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

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Abstract

Pesticide seed treatments are applied to the majority of maize planted in the United States, yet their consequences for beneficial soil symbionts remain incompletely characterized. Arbuscular mycorrhizal fungi (AMF) colonize the roots of most crop species and, in addition to improving mineral nutrient acquisition, frequently confer resistance to generalist chewing herbivores. Because fungicides are known to suppress AMF colonization, a fungicidal seed coating may reduce herbivore resistance at the same time that it protects the seedling from soil pathogens. The present study tested this possibility in maize. Sixty plants were grown in a common substrate across six seed treatment groups: a non-inoculated control, an AMF-inoculated control, and four AMF-inoculated groups grown from commercially pre-treated seed (fungicide; neonicotinoid plus fungicide; diamide; diamide plus fungicide). After four weeks, plants were assessed for leaf and root biomass, profiled for secondary metabolites by HPLC-DAD, and used in feeding assays with individually weighed *Spodoptera frugiperda* larvae. AMF-inoculated plants sustained approximately half the leaf area loss recorded on non-inoculated plants (1.98-fold difference; Holm-adjusted $p = 0.010$). Fungicide seed treatment abolished this difference, returning damage to the non-inoculated level ($p = 0.585$). Fungicide-treated plants produced approximately 25% greater leaf biomass ($p = 0.033$). Whole metabolite profiles did not differ significantly among treatments, although two individual chromatographic peaks were treatment-responsive. These

results indicate that fungicidal seed treatment can attenuate a biological pest-resistance service while simultaneously releasing a growth constraint.

Problem Statement

Pesticide seed treatment represents one of the most extensive changes in pest management practice of the past two decades. In place of scouting a field, identifying a pest population, and applying a targeted intervention, the grower purchases seed to which insecticide and fungicide have already been applied by the supplier. The decision is therefore made before planting and is applied uniformly, irrespective of whether a pest population is subsequently present. By 2011, between 79% and 100% of maize hectares in the United States were planted with neonicotinoid-treated seed (Douglas and Tooker, 2015). Treated seed has consequently become the default condition of the crop rather than a discretionary intervention.

The economic rationale is well established: treatment costs are modest relative to the value of an established stand, application is performed by the supplier, and protection is conferred during the interval in which seedling loss cannot be remedied by replanting. The rationale is therefore one of insurance rather than of targeted control. What this framework does not accommodate is that the treated seed germinates not into an inert substrate but into soil, which supports a dense biological community whose constituent organisms are not uniformly antagonistic to the crop.

Among the most functionally significant of these organisms are arbuscular mycorrhizal fungi. AMF colonize the roots of most terrestrial plant species, extend hyphal networks into soil volumes inaccessible to root hairs, and transfer phosphorus and other mineral nutrients to the host in exchange for photosynthetically fixed carbon. In many species they also alter the plant's interactions with insect herbivores, such that colonized plants sustain less feeding damage from

leaf-chewing insects. This phenomenon, termed mycorrhiza-induced resistance, constitutes a form of biological pest suppression that is not purchased and is not accounted for in conventional input decisions.

Fungicides are, by definition, compounds selected for activity against fungi, and they do not discriminate between the pathogenic fungi that constitute their intended target and the mutualistic fungi already established in the soil. This raises a testable proposition that has received limited direct examination: the fungicidal component of a commercial seed coating may attenuate a pest-resistance service that the crop would otherwise obtain without cost. The relevant pest pressure is substantial. The fall armyworm, *Spodoptera frugiperda*, was restricted to the Americas until 2016, when outbreaks were first recorded in West and Central Africa (Goergen et al., 2016); it subsequently spread across sub-Saharan Africa and into Asia within several years and is now among the principal biotic constraints on global maize production. It is also a generalist chewing herbivore, the feeding guild against which mycorrhiza-induced resistance is most consistently reported.

The present study tested this proposition under controlled conditions and determined whether any change in herbivore damage was accompanied by measurable shifts in maize secondary chemistry.

