Effect of Seed-Based Agrochemical Treatments on AMF-Mediated Maize Growth and Herbivore Performance

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Introduction

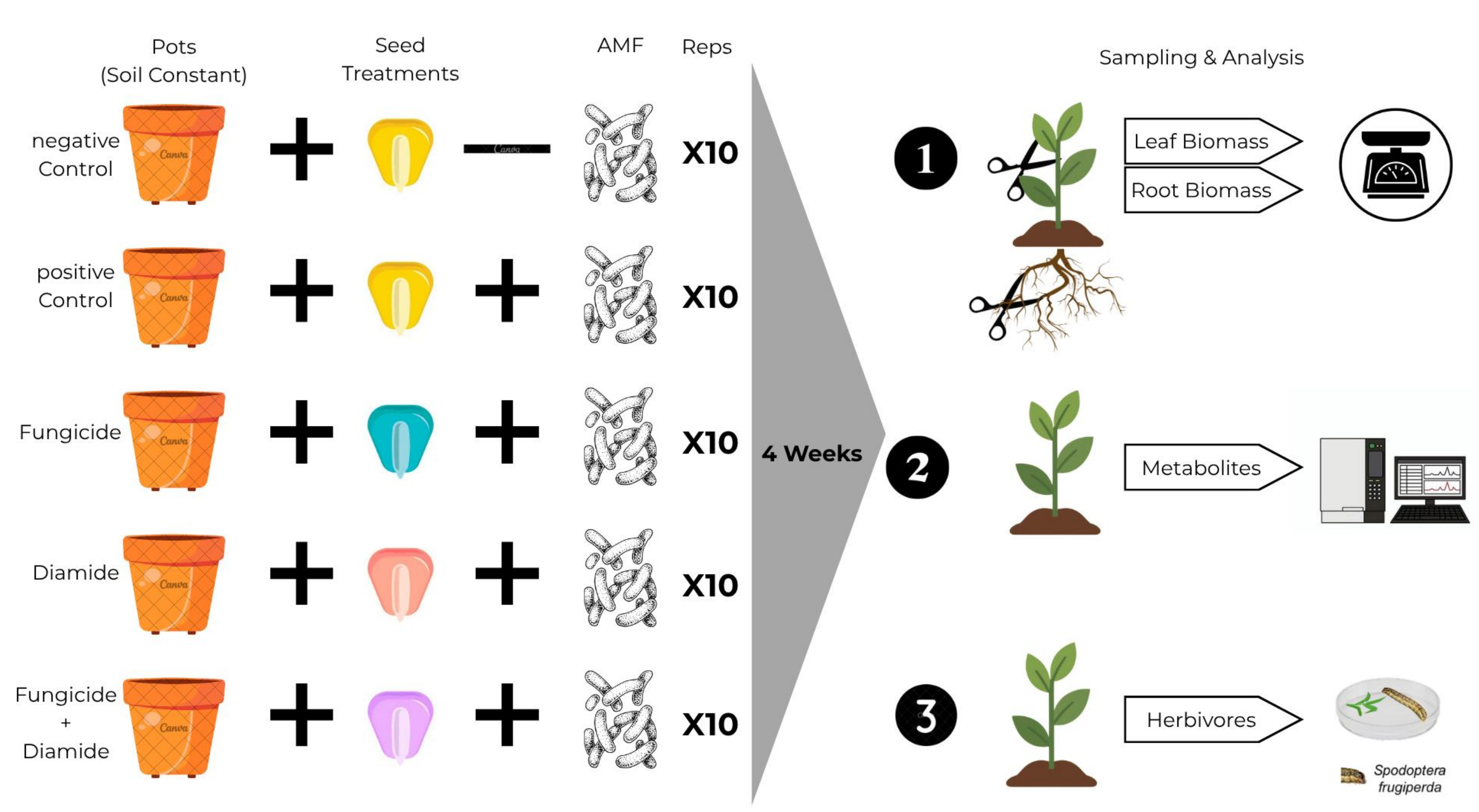
- Seed-applied fungicides and insecticides are ubiquitous in maize, but their effects on beneficial soil symbionts like arbuscular mycorrhizal fungi (AMF) are less understood[1].
- AMF typically reduce damage from generalist herbivores (mycorrhiza-induced resistance), but this benefit may be eroded by agrochemicals that suppress the symbiosis [2, 5].
- Maize benzoxazinoids are well-characterized chemical defenses against the fall armyworm (*Spodoptera frugiperda*), a major generalist pest, making this an ideal system to test whether agrochemicals disrupt AMF-mediated chemical defense [3].
- Fungicides directly target the fungal symbiosis (a long-exploited method for experimentally removing AMF), while insecticides like neonicotinoids and diamides are not necessarily neutral, with documented but inconsistent non-target effects on soil and plant-associated microbial communities [4]
- This study combines a fall armyworm bioassay with HPLC-DAD metabolite profiling to test how four seed treatments (Fungicide, NN+F, Diamide, D+F) interact with AMF status to shape maize growth, herbivore resistance, and secondary chemistry.

