

Article

Coating Kernza[®] Seeds with Hydrophilic Polymers and Mycorrhizal Inoculants Affects Seedling Emergence and Growth in Drought

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Abstract

Perennial grains, such as Kernza[®] (*Thinopyrum intermedium*) show promise in their ability to produce grain crops while withstanding drought and temperature extremes. However, Kernza establishes slowly, particularly in dry soils. Seed coat treatments that retain water around the germinating seed (superabsorbent polymers, or SAPs) or assist water uptake in the seedling (arbuscular mycorrhizal fungi, or AMF) could improve Kernza establishment. We conducted a greenhouse study to test the efficacy of two SAPs, one AMF inoculant (*Rhizophagus intraradices*), and each SAP $\times$ AMF combination as seed coats, on Kernza germination and growth under well-watered versus drought conditions. Two AMF treatments had higher proportion emergence under drought conditions than non-drought. Aboveground seedling biomass was significantly lower in four of the five seed coat treatments compared to their non-drought counterparts, but control plants did not exhibit this size reduction. Plant root colonization by AMF increased in all non-drought treatments regardless of seed coat treatment. Contrary to expectations, both SAPs tested did not improve germination and actually hindered growth under drought conditions. The AMF inoculant sometimes improved germination, but not seedling development. Future research on improving Kernza seed establishment should target more species-specific AMF inoculants or nutrient supplements as seed coats, rather than SAPs.

Keywords: *Thinopyrum intermedium*; superabsorbent polymer; arbuscular mycorrhizal fungi; *Rhizophagus intraradices*; perennial grain; seed germination; aboveground biomass; drought tolerance

1. Introduction

Recent changes in climate introduce significant challenges in twenty-first century agriculture. Increased variability in moisture and temperature regimens [1], increased soil degradation [2], and shifting pest and disease ranges and lifecycles [3] represent only a few of these challenges. While the impacts of climate change vary significantly by region, many already food insecure countries will face an increased risk of food insecurity moving forward [4]. Wheat alone represents ~20% of both daily calories and protein globally, and its production is split roughly evenly between the developed and undeveloped world [5].

One of the strategies employed to address these threats to global food security has been the development of perennial grain agriculture, including the breeding of novel grain, legume, oilseed, and forage crops [6,7]. Given their ability to provide multiple growing seasons' harvest with a single planting, perennial grains offer the potential for decreased soil disturbance and lower energy, labor, and resource inputs [8]. Additionally, given their establishment of deep, complex root systems, they provide increased nutrient retention,



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carbon sequestration, and water infiltration, as well as protection from soil erosion of improved soil structure [9]. In particular, the recent development and commercialization of perennial rice varieties with yields comparable to their annual counterparts represents incredible strides toward improved livelihoods for farmers, lowered inputs maintaining historical yields, and increased soil and water quality [10].

Another perennial grain recently released for commercial cultivation is the cool-season intermediate wheatgrass Kernza® (*Thinopyrum intermedium* [Host] Barkworth & D.R. Dewey), a perennial dual-use crop being bred and grown both as forage and as an annual wheat grain analog [11,12]. Despite its potential to provide many of the aforementioned ecosystem services of perennial agriculture, Kernza still faces multiple agronomic challenges. One such challenge is its slow early establishment relative to annual crops [13], particularly under dry conditions. One strategy to aide in the early establishment of direct seeded crops in arid environments has been seed coating [14].

Pedrini et al. [15] define “seed coating” as “the process of applying exogenous materials to the surface of the natural seed coat”. This procedure is employed for one or both of two reasons: to alter the physical properties of the seed, and/or to apply chemical, biological, or biochemical treatments to the outside of seeds [15,16]. While this technology has existed since the 1800s, only in recent decades has it been expanded to mainstream precision agriculture not only to improve seed handleability, but also to treat seeds with enhancing agrichemicals particularly pesticides and fertilizers [17]. However, there is growing interest in expanding coated seed treatments beyond these categories.