Literature Review

The arbuscular mycorrhizal symbiosis is among the oldest known plant associations. Fossil and phylogenetic evidence places its origin at more than 450 million years before present, indicating that it predates the evolution of true roots and has been a component of terrestrial plant life since the colonization of land (Smith and Read, 2008). Although its nutritional function is the most extensively documented, Jung et al. (2012) synthesized evidence that colonization also

reorganizes host defensive physiology. Colonized plants are not merely better provisioned; their defensive responses are primed, such that induction occurs more rapidly and at greater magnitude upon attack without the metabolic cost of maintaining those defenses constitutively.

The consequences for herbivores are documented but not uniform. Koricheva, Gange and Jones (2009) conducted a meta-analysis of the available literature and reported that the effect of mycorrhizal colonization on insect performance depends substantially on herbivore feeding guild. Generalist chewing insects, which exploit many host species and are not adapted to the chemistry of any one of them, generally perform more poorly on mycorrhizal plants, whereas specialists frequently perform better. The fall armyworm belongs to the former category, which makes it an appropriate test organism for detecting a protective effect should one be present.

The chemical basis of maize resistance to lepidopteran herbivores is comparatively well resolved. Benzoxazinoids, a class of compounds stored in an inactive glycosylated form and enzymatically activated upon tissue disruption, constitute the principal deterrent (Wouters et al., 2016). This is methodologically consequential, since the identity of the relevant defensive chemistry permits changes in herbivore performance to be examined against measurable metabolite variation rather than treated as unresolved.

Evidence regarding pesticide effects on the symbiosis is asymmetric. On the fungicide side, Hage-Ahmed, Rosner and Steinkellner (2019) reviewed the substantial literature demonstrating that fungicides suppress AMF colonization. This suppression is sufficiently reliable that fungicide application has long been employed as an experimental method for removing mycorrhizae from a system. Evidence concerning insecticides is less settled. Parizadeh, Mimee and Kembel (2021) demonstrated that neonicotinoid seed treatments significantly restructure both soil and phyllosphere bacterial communities, indicating that

compounds directed against insects are not necessarily neutral toward other components of the plant-associated microbiome. Whether such non-target activity extends to AMF, and whether it carries consequences for defense, has not been resolved.

Work conducted in the laboratory in which the present study was carried out has established the causal sequence under examination here. Jordan and Kessler (2026) demonstrated that intercropping and cover cropping alter maize secondary metabolism and, through it, herbivore resistance. That study establishes the pathway from management practice to plant chemistry to insect performance for a different management variable within the same system.

These literatures have seldom been integrated. Substantial evidence exists on the modification of plant defense by AMF, on the suppression of AMF by fungicides, and on the scale of seed treatment adoption. What has been lacking is an experiment crossing a realistic panel of commercial seed treatments with mycorrhizal status while measuring growth, secondary chemistry, and herbivore damage in the same plants.

Methods

Sixty maize plants were grown in a Cornell University greenhouse. All pots received the same growing substrate, amended with bone meal and homogenized as a single batch prior to filling, so that nutrient status would not vary systematically among treatment groups. Plants were assigned to six groups of ten. The Negative Control received untreated seed and no fungal inoculum. The Positive Control received untreated seed and AMF inoculum. The four remaining groups all received AMF inoculum and differed only in seed treatment: fungicide alone; neonicotinoid plus fungicide (NN+F); diamide insecticide alone; and diamide plus fungicide (D+F). Seed was commercially pre-treated, such that the treatments correspond to products

available to growers rather than to compounds applied manually at an experimentally selected rate. Inoculum was homogenized into the growing medium at 6.5 g per pot.

