with benoxacor, metolachlor decays 1.5 times faster (k = 0.00046 h^{-1} ± 0.00005) than during direct photolysis. This trend did not hold true in the 1:0.5 ratio, but more testing needs to be done in this lower range.
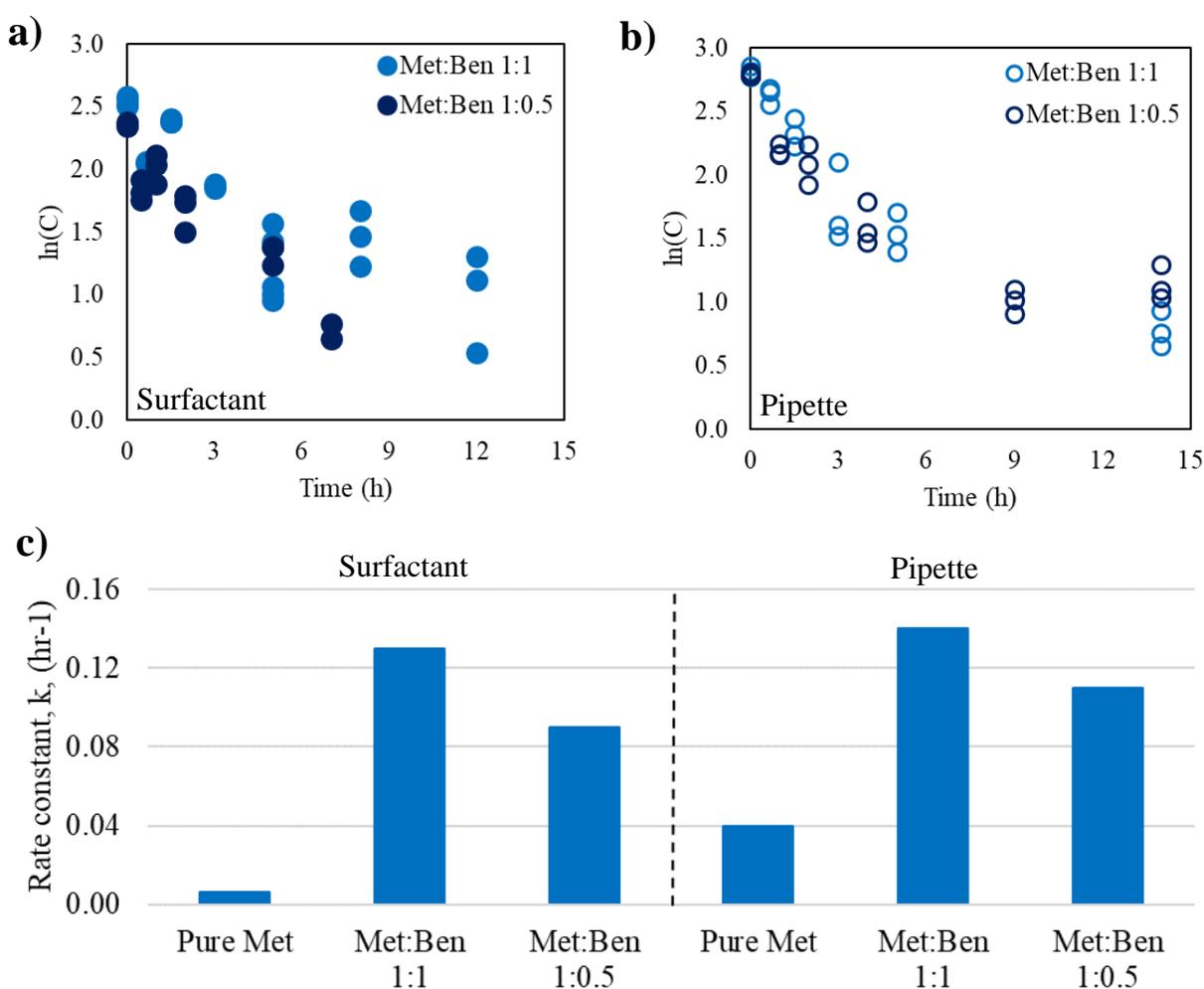
Surface.

