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Examination of the Soil Bacteria Responsible for the Decomposition of Ailanthone, an Inhibitory Chemical in *Ailanthus altissima*

Acknowledgements

Faculty Advisors: Dr. Justin Maresh, Chemistry Department and Dr. Judith Bramble, Department of Environmental Science and Studies

Examination of the Soil Bacteria Responsible for the Decomposition of Ailanthone, an Inhibitory Chemical in *Ailanthus altissima*

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ABSTRACT *Ailanthus altissima* produces the inhibitory chemical ailanthone, which is primarily found in the roots and bark of maturing trees. The goal of this project was to determine under what soil conditions ailanthone decomposes. Ailanthone broke down in slightly basic conditions and non-sterile soil while remaining stable in sterile soil. This suggests that ailanthone is broken down by soil microbes. The next phase in this project was isolating the soil bacteria that may be responsible for ailanthone decomposition in soil. Soil bacteria were successfully cultured from soil samples near *A. altissima* trees using a structurally similar compound to ailanthone, quassin, for selection.

INTRODUCTION

Invasive species can have many negative effects on urban ecosystems, but a pressing issue in cities like Chicago is the threat to urban biodiversity. Invasive species can produce secondary metabolites that provide a competitive advantage over native plants; allelopathic chemicals are such compounds that affect the growth of other vegetation (Callaway, 2004). Native plants often times have not adapted ways to defend against these chemicals, causing native populations to decline. Studying these allelochemicals allows us to understand the role allelopathic invasive plants play in urban biodiversity.

Ailanthus altissima is a fast growing, invasive tree species native to China and is abundant throughout Chicago. It is a known producer of the inhibitory chemical ailanthone that has been shown to affect seed

germination and growth under laboratory conditions; however, the impact of *A. altissima* in nature is not fully understood (Heisey, 2003). Ailanthone may be produced as an allelochemical to outcompete other plants, or it may be produced as an anti-herbivore compound meant to deter insects from consuming its leaves. If *A. altissima* is indeed an allelopathic plant, then its presence in Chicago could reduce urban biodiversity. If ailanthone is an anti-herbivore compound or if it does not accumulate in soil concentrations high enough to affect plant growth, then it could potentially have a positive impact on urban biodiversity. *A. altissima* thrives in unlikely places and grows quite rapidly; it is commonly found in alleys or areas with poor soil and it can grow multiple feet each year. This tree has the potential to be utilized to increase the biodiversity in areas of Chicago that are barren of flora.

If ailanthone is allelopathic, it should be released from the roots and present in the soil. Prior research has shown very high levels of inhibition from extracts made

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from *A. altissima* roots, but very little inhibition from soil collected around the roots. One of the goals of this project was to determine under what conditions ailanthone breaks down in the soil. The soil conditions that lead to the decomposition of ailanthone will reveal whether ailanthone can accumulate in soil in concentrations high enough to affect the growth of plants in nature. Isolating the soil bacteria responsible for the breakdown of ailanthone comprised the second half of this project; the methods for this part were adapted from a procedure used to isolate soil bacteria that could survive on antibiotics (Dantas, 2008). Quassin, a structurally similar and inexpensive compound, was used to isolate bacteria instead of ailanthone (Figure 1). The isolated soil bacteria can be used in decomposition rate experiments to determine the length of time ailanthone remains in non-sterile soil.

METHODS

BREAKDOWN OF AILANTHONE IN VARYING SOIL CONDITIONS

Ailanthone isolated from local *A. altissima* trees was used in the decomposition experiments. Four different pH buffers were prepared (2.2, 4.6, 7.0 and 10.0) and ailanthone (2.02 mM) was added to each buffer solution. To quantify the ailanthone over the course of the experiment, 1 mL samples were extracted every day for six days and 10 μ L were analyzed by high performance liquid chromatography (HPLC, Waters Acuity) with a 2.1x100 mm ACQUITY BEH C 18 1.7 μ m column with a gradient of 0.1% trifluoroacetic acid (TFA) and acetonitrile. The ailanthone peak was identified by UV spectrum, and the area of the peak was recorded at the absorbance maximum wavelength of 244.6 nm. The peak for ailanthone on the HPLC had a retention time of 2.33 minutes.

