AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
FUNDING CYCLE 2019 – 2021

TITLE: Nanoscale delivery of pesticides to replace soil fumigation

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EXECUTIVE SUMMARY: Pesticides play an important role in agriculture by preventing the loss of crops due to pests. Soil fumigants are commonly used to control devastating soil-borne diseases such as root-knot (*Meloidogyne*) and cyst (*Heterodera* and *Globodera*) nematodes as well as weeds (Oka, Koltai et al., 2000). Fumigants differ from many other forms of pesticides in that they are volatile gases at room temperature which allows the gas to diffuse through the soil pores and treat a large surface area of soil. Since soil fumigants become gaseous pesticides that diffuse out of the soil over time, they are capable of being inhaled by workers who enter the treated areas too soon or when tarps leak and the fumes drift off target. Exposure and health impacts of fumigants to bystanders and the environment has led many fumigants to be phased-out or suspended; however, efficacious replacements are not readily available as most pesticides bind tightly to soils and are not able to reach into the zones plagued by soil pests. Nanotechnology-based delivery of pesticides is a viable path to overcome these limitations and reduce risk associated with fumigation practices. Engineered nanoscale delivery systems allow for the dispersion of non-volatile, hydrophobic pesticides in water. Their small size and Brownian motion enables them to migrate into even very compact soil pore spaces allowing for the treatment of similar surface areas as fumigants. Given the significant need for effective pest management in food security and our obligation to replace current fumigation practices, our long-term goal is to improve pesticide delivery to target soil pests while minimizing off-target impacts on essential soil microbes and crops. In this project, we will leverage commercially available products to engineer novel nanoscale pesticides to optimize soil dispersion with the goal of replacing soil fumigants. The aim of this application is to determine the principle physicochemical features (size, surface chemistry, aqueous stability, durability and composition) that govern the soil mobility of nanoscale delivery systems.

OBJECTIVES: Building upon our preliminary findings, we are well-positioned to successfully accomplish the goal of our project. Given our overarching research hypothesis that the size, surface chemistry and composition (encapsulated or particulate form) govern the soil mobility of nanoscale delivery systems, we aim to accomplish the following specific research objectives:

Objective 1: Characterize the important properties [size, surface chemistry, aqueous stability, durability, and composition (capsule or particulate form) of the nanomaterial] and soil transformations of nanoscale delivery systems that contribute to the soil mobility.

Objective 2: Investigate the soil mobility, dispersion and surface area coverage in soils of nanoscale delivery systems. Soil mobility will be determined using a flow-through soil column and measures of soil depth penetration and plume movement. Dispersion potential will be determined by measures of hydrophilicity and particle stability in water. Surface area covered by the treatment will be calculated based on the concentrations distributed in soil and compared to predicted distributions from fate and transport models of fumigants.

Objective 3: Establish a decision matrix to determine when nanoscale delivery systems may be able to replace current fumigant practices based on the defined relationship between the delivery system physicochemical properties and their soil mobility and impacts on agriculture. Multi-criteria decision analysis will be used to translate our primary research findings into a decision tool useful for the broader agricultural community.

PROCEDURES: Commercially available encapsulated and particulate pesticides will be fractionated and characterized for their primary particle size, surface charge, durability, and the size of their agglomerates in solution (Objective 1). Based on these properties and their relationship to soil mobility (Objective 2), we will then map the nanoscale delivery systems to potential optimization strategies to replace fumigants in agricultural fields (Objective 3).

<u>Selection of model nanoscale pesticide delivery systems:</u> Based on our survey and experience working with nanoscale pesticides, we selected a matched set of encapsulated (Cyonara 9.7) or milled (Tempo SC) pyrethroids that can be fractionated into nano-scale size fractions and can be further modified with the addition of polyethylene glycol. Tempo SC is a suspension concentrate of milled AI (β-cyfluthrin); whereas, Cyonara 9.7 is a dispersion of encapsulated AI (λ-cyhalothrin). Both are synthetic pyrethroids and λ-cyhalothrin functions as an effective nematicide, a key target soil pest; blocking 100% nematode egg hatch, but not affecting root weight of cowpea treated with λ-cyhalothrin (Ononuju and Nzenwa, 2011). In addition, pyrethroids are broken down by pyrethroid-degrading microbes in the environment and thus, should not significantly alter the microbiome (Thatheyus and Selvam, 2013).