Our study examines the efficacy of three active seed treatments when applied as a seed coating: two superabsorbent polymers (SAP) and a mycorrhizal inoculant. SAPs are synthetic or natural hydrophilic gels that form networks of polymer chains capable of reversibly absorbing and retaining water hundreds to thousands of times their dry mass in water [18], and have been used in multiple capacities, including construction materials, hygiene products, and soil amendments. These hydro gels have been used to draw moisture from the surrounding soil into closer proximity to the seed, thereby increasing imbibition and aiding in germination [19]. For plants growing in water-stressed conditions, SAPs have been found to increase the plant-available water in soils, induce faster growth of plants, and prolong plant survival under periods of water stress [20]. As soil amendments, these compounds are incorporated into the soil or directly coated on exposed roots in transplanting [21,22]. However, the potential to apply SAPs with precision via seed coating has yet to be explored. Terra-Sorb® and Soil Moist™ are both polyacrylates that are specifically marketed for horticultural use. Both are fully synthetic and of petrochemical origin, as are the vast majority of SAPs [18]. Several studies examining the performance of growing plants under drought conditions with Terra-Sorb have shown that its use is generally effective; however, some long-term results remain variable [23–25]. We were unable to find a published study on the effects of Soil Moist; however, literature reviews on synthetic acrylic polyacrylamides broadly show that they hold significant potential for increasing field germination, plant-available soil moisture, and crop yield, as well as increasing fertilizer efficiency [26,27]. This study examines the early impacts of two different SAPs, Terra-Sorb and Soil Moist, as well as their effects in conjunction with an inoculant of arbuscular mycorrhizal fungi (AMF).

The AMF species most commonly used in commercial inoculants is *Rhizophagus intraradices* ([Dangeard] Walker and Schüßler). The colonization of AMF in the root tissues of agronomic plants is well documented as increasing plant growth, resilience, and production as the hyphal association provides massively increased surface area with which to provide the host plant with water and mineral nutrients (particularly phosphorus) in exchange for photosynthates [28,29]. Additionally, these associations have been found to provide host

plants, and specifically annual wheat (*Triticum aestivum* L.), with increased drought resistance [30–32]. Many agricultural fields under conventional management have significantly diminished AMF diversity and abundance compared to native or organically managed soils [33–36]. Historically, Kernza has not exhibited high AMF responsiveness [37]; however, newer accessions of Kernza have shown an increased responsiveness to AMF [38]. Successful studies in applying rhizobia seed coat inoculants to legumes [39,40], and AMF to legumes and wheat [41–43], offers hope that similar results could be achieved in coating Kernza seeds with AMF.

One challenge in using SAPs as a soil amendment is that they are most effective with multiple applications over time, and at higher rates than in a single seed coating [44]. This poses a concern of longevity in the benefit of using SAPs as a seed coat and whether a targeted, small dose would be enough to ensure seedling establishment. Meanwhile, AMF take weeks to fully colonize a new plant host [45]—after which the AMF provides a long-term advantage to resource acquisition [25,26,46]. However, the utilization of both treatments together could allow the SAP to provide moisture for initial seedling germination and establishment, with its effects diminishing just in time for the AMF to colonize the seedling and continue to provide it with moisture as it grows. Amending field soil with Terra-Sorb upon planting Kernza has already been an established practice at The Land Institute [47], but Soil Moist remains untested. Additionally, no studies to date have been conducted testing either of these SAPs or other substances as seed coatings for Kernza.

The aim of this study was to determine whether SAP or AMF inoculants can be applied as a seed coat to Kernza to increase its establishment under drought conditions. We hypothesized that (1) adding SAP directly to the seed will pull moisture from the soil to the seed, aiding in germination and early establishment, and (2) adding AMF inoculation will increase AMF colonization rates in young seedlings, aiding in more vigorous growth and better establishment after germination. We held three predictions for these hypotheses: (1) Adding SAP to the seed coat will increase emergence rates, (2) adding an AMF inoculant to the seed coat will increase AMF colonization in seedling roots, and increase seedling growth and development over a longer span of time, and (3) adding both an SAP and AMF inoculants to the seed coat will both increase seedling emergence and later stage growth and development.

2. Materials and Methods

2.1. Study Times and Growing Conditions

This study was conducted in two rounds. The first round was conducted from 16 December 2022 to 17 February 2023 and the second round from 27 February 2023 to 1 May 2023. Both rounds were conducted in a climate-controlled greenhouse located at The Land Institute in Salina, KS (38.766949° N, −97.566082° W) maintained at mean climate temperature of 20 °C and relative humidity of 39%.