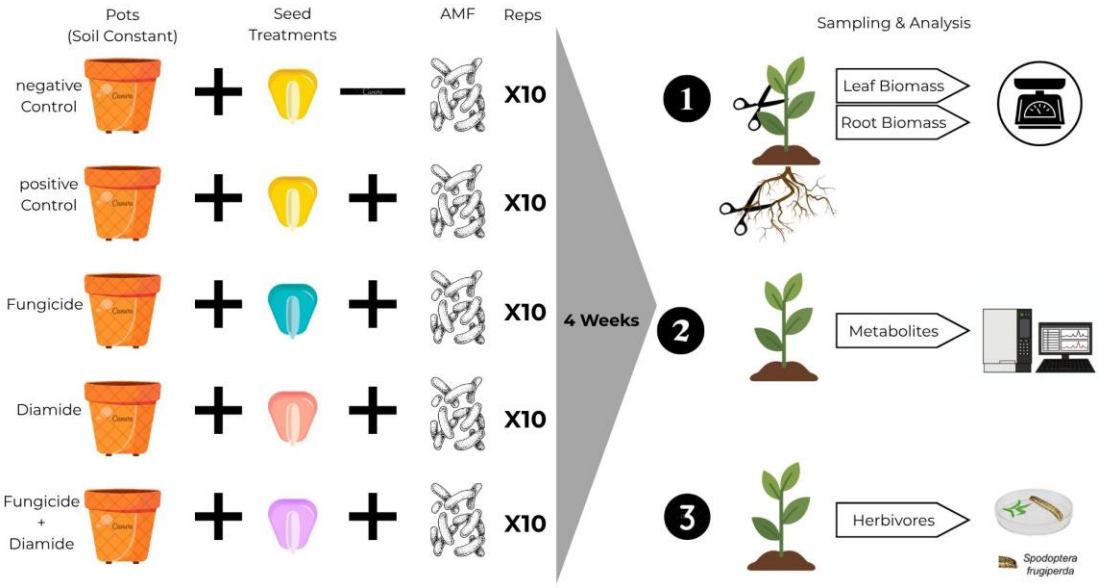

Figure 1. Experimental design. Each pot received a common substrate, a seed treatment, and, except in the Negative Control, AMF inoculum. After four weeks, plants were harvested for leaf and root biomass, profiled for secondary metabolites, and used in fall armyworm feeding assays. A sixth group, neonicotinoid plus fungicide, is not represented in this schematic but was included in the experiment.

After four weeks of growth, each plant was used in a feeding assay. A single fall armyworm larva was weighed individually and presented with maize leaf tissue of a standardized initial area (0.274 in², approximately 1.77 cm²). After four days, the remaining tissue area was measured and the larva was reweighed. Consumed leaf area, calculated as the difference between initial and remaining area, constitutes the primary damage response reported here. Plants were subsequently harvested destructively for leaf and root fresh biomass, and leaf and root tissue was profiled by high-performance liquid chromatography with diode-array detection (HPLC-DAD). Chromatograms were processed in *chromatographR* for retention-time alignment, peak detection, and peak table assembly. Whole chemical profiles were compared among treatments by PERMANOVA and visualized by non-metric multidimensional scaling (NMDS). Individual

treatment-responsive peaks were identified using a random forest variable-selection procedure (Boruta).

Damage data were analyzed on a logarithmic scale, which satisfied assumptions of normality and homogeneity of variance (Shapiro-Wilk $p = 0.52$; Levene $p = 0.97$). A one-way ANOVA was conducted across all six groups, followed by three planned contrasts specified in advance (mycorrhizal against non-mycorrhizal, fungicide against mycorrhizal, and fungicide against non-mycorrhizal), with Holm correction applied for multiple comparisons. The four groups that shared AMF inoculation and differed only in seed chemistry (Positive Control, Fungicide, Diamide, D+F) constitute a complete two-by-two design and were analyzed separately as a fungicide by diamide factorial using Type III sums of squares. The NN+F group could not be incorporated into this factorial, as no neonicotinoid-only group exists to complete the design, and is therefore reported descriptively. Final larval weight was analyzed by ANCOVA with initial larval weight as a covariate, since initial size strongly predicts final size and varied among groups by chance.