Fig 6. Indirect photolysis of metolachlor (Met) induced by benoxacor (Ben) in 1:1 and 1:0.5 ratios loaded by **a)** surfactant or **b)** pipette, and **c)** rate constants of those ratios loaded by surfactant or pipette as compared to direct photolysis of metolachlor

Lastly, indirect photolysis of metolachlor induced by benoxacor was examined on the surface, loaded by either surfactant or pipette. Figure 6 demonstrates that metolachlor experienced faster decay when loaded by pipette than when loaded by surfactant, regardless of whether direct or indirect photolysis was being tested and regardless of amount of benoxacor added, although the affect was slight. It was also found that using a higher ratio of benoxacor resulted in a faster rate of decay by indirect photolysis of metolachlor, independent of loading technique. For a 1:1 ratio of metolachlor to benoxacor, the rate constant was $k = 0.14 \, h^{-1}$ when loaded by pipette and $k = 0.13 \, h^{-1}$ when loaded by surfactant, and for a 1:0.5 ratio $k = 0.11 \, h^{-1}$ by pipette loading and $k = 0.09 \, h^{-1}$ by surfactant loading. There was some variation in the data that should be considered, as the rate constant would change based on the length of irradiation time, and further experimentation is required to solidify analyses made from these results. This variability is likely due to photoproducts that were detected by the HPLC. Peaks that did not match the retention time of metolachlor or benoxacor were consistently apparent in the HPLC data and these photoproducts are also potential sensitizers of the reaction.

Conclusion

Direct photolysis experiments both in water and on the soil surface demonstrated that the safener, benoxacor, decays much more quickly than its paired herbicide metolachlor. Metolachlor was seen to be stable under sunlight. This result was corroborated by the molar extinction coefficients of the two compounds and the greater overlap of benoxacor with the solar spectrum. This suggests that it is possible that benoxacor could act as a sensitizer to metolachlor and cause it to decay under sunlight when applied together to a crop in a formulation such as Dual II Magnum.

Benoxacor and nitrate both exhibited a sensitizing effect on metolachlor in water samples. The strength of benoxacor as a sensitizer was comparable to that of nitrate, which is a sensitizer that is already commonly found in surface waters. This suggests that indirect photolysis of metolachlor induced by benoxacor should be studied, as benoxacor has a similar sensitizing effect as nitrate. Further study into the indirect photolysis of metolachlor in water demonstrated that it decayed more quickly when paired with Benoxacor in 1:1, 1:10, and 1:100 ratios. This confirms that benoxacor does induce indirect photolysis in metolachlor in water, at least in these larger ratios.

These results were mimicked with indirect photolysis experiments on the quartz surface. Although using smaller ratios did not produce dramatic results in water samples, when applied to the surface using a pipette the decay of metolachlor occurred at least 15 times faster when paired with benoxacor in 1:0.5 or 1:1 ratios. Overall, metolachlor decay induced by benoxacor was faster when loaded by pipette than by surfactant, and rate of decay increased with increasing benoxacor concentration. These surface experiments demonstrate that benoxacor has an even

more pronounced sensitizing effect when applied with metolachlor on the surface than in water

samples, which was anticipated from existing research.

More experiments need to be done, specifically in the lower metolachlor to benoxacor

ratio range, in order to draw further conclusions on the environmentally and agriculturally

relevant sensitizing effects of benoxacor. In commercially available products such as Dual II

Magnum, the metolachlor to benoxacor ratio is approximately 1:0.05.[29] Although this ratio is

lower than those covered in these experiments, the preliminary results show an important trend

of increased metolachlor decay when paired with benoxacor both in water and on soil. The next

steps would include examining metolachlor decay induced by benoxacor on a kaolinite surface to

better model soil before eventually experimenting on an actual soil surface. In addition, the

mechanistic pathways that lead to metolachlor decay when paired with benoxacor should be

researched, as they are currently unknown. Suspected photoproducts appeared in the HPLC

results and they have the potential to further sensitize the decay of metolachlor and could

significantly impact the pseudo-first order rate constants. These photoproducts need to be

characterized to understand their role in the decay of metolachlor under indirect photolysis and to

understand the nature of the chemicals that are being formed in the environment as a result of

herbicide use.

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