2.2. Plant Material and Seed Treatments

For both rounds we used Kernza® seed from accession 704, the most recently developed commercial accession at the time of this study. Two SAPs, Terra-Sorb® (Lebanon Seaboard Corporation, Cleveland, OH, USA) and Soil Moist™ (JRM Chemical, Inc., Lebanon, PA, USA), and the AMF inoculant Mykos (*Rhizophagus intraradices*) (Sierra Re-forestation Company, Gilroy, CA, USA) were tested in five distinct seed coat treatments: Terra-Sorb®, Soil Moist™, Mykos®, Terra-Sorb® + Mykos®, and Soil Moist™ + Mykos®. A sixth control group consisted of seed to which a seed coat was applied without any SAP or AMF inoculant. The Mykos blend contained 300 propagules/gram, a value periodically

assessed by the manufacturer via quality testing [48]; however, we did not independently assess spore counts of the inoculant in this study.

2.3. Soil Collection and Preparation

For each treatment in a round, plants were grown in field soil to better mimic wetting-drying cycles and presence of natural microbes (including resident AMF species) in field conditions, thus providing a more accurate assessment of how the seed coat treatments may perform in the field while also accurately controlling the water each plant received. The field soil for both rounds was a Cozad silt loam [49] collected from a tilled field in Salina, KS, USA (N 38.772642, W −97.590071). Field-collected soil was screened for insects using a 0.6 cm sieve, and dried for at least three weeks at in a Munters® desiccant dehumidifier (model# HCD-2250-EA-SFCS, Amesbury, MA, USA) at a range of 20–22 °C and 8–15% humidity. The soil was kept in dark conditions to preserve any mycorrhizal spores present. While we did not test this specific soil for AMF composition, previous research within 2 km of this field site has shown the local agricultural soil to contain ~30 species, on average, of Glomeromycota [50].

2.4. Seed Coating Process

A separate set of seeds was coated for each round of the experiment. We developed a novel seed coating protocol for Kernza® by adapting the procedure by Pedrini et al. [51]. We used a Rotary-4 Lab Seed Coating & Treating Unit (AgInnovation®, Walnut Grove, CA, USA) to encrust 3.5 g batches of Kernza® seed with 116 µL polyvinyl acetate (PVA) liquid adhesive, 2.0 g powdered talc as a bulk filler, and 0.035 g Terra-Sorb (mixed with 0.385 g talc), 0.035 g Soil Moist, and 0.05 g Mykos. PVA and talc were chosen for the binding agent and the filler, respectively, since both substances are commonly used in seed coating [17] and the materials were readily available. Each powdered treatment was run through a Krups coffee grinder (Ref# F2034251, Groupe SEB Deutschland GMBH, Frankfurt, Germany) until fine enough to pass through a 45 µm sieve (No. 325, Cole-Palmer Instrument Company LLC, Vernon Hills, IL, USA). Each batch underwent alternating layers of powder and binder at 75% maximum speed for ~10 s per layer. The first layer was composed of 0.0083 mL liquid PVA binder (Free Flow®, AgriLead®, Russel, KS, USA), dispensed in the center of the rotating bottom disk of the seed coating unit with a micropipette to atomize the liquid. This step was followed by one of the three powdered treatments. In the case of batches including more than one treatment, the SAP was applied before the AMF inoculant as consecutive layers. The subsequent layers consisted of binder and talc until eleven total layers were achieved. The talc layers provide an encrusted layer that protects the underlying treatment layer, increase seed weight (on average from 3.5 g uncoated seed to 5 g coated seed), and produce a more uniform seed shape. The final three layers consisted only of 0.0083 g each of the PVA binder Custom Coverage Red® (AgriLead®, Russel, KS, USA). This final step served not only to clearly identify seeds as treated, but also to help protect the underlying layers and increase flowability. Seeds finished treatment averaging a 60% increase in mass. Treated seeds were then left to open-air dry at room temperature for at least 48 h.

2.5. Experimental Design

Thirty 815 mL plant pots were filled with 700 g air dried field soil and treated with a Pylon TR insect bomb [4.5% Chlorfenapyr] prior to seeding. Total soil water holding capacity was calculated at 34% increase over dry mass. Each pot was planted at a depth of 0.5 cm, with four seeds evenly spaced toward the corners of the pot, for a total of 1140 plants in 360 pots per round. Each round was arranged in a greenhouse by watering regimen, with three blocks of the six different coating treatments per watering regimen.