Hypothesis & Prediction

Hypothesis: AMF colonization reduces fall armyworm feeding damage via induced changes in maize secondary chemistry, and this protection is disrupted when AMF is suppressed by seed-applied fungicide

Predictions: Mycorrhizal plants will sustain less leaf damage than non-mycorrhizal plants
• Fungicide-treated plants will lose AMF protection and show damage levels resembling non-mycorrhizal controls
• These differences will be reflected in measurable shifts in benzoxazinoid-class metabolites between treatments

Methods



1. Maize seedlings were grown in five seed treatment groups (Negative Control, Positive Control, Fungicide, Diamide, Fungicide + Diamide), each crossed with AMF inoculation status, 10 replicates per group.
2. After 4 weeks, plants were destructively sampled for leaf and root biomass, secondary metabolite profiling (HPLC-DAD), and fall armyworm (*Spodoptera frugiperda*) feeding bioassays.
3. Metabolite peak tables were assembled in chromatographR (retention time alignment, peak detection, peak table assembly) and compared across treatments using PERMANOVA and random forest (Boruta) variable selection
4. Biomass and herbivory data were analyzed using factorial ANOVA with planned pairwise contrasts (Holm-adjusted)
5. Larval weight gain was assessed both as raw final weight and with initial weight included as a covariate, to distinguish treatment effects on growth from pre-existing differences in starting size

Results

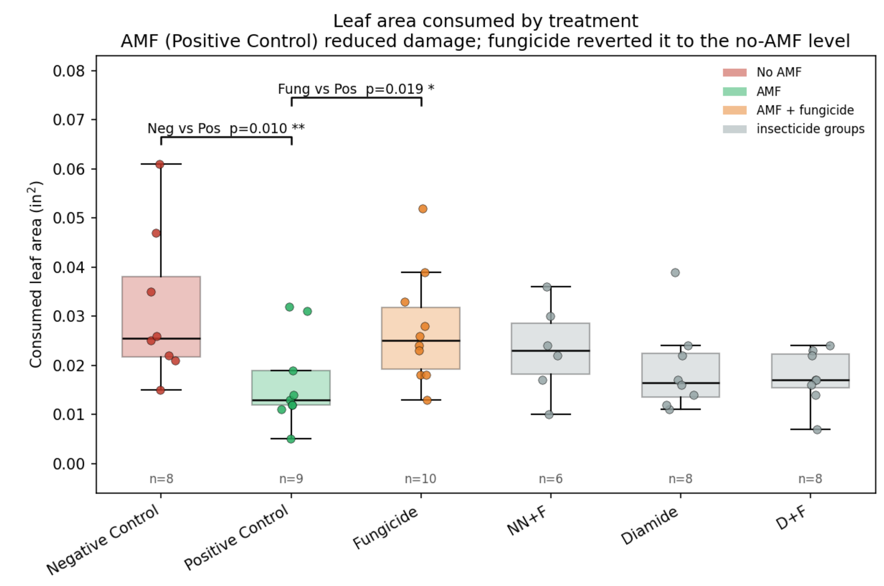


Fig. 3: Leaf area consumed by fall armyworm larvae across all six treatments, showing AMF (Positive Control) reduced damage relative to Negative Control, an effect reversed by fungicide co-treatment.