Attrition reduced the available dataset. Of 60 plants sown, 51 yielded complete plant and larval data and 49 yielded herbivory data. Group sizes for the damage analysis were as follows: Negative Control, 8; Positive Control, 9; Fungicide, 10; NN+F, 6; Diamide, 8; D+F, 8. All analyses were conducted on complete cases, and no values were imputed.

Findings

Mycorrhizal plants sustained substantially less feeding damage than non-mycorrhizal plants. Larvae removed a mean of 0.0315 in^2 of leaf area from non-mycorrhizal plants and 0.0166 in^2 from mycorrhizal plants, approaching a two-fold difference. Treatment was a significant predictor of damage overall ($F(5,43) = 2.91$, $p = 0.024$).

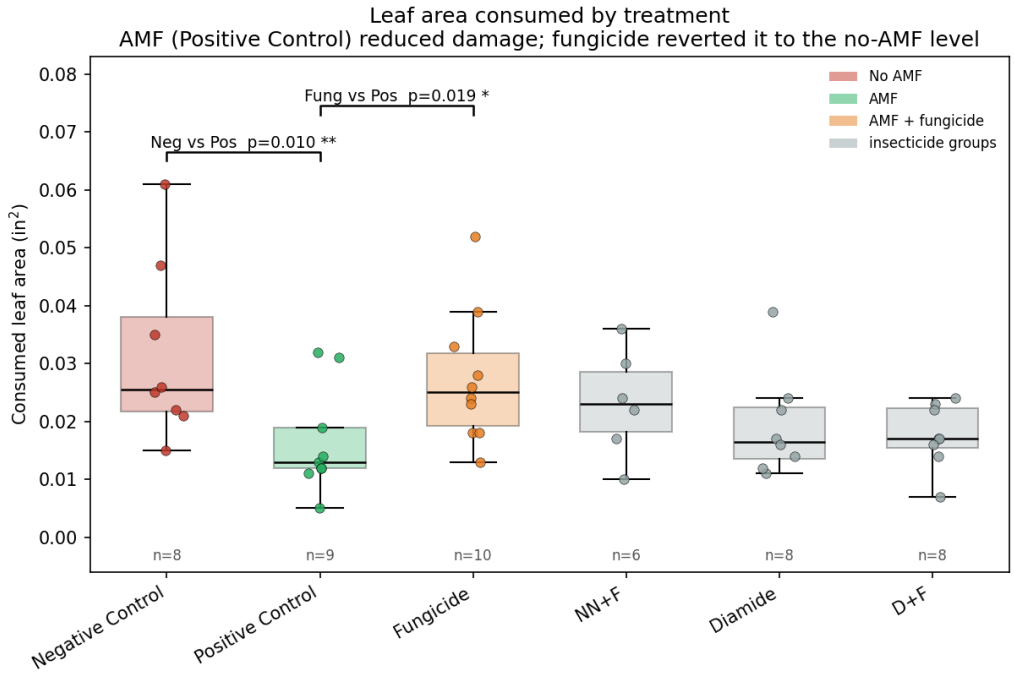

Figure 2. Leaf area consumed by fall armyworm larvae across all six treatments. AMF-inoculated plants (Positive Control) sustained approximately half the damage recorded on non-inoculated plants (Negative Control), and fungicide-treated plants returned to the non-inoculated level.

The planned contrasts resolved this pattern further. Non-mycorrhizal plants sustained 1.98 times the damage recorded on mycorrhizal plants (Holm-adjusted $p = 0.010$). Fungicide-treated mycorrhizal plants sustained 1.76 times the damage recorded on untreated mycorrhizal plants ($p = 0.019$). Fungicide-treated plants and plants that received no inoculum were statistically indistinguishable ($p = 0.585$). Within the resolution of this experiment, the fungicide treatment did not merely reduce the protective effect but eliminated it.