Next, a sterile soil solution was prepared by syringe filtering a mud solution (10 g soil/100 mL H₂O) into seven individual sterile vials in a sterile hood. A non-sterile soil solution (10 g soil/ 100 mL H₂O) was prepared without filtration along with a pH 7.2 solution.

Ailanthone was added to each solution (2.02 mM). For seven days, 1 mL was extracted every day and syringe filtered to remove any soil debris. 10 μ L were analyzed on the HPLC, and the area of the peak was recorded at 244.6 nm, as described above.

ISOLATION OF SOIL BACTERIA

Liquid media was prepared by mixing 1 mg/mL of quassin into Single Carbon Source media containing 50g (NH₄)₂SO₄, 30g KH₂PO₄, 5.0g MgSO₄·7H₂O, 191mg Na₂EDTA, 45mg ZnSO₄·7H₂O, 45mg CaCl₂·2H₂O, 30mg FeSO₄·7H₂O, 10mg MnCl₂·4H₂O, 10mg H₃BO₃, 4mg Na₂MoO₄·2H₂O, 1.92mg CuSO₄, 3mg CoCl₂·6H₂O and 1mg KI in one liter of Milli-Q water. The pH was adjusted to 5.5 using NaOH and sterilized using a 0.22 μ m filter. Solid media was prepared by mixing 15g agar per liter of SCS media and then sterilized with autoclaving (Dantas, 2008). Quassin (1 mg/ mL) was added to the solid media after autoclaving, providing a medium for bacterial growth in which quassin is the only carbon source.

To isolate quassin-dependent bacteria, a mud extract was prepared with 50 mg soil/100mL water. 100 μ L of the mud extract was placed in sterile tubes with 1X Single Carbon Source (SCS) media (5 mL). After seven days of growth in an incubator (26.8 °C) with shaking (145 rpm), 2.5 μ L of each solution was placed in a new sterile tube with 1X SCS (5mL) media. This dilution was repeated two more times, each after seven days of growth. After the third dilution, 100 μ L of each liquid culture was placed on solid SCS media with quassin. After one week of growth in an incubator (22 °C) without shaking, bacteria were restreaked onto new solid SCS media plates. Single colonies from the restreaked plates were isolated after two weeks of growth and placed into 5mL of 1X SCS liquid media with quassin. All colonies remaining were bacteria that could survive exclusively on quassin.

RESULTS

Ailanthone remained stable in the pH buffers of 2.2, 4.6 and 7.0 over the span of six days while ailanthone in pH 10.0 buffer had a half-life of about 2.5 days (Figure 2). Ailanthone remained stable in the sterile mud solution and the pH 7.2 buffer over seven days while it decomposed with a half-life of 4 days in the non-sterile mud solution with a pH of 7.2 (Figure 3).

Soil bacteria was successfully isolated from the soil solution and single bacteria colonies that survived on quassin as the only carbon source were successfully isolated on solid SCS media. The single colonies were then successfully cultured in liquid media with quassin as the only carbon source (Figure 4).

