Developing a Soil Bioassay for Alfalfa Autotoxicity

Kim Cassida, Michigan State University

Alfalfa autotoxicity is a well-known phenomenon that has never been fully explained. What we know is alfalfa contains a water-soluble compound or compounds toxic to new alfalfa seedlings. While there are several leading candidates, specific toxins have never been positively 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 injury to taproots on seedlings appearing to have established successfully. Plants can never completely compensate for lost taproots, so this damage causes reduced persistence and yield over the entire stand life.

Alfalfa seedlings growing in soil from grass (control) or alfalfa plots.

The degree and duration of autoxicity 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 stand termination, dissipate faster from sandy than fine-textured soils, wash out of soil with precipitation, and be reduced by tillage after alfalfa termination. Genetics influence autotoxicity, but it is not clear whether this effect is related to reduced toxin concentration, increased tolerance, or both.

Much of our knowledge on autotoxicity was obtained from laboratory bioassays using extracts of plant material. 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, genetic, and management factors. Best management practices for the appropriate planting delay after termination of an existing alfalfa stand are based on relatively few field studies and range from two weeks to two years. This large range of waiting period leaves farmers in limbo, reluctant to risk expensive seed on trial and error, and may contribute to a decline in alfalfa acreage if it seems less risky to just grow something else. Farmers need an answer to the question – "Is it safe to replant alfalfa, right now?" From this practical perspective, it is not necessary to quantify all factors contributing to autotoxicity of particular soil, but simply to answer "yes" or "no" to alfalfa planting decisions. We obtained funding from the Alfalfa Checkoff program to begin development of a soil bioassay that can potentially be used to evaluate the autotoxicity status of a field and assist farmers with alfalfa planting decisions. The ideal test will be affordable and fast and use soil samples collected in the same manner as soil fertility samples. This assay can be offered through Michigan State University Plant Diagnostic Services to farmers across the country or adopted by other diagnostic services.

We adapted a soil-on-agar (SOA) method for our initial test. In this method, alfalfa seeds are germinated in a layer of field soil placed over a layer of clear agar in a small flask. This allows us to evaluate root growth and structure as the radicles grow down into the agar. Differences were detected after only four days of growth, making this method acceptably rapid. At this stage of test development, we are attempting to demonstrate proof-of-concept for the bioassay, and not to definitely answer all questions about autotoxicity. We did identify differences among soil sources, alfalfa stand ages, still alive vs. recently terminated alfalfa, and genotype. Variety of the seed grown in the field soils proved to be an important consideration. Plants grown in the greenhouse in selected soils over a three-week period confirmed results predicted by the bioassay.

While the bioassay can detect differences in alfalfa seedling development among soils that are consistent with autotoxicity, there are still many questions to answer. The next steps in bioassay development are to refine the technique and validate results using longer-term greenhouse studies and soil samples from farms. These validation efforts will require several growing seasons. At the beginning of 2020, we optimistically thought a test might be

ready to deploy within two years. The current disruption of university research operations due to the pandemic will almost certainly delay that. Once a validated bioassay is available, it should be a useful tool to help farmers determine when it is safe to replant that alfalfa field. It can also be used to study the incidence of autotoxicity on a farm scale, which should help clarify management methods to help mitigate the problem. *(A full report of the results of this project can be found at alfalfa.org/pdf/USAFRI/Final%20Reports/2017/17Cassida-2.pdf.)* Funding for this study was provided by the U.S. Alfalfa Farmer Research Initiative of the National Alfalfa & Forage Alliance.