The two watering regimens were non-drought (soil maintained at 70% water holding capacity by weight, every day) and drought (soil watered to 20% water holding capacity by weight, every other day). A pre-randomized subset of five pots from each seed coat treatment was weighed before watering. The mean weight of those five pots was calculated and used to determine the water mass to be added to all pots of the treatment to maintain the appropriate percent water holding capacity.

2.6. Trait Measurements

Four seedling growth response metrics were measured: rate of emergence, above-ground biomass, length of time needed to reach the V5+ stage of development (five or more leaf collars visible), and root AMF infection rates. All seedling emergence and growth response metrics were averaged by pot, so that each pot represented a single replicate. Emergence data were recorded per pot daily for the first 34 (Round 1) or 42 (Round 2) days. After this time had elapsed, pots with no seedlings were removed from the study. In the remaining pots, the largest plant with V5 or greater development stage was harvested and rinsed to remove any soil. Aboveground biomass of the harvested seedling was then dried in the Munters desiccant dehumidifier and weighed, while the root mass was preserved in 70% ethanol for future AMF colonization assessment.

If a pot did not have any V5 plants, the pot was thinned to the single most vigorous seedling. This seedling was allowed to continue growing until it reached V5 stage, then harvested. At Day 63 (Rounds 1 and 2), all remaining seedlings were collected and processed for aboveground biomass and roots regardless of their development stage.

We used the method developed by Vierheilig et al. [52] to stain the AMF colonization in collected root tissue. Roots from each sample were cut into 2 cm long sections, which were placed in a tissue embedding cassette and tied in nylon sock liners to contain fine root tissues. Samples were boiled in a 10% KOH solution for 5 min before rinsing and soaking them in a 5% boiling Sheaffer® black ink (Sheldon, CT, USA)—vinegar solution for an additional five minutes. Dyed samples were rinsed to remove excess ink, then stored in a 50% vinegar solution at room temperature. We determined the proportion of root intersections colonized by AMF for each sample by examining randomly selected stained root segments on wet slides under 100× magnification for 100 frames (root intersections) and counting the number of frames that contained arbuscule or vesicle structures [53].

2.7. Statistical Analyses

All analyses, with the exception of aboveground biomass, were performed using R Statistical Software, v 4.2.2 [54]. We conducted separate mixed model analyses using emmeans [55] and nlme [56] packages on the fixed effects of seed coat treatment and water regimen, as well as the interaction between seed coat treatment and water regimen, on the dependent variables of proportion seedling emergence per pot and percent of root intersections colonized with AMF colonization, with study round included as a random effect. For each significant model effect, we performed a follow-up pairwise comparisons analysis.

For aboveground biomass of plants that had developed at least five true leaves (V5+), the dataset did not meet the assumptions of normality or homoscedasticity, and sample size was unbalanced due to low numbers of plants of sufficient size in two treatment combinations (SoilMoist in drought, sample size = 9; Terra-Sorb + Mykos in drought, sample size = 1). Therefore, we used the “proc npar1way” procedure in SAS [57] to perform a nonparametric Kruskal–Wallis analysis, comparing biomass between water regimens, among seed treatments, and among all water regimen + seed coat treatment combinations.

Pairwise comparisons among water regimen + seed coat treatment combinations were assessed using the Dwass–Steel–Critchlow–Fligner procedure.

For development rate, we used the survival package [58] survival analysis to analyze the effect of water regimen, seed coat treatment, and the interaction of these two effects on the time needed for seedlings to develop to V5 stage. Using survival analysis instead of a mixed model allowed us to incorporate both left-censored data (replicates in which no seedlings emerged) and right-censored data (seedlings that emerged, but did not attain V5 growth stage by the end of the study). A Cox regression analysis was performed using the survminer [59] package, and figures were generated using ggplot2 [60], ggsurvfit [61], and survminer [59] packages.

3. Results

3.1. Emergence

There was a significant interaction effect between water regimen and seed treatment on emergence (Table 1). Under drought conditions, higher rates of proportion emergence were observed in seeds coated with the Mykos or Soil Moist + Mykos compared to the same seed coat grown under non-drought conditions (Figure 1). The only significant difference observed among seed treatments within each watering regimen was between Mykos versus Soil Moist under drought conditions (Figure 1).

Table 1. Mixed model results for effects of water regimen, seed coating treatment, and the interaction of these main effects on proportion seedling emergence and percent of root intersections colonized by AMF.