<u>Fractionation:</u> The initial step in separating different size fractions of encapsulated or milled pesticides is removal of the “other” ingredients from the suspensions. To do this, we will load 5 mL of 100 ppm solution into a falcon vial and mark the vial for precisely 5 mL. Samples will be centrifuged and the supernatant will be separated from the pellet formed at the bottom of the vial by carefully and slowly decanting the solution, leaving the pellet at the bottom. The 100 ppm solution will be centrifuged, decanted and repeated 3 times before the vial is replenished to the 5 mL mark with pure water and vortexed to disperse the pesticide delivery system. The next step is to separate the capsules into micron scale and nanoscale size fractions by

centrifuging the cleaned, 100 ppm solution more slowly. The pellet will be comprised of the fraction that is larger than 1000nm while the supernatant will contain only the nanoscale fraction. Iterative fractionation will be conducted until we obtain the sample volumes and concentrations needed for the project.

Surface chemistry modifications: Surface modification with polyethylene glycol (PEG) is a widely used technique to improve nanoparticle stability in aqueous suspensions. Any number of ethylene glycol repeats can be used to achieve variation in particle stabilization. We will use established procedures for linking some common PEG moieties with molecular weight average molar masses (M_W) of 2000, 5000, and 10,000 Daltons (Nobs et al., 2004). Surface charge and hydrophobicity adjustment of non-encapsulated AI will be performed using a common approach for noncovalent PEGylation (Hong et al., 2006; Liu et al., 2008). The hydrophobic NP surface will be coated with a lipid-PEG conjugate as PEGylated phospholipids with linear or branched PEG chains bind to the hydrophobic surface of the active ingredient in such a way that hydrophilic PEG groups are facing the aqueous exterior creating a hydrophilic PEG corona around the nanoparticle. Purification of the PEG-NP conjugate is then performed through a series of centrifugation and washing steps followed by dialysis.

SIGNIFICANT ACCOMPLISHMENTS: The increased use of plastics in everyday and industrial products has sparked an investigation into how micro and nanoplastics may affect the environment. To achieve this goal, it is important to understand how nanoplastics move and behave in different environments. In this experiment, we investigated the movement of nanoplastics through different depths of soil using 45 nm fluorescent nanoplastic beads. We first determined the brightest fluorescent color type by comparing yellow, orange, red and dark red Fluospheres against soil leachate (**Table 1**).

Table 1. Fluorophore colors used and their wavelengths

	Excitation	Emission
Yellow	490	514
Orange	540	560
Red	580	608
Dark Red	650	683

Soil columns containing 5g (dry weight) sandy loam soil were first wetted with 10 ml water then leached with 12ml of water containing 20mg/L of fluorescent nanoplastic spheres. The fluorescence in the leachate was measured at the appropriate wavelength for each fluoropshere. The orange beads were found to be 1.3X brighter than the yellow, 1.6x brighter