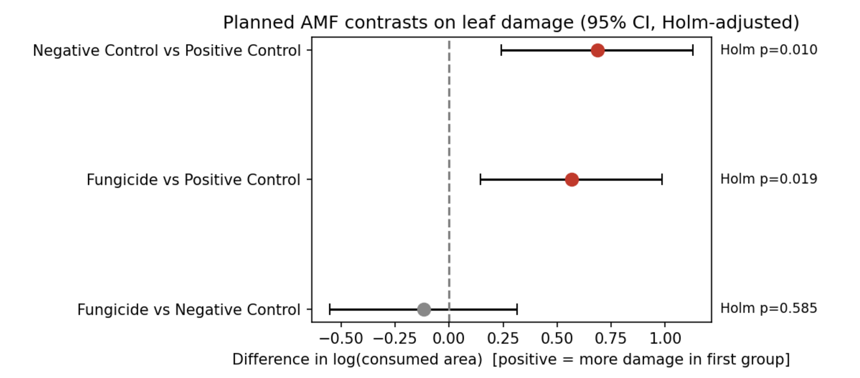


Fig. 4: Holm-adjusted pairwise contrasts in log(consumed leaf area) showing reduced damage on AMF-inoculated (Positive Control) plants relative to Negative Control and Fungicide.

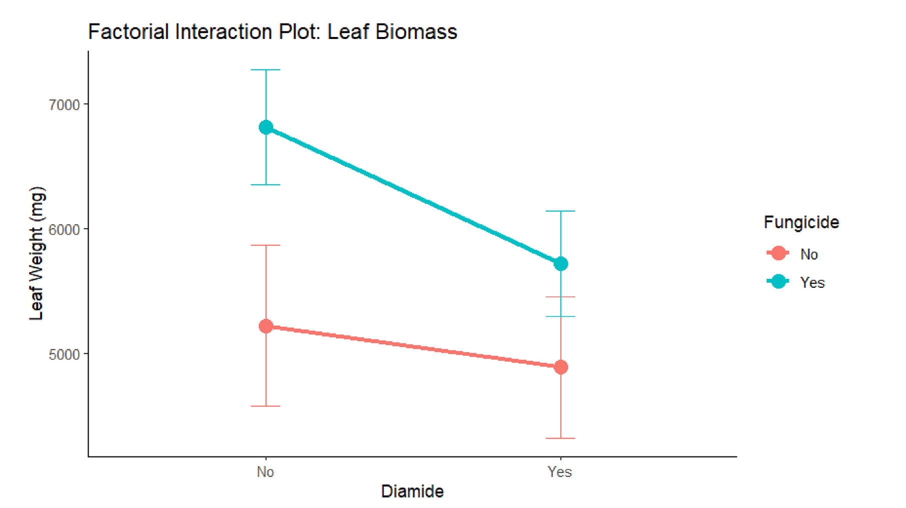


Fig. 5: Factorial interaction plot of leaf biomass by fungicide and diamide treatment, showing fungicide increased biomass regardless of diamide status.

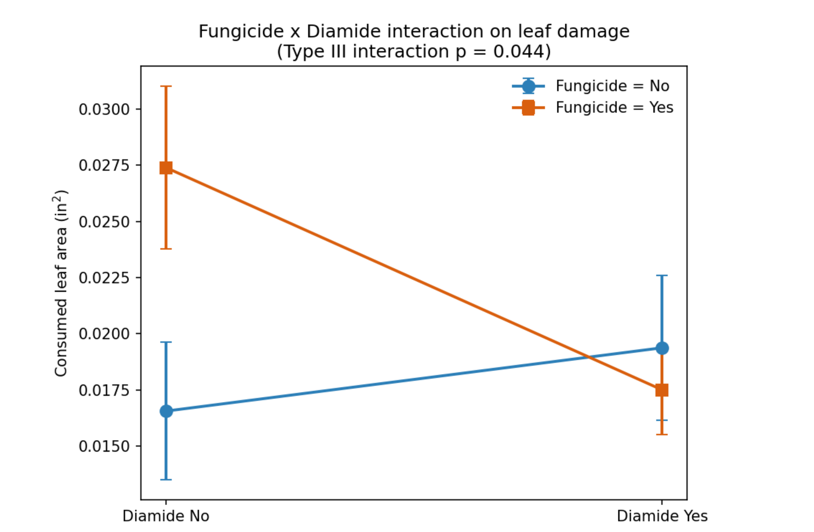


Fig. 6: Interaction plot of consumed leaf area showing that fungicide increased herbivory when diamide was absent but not when diamide was present (Type III interaction p = 0.044).