Model Effect	Emergence			AMF Colonization		
	df	F	p	df	F	p
Water	1, 706	2.7683	0.0966	1395	36.1396	<0.0001 **
Seed Treatment	5, 706	3.7392	0.0024 *	3395	1.4476	0.2285
Water:Seed Treatment	5, 706	3.1044	0.0088 *	3395	0.4462	0.7201

* = $p < 0.01$, ** = $p < 0.0001$.

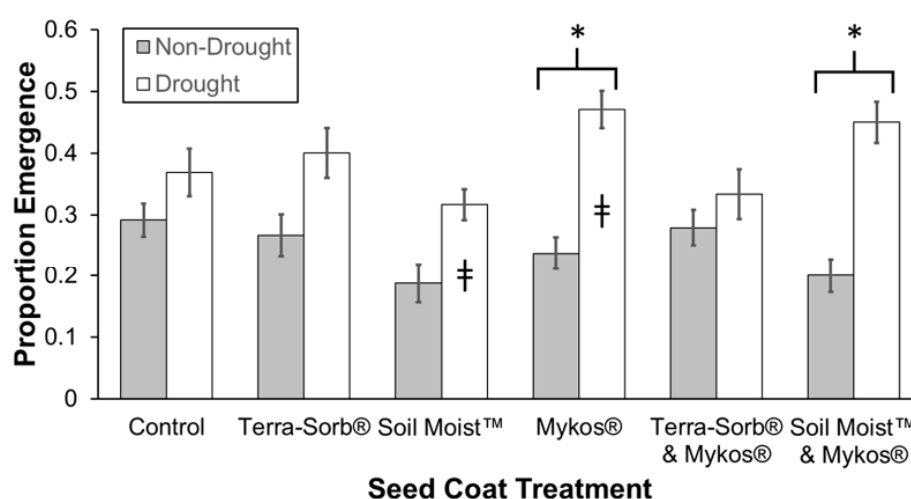


Figure 1. Mean $\pm$ SE proportion of Kernza® seedling emergence per pot by seed coat treatment and water regimen. Statistically significant differences (as assessed by mixed model pairwise comparisons) of seed coat treatment between watering regimens are marked with a *, while significantly different seed coat treatments within water regimen are marked with a †. Unmarked bars do not statistically differ.

3.2. Biomass

Aboveground biomass at the V5+ growth stage was significantly larger in non-drought vs. drought plants (Kruskal–Wallis $\chi^2 = 57.36$, $df = 1$, $p < 0.0001$), and differed among seed treatments (Kruskal–Wallis $\chi^2 = 17.116$, $df = 5$, $p = 0.0043$). There were also significant differences among water regimen + and seed coat treatment combinations (Kruskal–Wallis $\chi^2 = 70.72$, $df = 11$, $p < 0.0001$). Seedlings developed from seeds treated with Mykos and Soil Moist + Mykos had greater biomass when grown under non-drought conditions versus drought treatments (Figure 2). No significant differences were found among seed coat treatments within each watering regimen ($p > 0.05$).

