

2020 USAFRI Research Project Objectives

Developing a Soil Bioassay for Alfalfa Autotoxicity Michigan State University - Cassida

Project Award: \$92,662

Justification:

- Alfalfa autotoxicity is a well-known phenomenon that has never been fully explained. What we know is that alfalfa contains a water-soluble compound or compounds that are allelopathic to new alfalfa seedlings. While there are several leading candidates, the specific chemical entities have never been definitely identified, thus confounding any attempt to measure them directly. Autotoxicity causes direct failure of germination and seedling establishment in some cases, but the most damaging effect is permanent damage to root systems on seedlings that appear to have established successfully. This root damage causes reduced persistence and lifetime yield for the stand, a phenomenon called autosuppression or autoconditioning.
- The degree and duration of autotoxicity and autosuppression are influenced by a complex mix of environmental, genetic, and management factors. The problem is believed to: increase with age and density of the alfalfa stand, dissipate over time after alfalfa stand termination, dissipate faster from sandy than fine-textured soils, wash out of soil with precipitation, and be reduced by tillage after alfalfa termination (Undersander et al., 2015). Response to these factors is not always evident in field studies (Seguin et al., 2002). Genotype influences toxicity of alfalfa plant extracts in vitro (Chung and Miller, 1995), but it is not clear whether this effect is related to reduced toxin concentration, increased tolerance, or both.
- Field studies on autotoxicity are challenging because it can take several years simply to set up field plots with a range of stand ages, and it is difficult to control for all the possible interactions of environmental and management factors. Much of our knowledge on autotoxicity is obtained from laboratory bioassays using extracts of plant material. Best management practices for the appropriate planting delay after termination of an existing alfalfa stand range from two weeks (Tesar et al., 1993) to two years (Undersander et al., 2015). This large range in waiting period leaves growers in limbo, reluctant to risk expensive seed on trial and error, and may contribute to decline in alfalfa acreage if it seems less risky to just grow something else. Growers need an answer to the question, "is it safe to replant alfalfa, right now?"
- In 2018, we obtained checkoff funds to investigate feasibility of developing an affordable, rapid bioassay that can be used to evaluate the autotoxicity status in field soil and assist farmers with planting decisions for alfalfa. This assay can be offered through MSU Plant Diagnostic Services to farmers across the country, or adopted by other diagnostic services. The bioassay is based on the soil-on-agar (SOA) method (Voight et al., 1997). The SOA method requires minimal soil processing and consists of growing seeds in a half-inch layer of field soil on top of clear agar in an upright culture flask. This enables roots to grow in a normal orientation with good visibility for evaluating root length and morphology. We were able to detect differences in alfalfa seedling establishment and morphology when growing seed in soils potentially affected by autotoxicity, thus establishing proof of concept. We then obtained state funding to expand the work. With this continuation proposal to the alfalfa checkoff, we propose to help fund a graduate student and support expansion of method validation outside the state of Michigan.
- Our team at MSU is excited to continue this work. In 2019, Michigan grew 750,000 acres of alfalfa, comprising 76% of the hay and haylage acreage in the state (USDA- NASS, 2019). MSU's alfalfa research

program provides access to replicated plots across different soil textures with a range of alfalfa stand ages, locations, and alfalfa genetics. The PIs have well-equipped laboratory, greenhouse and growth chamber space. MSU Diagnostic Services was established in 1999, with diagnosticians specializing in plant pathology, entomology, nematology, and weed science to serve a wide variety of clients, ranging from regulatory agencies and agricultural businesses to commercial growers and homeowners. Diagnostic Services currently conducts a variety of bioassays as needed including soil bioassays to assess herbicide carryover. The addition of a bioassay testing alfalfa autotoxicity would be a natural fit within the clinic.

- The potential impacts of this project on the alfalfa industry are many. An accurate bioassay will help reduce risk of establishment failure and lifetime stand productivity losses to autosuppression, while reducing rotation time between alfalfa plantings. All these should improve profitability of growing alfalfa, thus encouraging its use. The bioassay will also provide a research tool that can assist in finally identifying the compounds responsible for autotoxicity. It can assess soils across a wide range of conditions, allowing faster progress in identifying trends than conducting controlled field experiments for every possible combination of factors. Preliminary data obtained in these trials will be used to support a larger proposal to USDA NIFA targeted at identifying the causes of autotoxicity.

Objectives:

- The objectives of this project are to 1) refine bioassay methodology in the laboratory; 2) compare autotoxicity in soils obtained from a diversity of alfalfa stands varying in environmental, genetic, and management factors; 3) evaluate ability of bioassay to identify autotoxicity response to alfalfa termination date; and 4) compare autotoxicity in farm fields before and after planting of new alfalfa seedings.