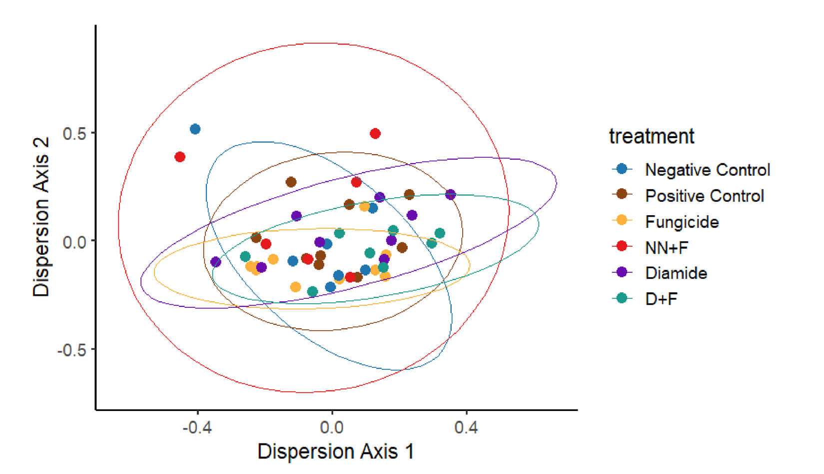


Fig. 7: NMDS-based dispersion of overall leaf chemical profiles by treatment, showing substantial overlap among groups consistent with the non-significant PERMANOVA result.

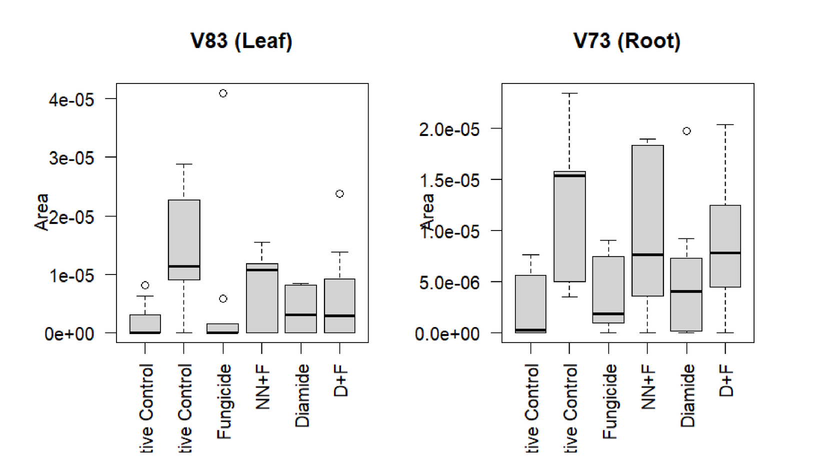


Fig. 8: Peak area of two treatment-responsive metabolites identified by random forest (Boruta), V83 in leaf and V73 in root, across the six seed treatments.

Discussion

- **AMF reduced herbivory roughly two-fold**, consistent with mycorrhiza-induced resistance against generalist chewers, and this protection was abolished by fungicide seed treatment, the same treatment that suppresses AMF colonization.
- This knockout pattern (fungicide damage ≈ no-AMF damage, both > intact AMF) supports a causal role for AMF, not just a correlate of inoculation.
- The **fungicide-driven increase in biomass without a corresponding herbivory benefit suggests the symbiosis carries a growth cost** that fungicide releases, while simultaneously removing the chemical defense benefit, a real biological trade-off rather than a uniformly negative effect.
- Whole-profile metabolite differences were subtle (non-significant PERMANOVA), but specific peaks (V83, V73) varied meaningfully by treatment, suggesting AMF/agrochemical effects act on a small number of defense-relevant compounds rather than reorganizing the full secondary metabolome, consistent with targeted modulation rather than broad metabolic shifts.
- Together, these results suggest **fungicide use can dampen biological pest-resistance service that AMF normally provide**, with consequences for how seed treatments are combined in integrated pest management

Limitations: Larval weight interpretation depends on whether initial weight is controlled (covariate-adjusted analysis shows no treatment effect); NN+F retained only 6 replicates after attrition; chemical identities of V83/V73 are putative pending standards-based confirmation

References & Acknowledgements

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