DISCUSSION

In the first part of this project, ailanthone was shown to decompose in basic buffer conditions while remaining relatively stable at acidic to neutral pH. This shows that pH affects the stability of ailanthone: ailanthone may breakdown in basic soil while remaining relatively stable in neutral soil conditions. Ailanthone was also shown to decompose in non-sterile soil while it remained stable under sterile soil conditions. This indicates that microorganisms present in the non-sterile soil are most likely to be responsible for the breakdown of ailanthone in the environment. The next part of this project successfully isolated soil bacteria using quassin as the only carbon source. Since quassin and ailanthone have very similar structures, we can generalize that the cultured bacteria colonies will survive utilizing ailanthone as its exclusive carbon source. The next steps will be to introduce the isolated bacteria to ailanthone to confirm that they can survive on it and then to perform decomposition rate experiments with ailanthone to determine how fast isolated bacteria breakdown ailanthone. The results presented will provide insight into whether or not ailanthone can accumulate in the soil at high enough concentrations to affect other plants.

The data from both parts of this project suggest that ailanthone is unstable under typical soil conditions. If ailanthone cannot accumulate in concentrations high enough to effect surrounding plants, then *A. altissima* may not have a negative allelopathic impact on surrounding plants in soils from the Chicago region. The question remains as to the benefit, to the plant, of producing this chemical, but it supports the hypothesis that *A. altissima* may have a positive effect on urban biodiversity. Future studies will need to verify that the isolated bacteria decompose quassin and ailanthone at rates comparable to those observed in soil.

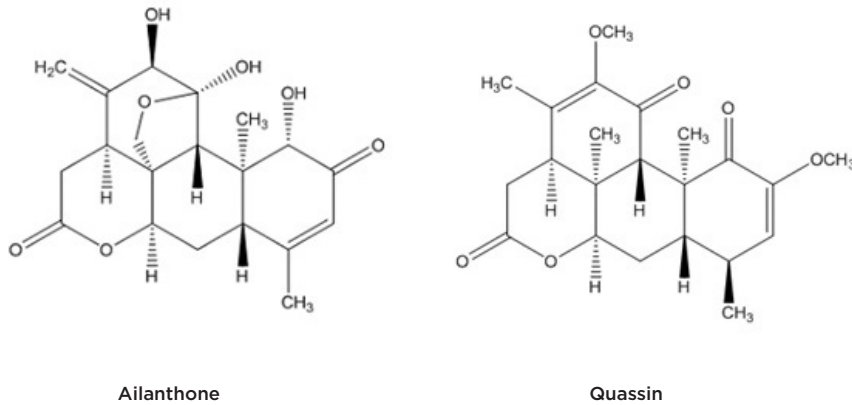


FIGURE 1

Structure of ailanthone and quassin.

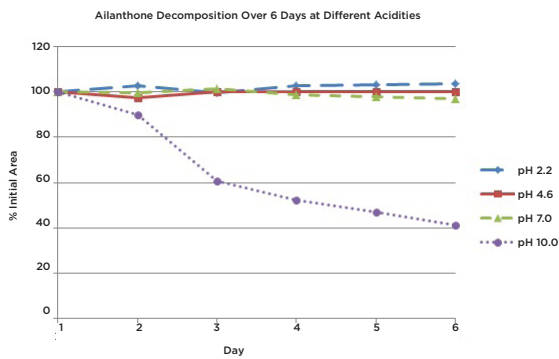


FIGURE 2

Percent initial ailanthone area over 6 days at different acidities measured on the HPLC at 244.6 nm.

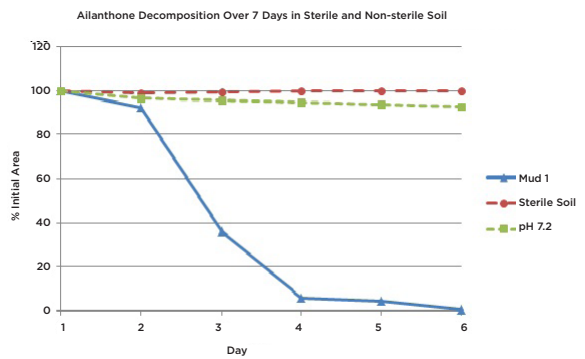


FIGURE 3

Percent initial ailanthone area over 7 days in sterile and non-sterile soil measured on the HPLC at 244.6 nm.