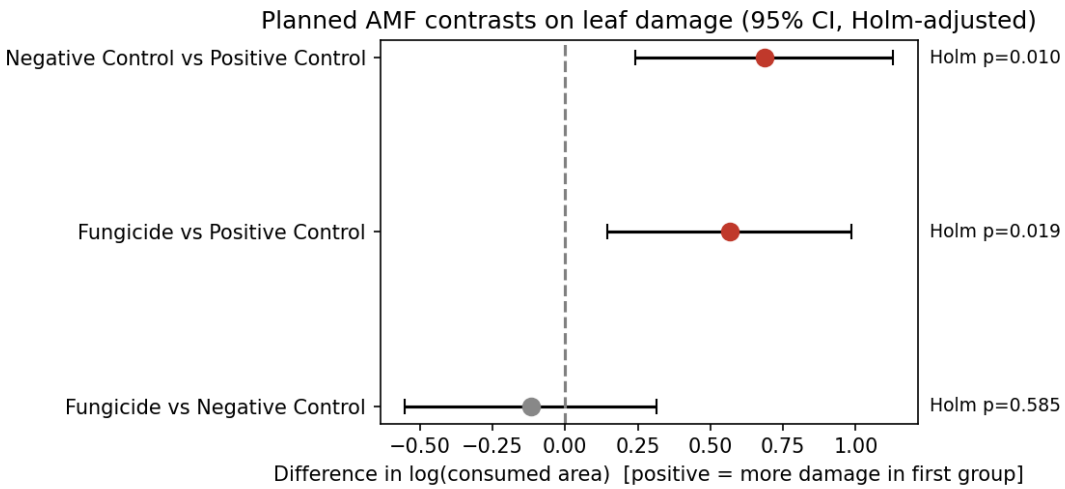

Figure 3. Planned contrasts on log-transformed consumed leaf area, with 95% confidence intervals and Holm-adjusted p-values. Positive values indicate greater damage in the first-named group. The confidence interval for the fungicide against non-inoculated contrast includes zero.

The insecticide treatments modified this relationship. In the two-by-two factorial, neither fungicide nor diamide produced a significant main effect on damage, but their interaction was significant ($F(1,31) = 4.41$, $p = 0.044$). Fungicide markedly increased damage in the absence of diamide (0.0166 to 0.0274 in²) but not in its presence (0.0194 to 0.0175 in²). The elevation in damage associated with fungicide treatment was therefore detectable only in plants that had not additionally received a chemical insecticide.

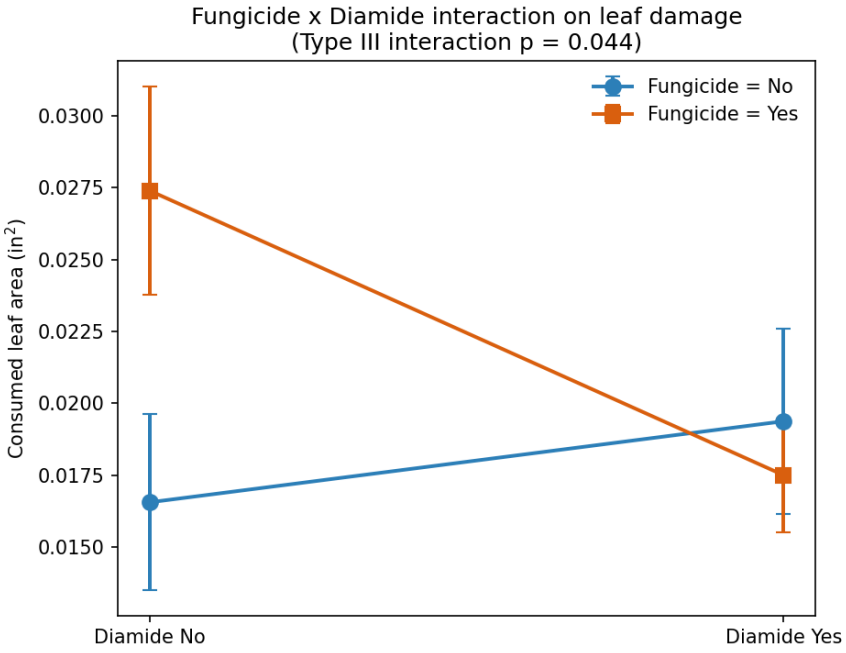

Figure 4. Fungicide by diamide interaction on consumed leaf area. The effect of fungicide on herbivory depended on the presence of a diamide insecticide (Type III interaction p = 0.044).