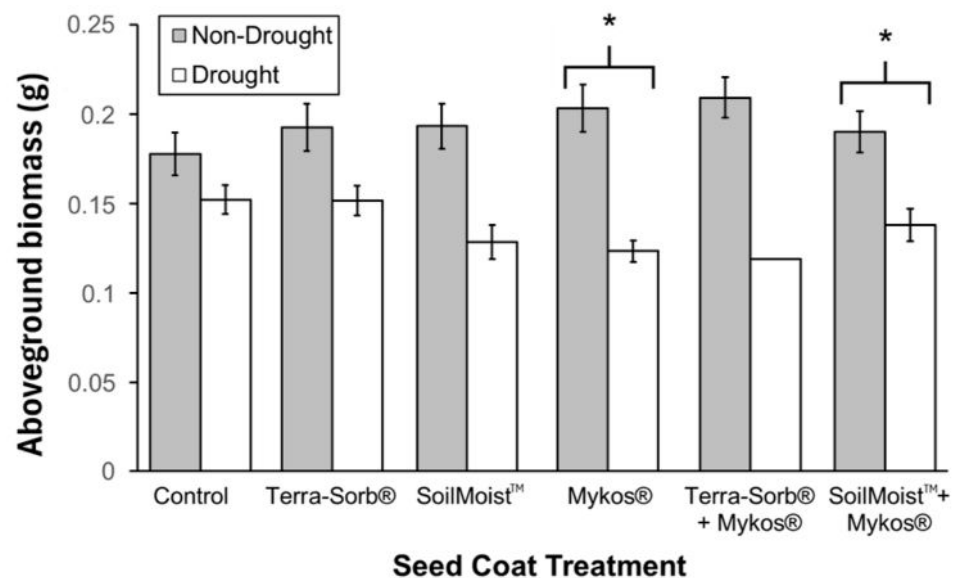


Figure 2. Mean $\pm$ SE dried aboveground biomass for Kernza® seedlings by seed coat treatment and water regimen. Only biomass of plants that had reached at least the V5 growth stage were included in this analysis. Statistically significant differences of seed coat treatment between watering regimens, as assessed by Dwass–Steel–Critchlow–Fligner pairwise comparisons, are marked with a *. Under drought conditions, SoilMoist (sample size = 9) and Terra-Sorb + Mykos (sample size = 1) treatments had low sample size.

3.3. Rate of Development

The probability of Kernza seedlings developing five true leaves significantly differed by watering regimen ($F = 0.0012$, $df = 1, 845$, $p < 0.0001$), seed coat treatment ($F = 0.0059$, $df = 5, 841$, $p < 0.0001$) and the interaction between water regimen and seed coat treatment ($F = 0.0059$, $df = 5, 841$, $p < 0.0001$). All treatments developed faster under non-drought conditions than their drought counterparts (Figure 3A,B). Additionally, under drought conditions, seeds coated with either Soil Moist or Terra-Sorb + Mykos developed slower than those of the control (Figure 3B). No treated seedlings under non-drought conditions fared differently from the control group.

3.4. AMF Colonization

A significant effect of watering regimen was observed on AMF colonization, with a higher percentage of root intersections colonized by AMF in plants grown under non-drought conditions versus drought conditions (Figure 4). However, within watering regimens, there was no significant variation in percent root colonization of seedlings between different seed coat treatments nor a significant interaction of seed coat treatment with watering regimen (Table 1, Figure 4).

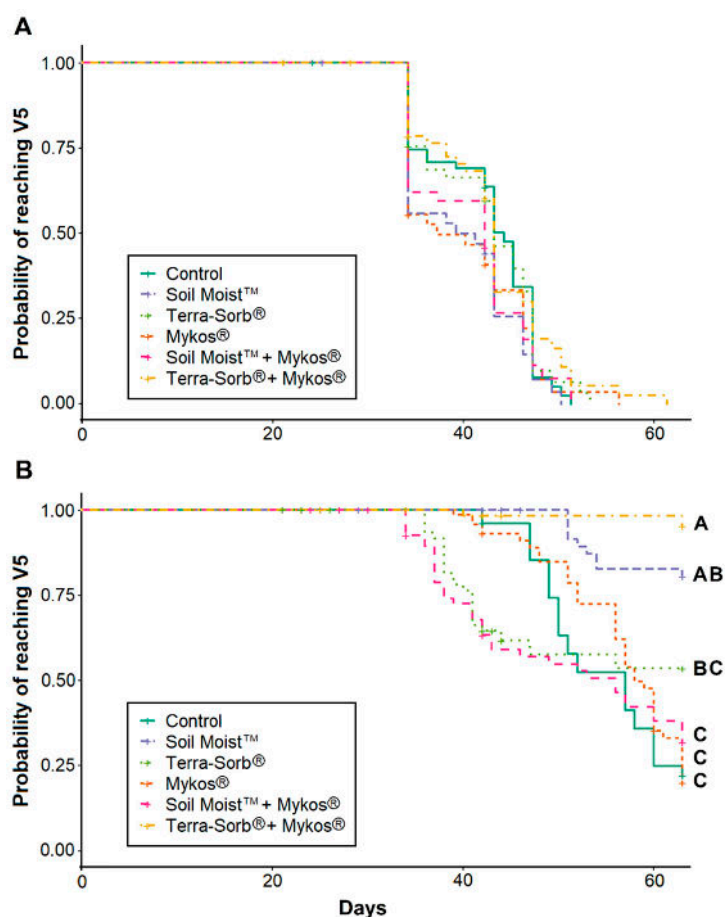


Figure 3. Kaplan–Meier curve of the probability of Kernza® seedlings from different seed coating treatments to develop five leaf collars (V5 stage) under (A) non-drought conditions and (B) drought conditions. All plants were terminated at 63 days regardless of development stage. Treatments in (B) that share a letter do not significantly differ from each other, while treatments that do not share letters are significantly different ($p < 0.05$).