FIGURE 4

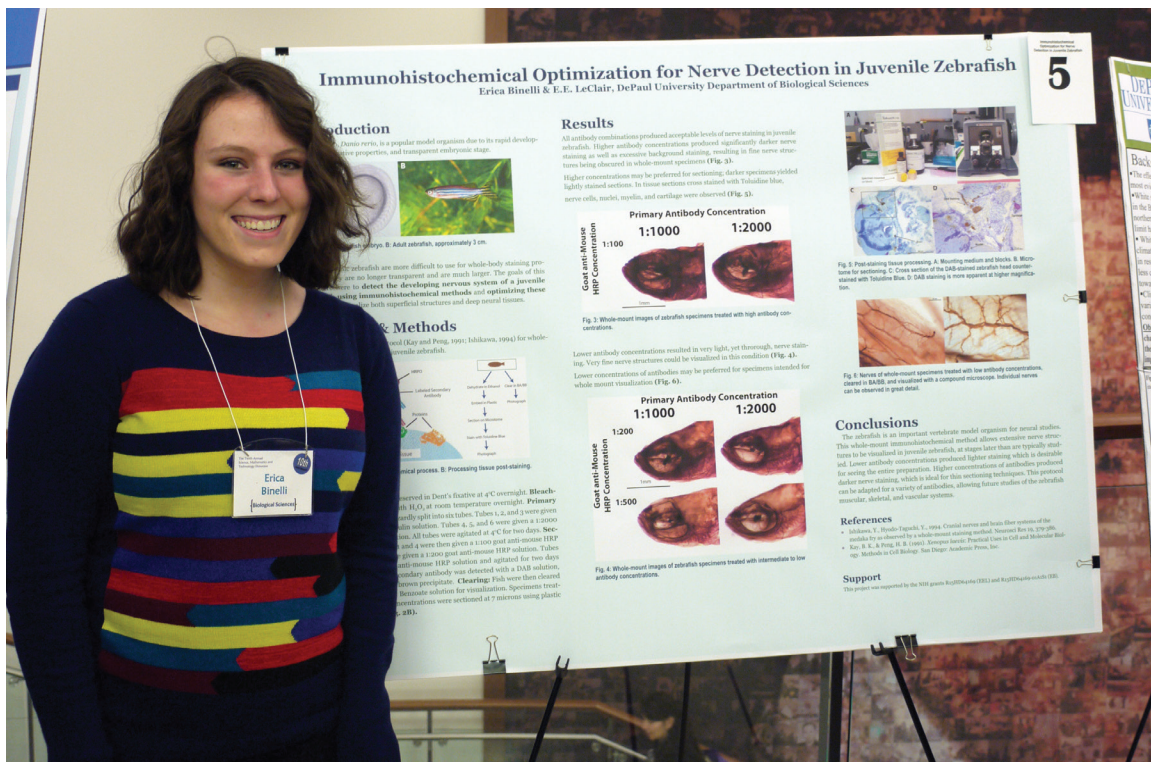
Single bacteria colonies isolated in 1X Single Carbon Source media with 1mg/mL quassin.

REFERENCES

Callaway, Ragan M., Wendy M. Ridenour. 2004. "Novel Weapons: Invasive Success and the Evolution of Increased Competitive Ability." *Front Ecol Environ* 2: 436-443.

Dantas, Gautam, Morten O. A. Sommer, Rantimi D. Oluwasegun, George M. Church. 2008. "Bacteria Subsisting on Antibiotics." *Science* 320: 100-103.

Heisey, Rod M., Teresa Kish Heisey. 2003. "Herbicidal Effects Under Field Conditions of *Ailanthus altissima* Bark Extract, Which Contains Ailanthone." *Plant and Soil* 256: 85-99.



Biology major Erica Binelli presents her research on zebrafish at the 10th Annual Natural Sciences, Mathematics, and Technology Showcase.