Plant growth responded in the opposite direction to defense. Fungicide-treated plants produced significantly greater leaf biomass than untreated plants (6323 mg against 5060 mg; $F(1,33) = 4.94$, $p = 0.033$), a difference of approximately 25%, with a marginal effect in the same direction for root biomass ($F(1,33) = 3.19$, $p = 0.083$). Root and shoot biomass were strongly correlated across the experiment ($r = 0.89$, $p < 0.001$, $n = 51$), indicating a whole-plant growth response rather than a change in biomass allocation.

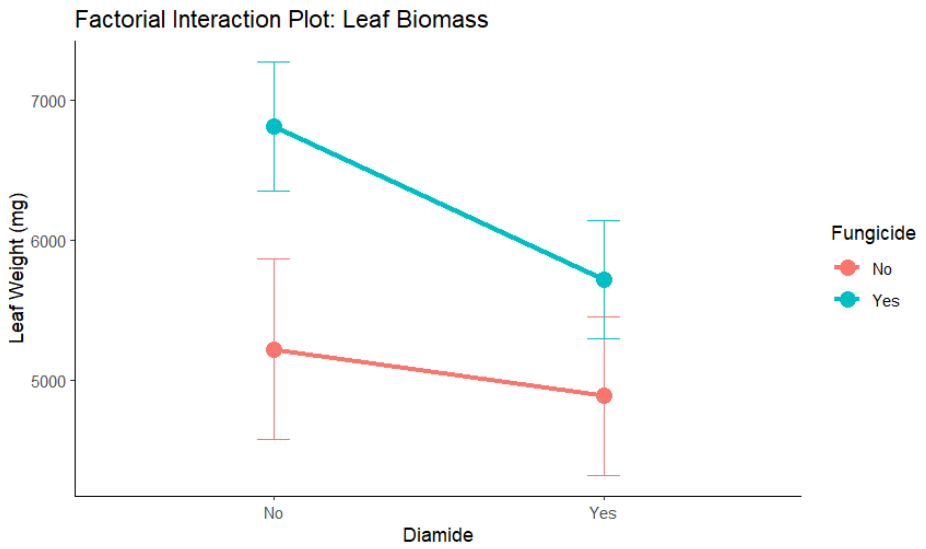

Figure 5. Leaf biomass by fungicide and diamide treatment. Fungicide-treated plants produced greater leaf biomass irrespective of diamide status.

Larval performance data proved less informative than anticipated. The great majority of larvae (96%) lost mass over the four-day assay, so final weight reflects feeding suppression rather than growth. Once initial weight was included as a covariate, and it was by a considerable margin the strongest predictor in the model ($F(1,44) = 218.0$, $p < 0.001$), treatment had no detectable effect on final larval weight ($F(5,44) = 0.97$, $p = 0.45$). A sequential model in which the treatment term is not adjusted for initial weight returns an apparently significant effect; that result is attributable to chance variation in larval starting mass among groups and is not interpreted here. Damage sustained by the plant, rather than mass change in the insect, is therefore the defensible response variable in this experiment.

Differences in secondary chemistry were subtle. PERMANOVA conducted on whole leaf metabolite profiles was not significant, and the NMDS ordination shows extensive overlap among treatment groups rather than separation.