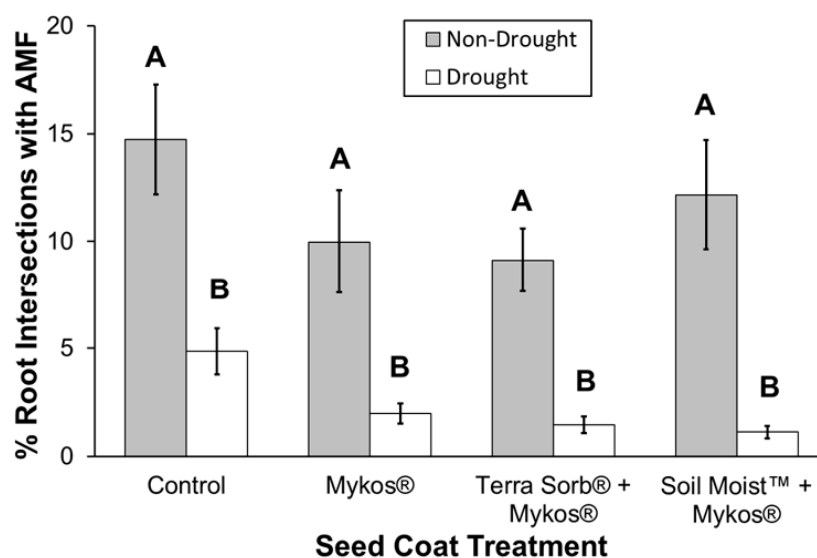


Figure 4. Mean + SE rate of mycorrhizal infection in Kernza™ seedlings under each combination of seed coat treatment and water regimen. The % Root Intersections with AMF represents the percent of 100 examined root intersections where mycorrhizal arbuscules or vesicles were present. Bars with shared letters do not significantly differ.

4. Discussion

This study was conducted to both develop a seed coating procedure for Kernza, as well as to test the feasibility of using seed coatings to increase drought tolerance in Kernza. We tested two hypotheses regarding SAPs and AMF treatments in their relation to Kernza[®] as a seed coat. Our first hypothesis, that SAPs would aid germination by increasing moisture availability to the seed, was not supported by our data. The AMF Mykos inoculant produced higher germination than the Soil Moist treatment, although neither treatment significantly differed from control. Interestingly, Mykos as a solo treatment and when combined with Soil Moist yielded higher germination rates under drought conditions than either treatment under well-watered conditions. The same pattern, although not significant, was observed in the Soil Moist treatment. Previous research has shown that SAPs can sometimes have negative effects on seed germination, particularly under non-drought conditions [62,63]. Retention of excess water around the seed reduces oxygen to the seed, thus inhibiting germination [62,63]. Though this mechanism has not been tested on AMF seed coats, it is possible that either the absorbent AMF spores themselves, or the medium in which the spores were placed in the Mykos blend, had a similar effect on the Kernza seeds.

The plant biomass data show an opposite, and stronger, pattern compared to the germination data. Plant biomass across all seed treatments, except Terra-Sorb, were significantly lower when exposed to drought compared to their non-drought counterparts. Gorim and Asche [64] found a paradoxical effect of seed coatings in barley, rye, and wheat, in that coated seeds that exhibited lower germination also had higher seedling biomass. In that study, seeds were kept under moist conditions at all times, similar to the well-watered plants in our experiment. Our study demonstrates that, under drought conditions, seed coats may have the opposite effect: increasing germination, but at the cost of reduced seedling growth. It should be noted, however, that this effect may depend not only on the type of seed coat, but also the thickness of the coating [64]. Regardless, these results run contrary to our prediction that seed coatings, particularly those containing AMF, would aid seedling growth.

Development time to the V5 growth stage followed a similar pattern as biomass. Growth did not differ among seed coat treatments under well-watered conditions. Under drought conditions, Terra-Sorb + Mykos and Soil Moist treatments developed to this vegetative stage than other treatments, when they managed to do so at all. These were also the two seed treatments that produced the lowest mean aboveground biomass under drought conditions. While the Mykos and Soil Moist + Mykos plants still managed to achieve similar V5 development time as the control treatment, even when exposed to drought, the plants were still physically smaller than they were for each of these seed treatments under well-watered conditions. The hypothesis that the AMF inoculant would improve seedling growth was not supported.