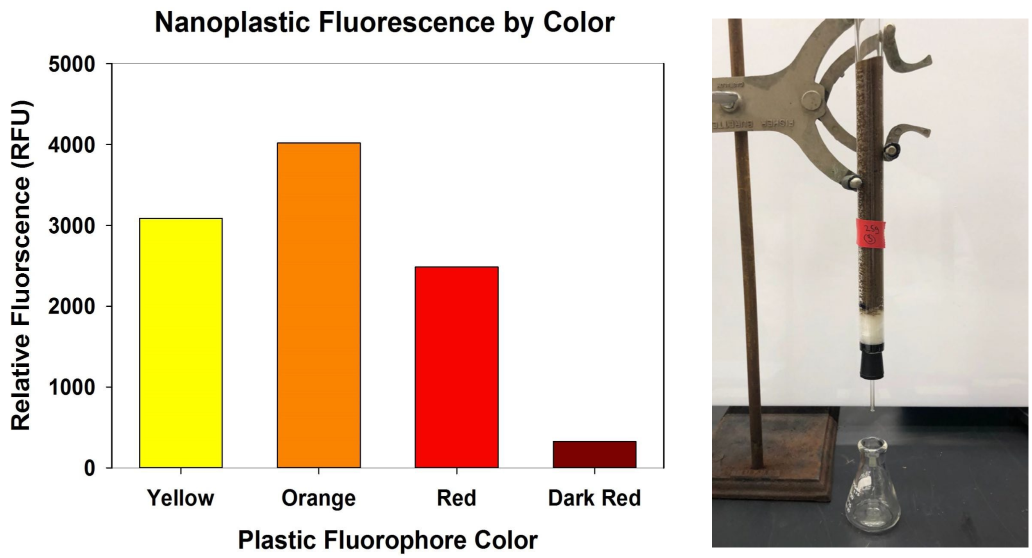

Figure 1. Fluorescence of various fluorophores in sandy soil leachate

than red and 12.4x brighter than the dark red; thus, the orange color fluorophores were selected for further experimentation (**Fig. 1**). Replicate soil columns containing 20-30 g of dried soil (both sandy and silty loam) were similarly wetted and then 10ml of the 20mg nanoplastic/L were added and the leachate was collected (**Figs. 2 and 3**, respectively). The columns were then flushed with 10 ml of water and that leachate was also collected until no detectable fluorescence was observed. The results show that there was similar recovery (82 to 87%) despite differing soil depths. This study contributes toward a better understanding of how nanoplastics may move in the environment by showing the potential for movement of particles through soil.

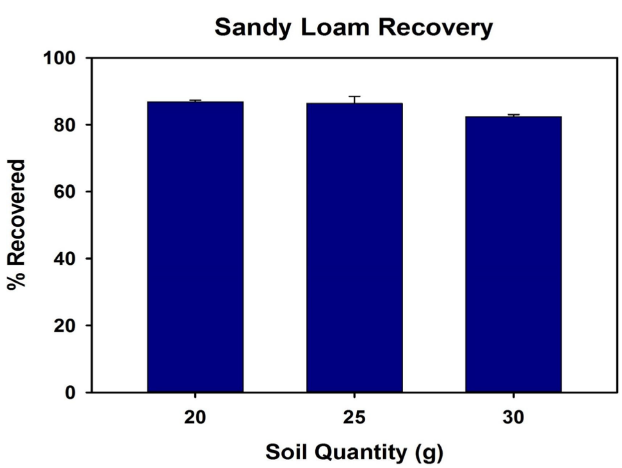

Figure 2. Sandy loam fluorescent plastic recovery with varying soil amounts.

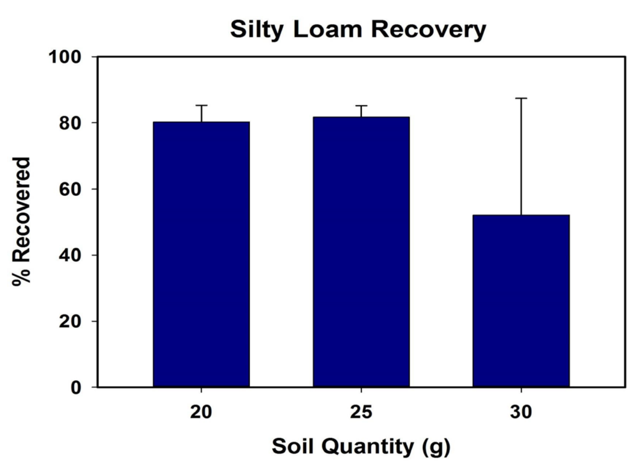

Figure 3. Silty loam fluorescent plastic recovery with varying soil amounts.

BENEFITS & IMPACT: We showed that any of the fluorescent nanospheres can be used to test against a soil background; however, longer wavelengths produce stronger signals. We also showed that nanoplastics do have the potential to move rapidly through both soil types and potentially contaminate groundwater. Much more work would be required to

The student who was supported on this project reported: "*The support provided by the Agricultural Research Foundation was a great opportunity for me to individually seek out professors for mentorship. When I heard about this research, I knew it would be a great experience both personally and professionally. Personally, I was discouraged previously from seeking professors as I thought I would be a bother. I have come to learn that there is a great necessity for undergraduate researchers in many laboratories.*"

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM: Proposal submitted to USDA/NIFA – not supported.

FUTURE FUNDING POSSIBILITIES: USDA/NIFA resubmit and a possible EPA Cooperative Agreement with the Pacific Ecological Systems Division in Corvallis, OR.