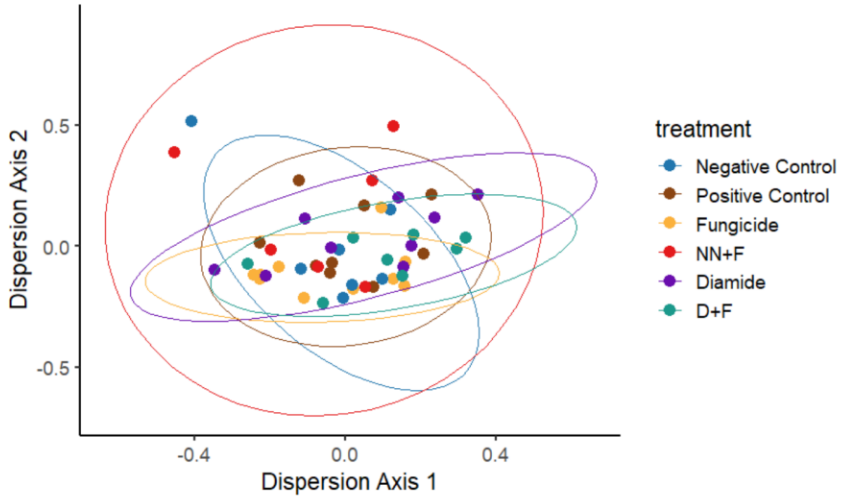

Figure 6. NMDS ordination of whole leaf chemical profiles by treatment. Groups overlap substantially, consistent with the non-significant PERMANOVA result.

Random forest variable selection nevertheless identified two chromatographic peaks that varied with treatment: one in leaf tissue (designated V83) and one in root tissue (V73). Both were highest in the AMF-only Positive Control and lower in the fungicide-treated groups, which is the direction predicted under mycorrhiza-induced resistance. The chemical identities of both peaks remain putative pending confirmation against authentic standards, and they are therefore reported as candidate markers rather than as named compounds.

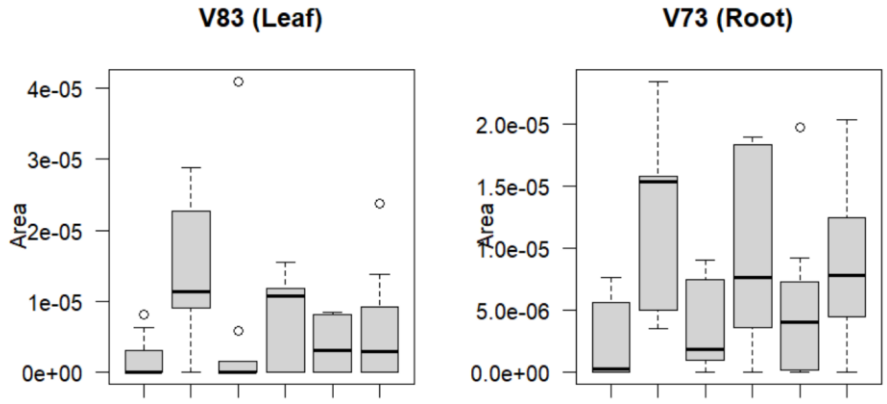

Figure 7. Peak areas of two treatment-responsive metabolites identified by random forest variable selection: V83 in leaf tissue and V73 in root tissue. The first two groups on each axis are the Negative and Positive Controls.

Discussion

The principal finding is that maize plants grown with mycorrhizal partners sustained approximately half the feeding damage from a major generalist pest, and that a commercial fungicide seed treatment eliminated this advantage. Both the seed treatment and the symbiosis constitute forms of crop protection; in this experiment, the purchased form displaced the biological one.

The equivalence between fungicide-treated and non-inoculated plants is central to the interpretation. Were mycorrhizal plants advantaged through some unspecified improvement in general vigor, fungicide-treated plants would be expected to occupy an intermediate position between the two controls. Instead they were statistically indistinguishable from the non-inoculated control, which is the pattern characteristic of an experimental knockout rather than of a correlate of inoculation.

