Alfalfa Autotoxicity Soil Bioassay Predicts Alfalfa Performance

Paige Baisley, Michigan State University
Kim Cassida, Michigan State University
Erin Hill, Michigan State University

Alfalfa autotoxicity – where compounds from one plant inhibit growth in another plant – sometimes prevents successful reseeding without rotating out of alfalfa. Site and management factors like soil texture, stand age, stand density, and rotation interval influence the presence and persistence of autotoxicity. Although longer rotation intervals up to two years increase reseeding success, the effective duration is uncertain. To address this uncertainty, we are developing a two-week soil-bioassay to predict the autotoxic potential of field soils based on alfalfa seedling performance. The bioassay is designed to function as a mail-in test through plant diagnostic services to help farmers make appropriate planting decisions.

Soils for the bioassay were collected at 15-cm depth, sieved to 6 mm, and stored at -80˚C. Field-moist soil was standardized to 160 g dry weight equivalent and added to cone-tainers (7.5 x 35 cm) in a greenhouse (17-21˚C; 16:8 light:dark). One alfalfa seedling was grown in each cone-tainer for 14 days with soil moisture maintained at 60% water-holding capacity and then destructively harvested to measure root morphology and root and shoot dry mass. To allow for comparison across runs, each run included three control cones with peat-based potting media and all bioassay measurements were expressed as a percent of the average control value within a run.

Bioassay validation used soils from an autotoxicity plot experiment designed to induce a range of autotoxic responses. Plots from a 5-year-old alfalfa variety trial were split and terminated 3 months or < 1 month before reseeding a single alfalfa variety, creating long and short fallow rotation intervals, respectively, with four levels of previous variety. Adjacent plots not previously in alfalfa served as controls. The experimental design included four replicates of each rotation interval-variety combination and four controls. Soils were collected from 30 treatment and four control plots directly before late summer reseeding, with three bioassay replicates conducted per sample. Alfalfa performance in plots was measured as first-cut biomass the year after planting. Biomass and root length were averaged by treatment combination (n = 4 plots; n = 12 bioassays). Bioassay root length was positively related to first-cut alfalfa biomass (R^2 = 0.66, $P < 0.05$), and both variables numerically ranked Short < Long < Control.

Additional validation used soils from production alfalfa fields and their grass borders to assess bioassay ability to consistently distinguish between grass and alfalfa soils and to identify site characteristics influencing bioassay performance. Site characteristics included soil pH, phosphorus, potassium, percent clay, and, at alfalfa sites, stand age and density. Soils (n = 105) were collected in fall 2024 and 2025 with four bioassay replicates per sample. Root length was shorter in alfalfa soils (56.8% of control) than in grass soils (83.7% of control) ($P < 0.05$). Within alfalfa soils, the best model for root length determined by backwards selection based on AIC was marginally significant ($P = 0.06$, $R^2 = 0.13$) and included pH ($P = 0.11$) stand age ($P = 0.04$) and stand density ($P = 0.08$). This is consistent with the understanding that autotoxicity increases in older stands. These results suggest the bioassay has potential as a commercial test once a root length suppression threshold for declaring planting-prohibitive autotoxicity is established.