The biomass data indicate that the effect is not uniformly detrimental. Fungicide-treated plants were approximately 25% larger. The mycorrhizal symbiosis imposes a carbon cost on the host, and suppression of the fungal partner releases that carbon for growth while the plant simultaneously forfeits the associated defensive benefit. The result is therefore a trade-off rather than a straightforwardly negative effect, and it accounts for the fact that seed treatments may appear effective on the basis of early seedling vigor while a cost accrues in a variable that is not routinely measured.

The chemical results are consistent with a targeted rather than a global mechanism. Had colonization reorganized maize secondary metabolism comprehensively, the ordination would be expected to separate the treatment groups and PERMANOVA to detect the difference; neither occurred. Two peaks nonetheless varied with treatment while the remainder of the profile did

not, consistent with modulation of a small number of defense-relevant compounds. This accords with a priming mechanism, under which the capacity to respond changes more than baseline constitutive chemistry. Given the putative peak identities and the non-significant whole-profile test, however, the chemical evidence supports this interpretation without establishing it.

The fungicide by diamide interaction carries the most direct practical implication. Fungicide increased herbivory only in plants that had not received a diamide insecticide; where the insecticide was present, it appears to have compensated for the loss of biological protection and held damage approximately constant. The combination of insecticide with fungicide-treated seed therefore conceals the attenuation of an ecological service rather than preventing it, since the service is lost irrespective of whether its absence is detectable in damage measurements. Given that fall armyworm populations have already developed resistance to multiple insecticide classes, a management system that reduces biological resistance while depending on chemical resistance carries a form of risk that is not readily apparent from field performance.

Several limitations constrain these conclusions. The most consequential is that AMF root colonization was not directly quantified. The inference that fungicide suppressed the symbiosis rests on the established literature and on the knockout pattern evident in the damage data rather than on a measurement obtained from these plants. Root staining and colonization scoring constitute the immediate next step, and until they are completed the causal claim is well supported but not established. Second, larvae lost mass across all treatments, so the assay addresses feeding suppression rather than insect growth. Third, the NN+F group retained only six replicates and could not be incorporated into the factorial design, so no conclusions specific to neonicotinoids follow from this experiment. Fourth, the study comprised a single greenhouse run on four-week-old seedlings in a common substrate; field soils support native AMF

communities, variable phosphorus availability, and competing microorganisms, any of which could amplify or attenuate the observed effect. Finally, the identities of V83 and V73 await confirmation against authentic standards.

An alternative explanation warrants consideration. Fungicide-treated plants were approximately 25% larger, and larger seedlings may present leaf tissue that differs in toughness, water content, or nutritional quality independently of any fungal effect. Consumption expressed per unit of initial larval mass followed the same pattern across the six treatment groups ($p = 0.063$), which is consistent with a defensive rather than a purely morphological explanation, but direct measurement of leaf toughness and carbon to nitrogen ratio would be required to exclude this alternative.

The question retains particular significance where treated seed and fall armyworm are expanding most rapidly. Treated seed is increasingly adopted in smallholder maize systems in South Asia and sub-Saharan Africa, which are also the systems in which armyworm damage is most severe, purchased inputs are least affordable, and an uncompensated biological pest-resistance service carries the greatest relative value. If a fungicidal coating exchanges that service for a modest gain in early growth, the resulting calculation differs substantially between production systems of differing scale and input intensity. Evaluation under field conditions, in soils supporting native fungal communities, represents the logical extension of this work.

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Acknowledgements

This research was supported by the Laidlaw Undergraduate Research and Leadership Programme, whose funding enabled the greenhouse and analytical work reported here. The author thanks Dr. André Kessler for his guidance and mentorship throughout the project, and Juan Pablo Jordán Dávalos for the foundational analytical framework and continued support. The author further thanks Ilian Vakarelov for processing the herbivory data, and the members of the Kessler Laboratory and the Cornell greenhouse staff for their assistance with plant care and experimental setup.