Colonization of plant roots by AMF showed an unexpected pattern. All seed treatments under well-watered conditions had significantly higher AMF colonization of plant roots than drought treatments. Since no AMF inoculant was applied to the control or SAP-only treatments, and since plants in different experimental treatments did not share a communal water source or drainage source, we hypothesize that the AMF that colonized that plants were from spores already present in the field-collected soil. This pattern is consistent with a recent meta-analysis [65], which found that plants in AMF inoculation studies had four times the level of AMF colonization in their roots when inoculated with field soil compared to commercial AMF inoculants. Furthermore, this study also found AMF inoculants to have effects on plant growth in nearly 70% of studies, even though only 16% of these studies had significant AMF colonization from those inoculants [65]. This

further supports the hypothesis that physical, chemical, or biological composition of the Mykos other than AMF colonization produced the effects we observed in our study.

The higher AMF colonization in well-watered conditions compared to drought conditions is also consistent with previous research. Arbuscular mycorrhiza species have shown reduced ability to colonize multiple plant species under drought conditions, including wheat [30], trifoliate orange [66], and Chinese fir [67]. Additionally, Kernza is less responsive to AMF than several annual and perennial crops [34], and as it has undergone domestication as a grain crop, it has increased its production of longer and finer roots [68]. Given these factors, we hypothesize that under drought conditions, Kernza may respond by reducing its affiliation with AMF in favor of increasing its own fine root growth. Future research is warranted to investigate whether either or both of these mechanisms are responsible for reduced AMF colonization in Kernza roots under drought conditions.

While it is possible that the SAPs or talc applied had negative effects on the AMF spores in the Mykos blend, this is unlikely. Acrylic polyacrylamides, the main ingredient in the SAPs used in this study, have been used to rear laboratory cultures of *Rhizophagus*, the AMF genus found in Mykos, as well as other AMF species [69]. Talc has been successfully used as a carrier medium for *Rhizophagus* inoculant for both grasses and legumes [70,71]. On the other hand, the use of the PVA coating could have interfered with AMF colonization. Research with PVA has shown that it can reduce seed germination when applied as a seed coat [72] and prevent adhesion of fungal conidia [73]. While no study, to our knowledge, has yet assessed the effect of PVA on AMF specifically, we hypothesize that the PVA binder in our seed coats may have interfered with AMF spores in the Mykos, inhibiting colonization by the inoculant. Later colonization by soil AMF could then have occurred as the seed coat dissipated and seedling roots extended into the soil. Future research is needed to determine how AMF and PVA binders specifically interact, and whether AMF seed coat inoculants can be effective if PVA or other substances are used as the binding agent.

It should be noted that, of all the seed treatments tested in this experiment, the control treatment, which contained a seed coat but no additional augmentation, had the most consistent emergence, biomass, and growth between the non-drought and drought watering conditions. This suggests that Kernza seeds are fairly resilient to drought conditions, and that access to water per se under such conditions may not be the biggest limiting factor to this crop's emergence and growth. As we previously noted, Kernza is slower to establish than many of its annual counterparts [13]; however, once established, it develops a rich rhizomicrobiome, particularly rhizobacterial species that are more commonly associated with grasslands than crop fields [74]. Seed coat treatments containing these beneficial rhizobacteria may facilitate Kernza establishment and growth more effectively than SAPs or an AMF inoculant designed for annual crops. Alternatively, coating seedlings with plant-derived enzymes that facilitate mineralization and uptake of organic N and P in the soil could also aid seedling establishment and growth. These enzyme-based seed treatments have demonstrated efficacy in annual wheat seedlings [75], but have yet to be tested on Kernza.

Our study is the first to produce a replicable method for coating Kernza seeds, and test the efficacy of SAPs and AMF fungal inoculants as seed coat treatments on Kernza emergence and early seedling growth. While none of the seed treatments tested yielded consistent improvements in Kernza performance under well-watered or drought conditions, the methodology developed can be used to test alternative seed treatments that may aid Kernza establishment. The ability to incorporate seed coat technology with breeding advances has great potential to advance Kernza's expansion on the agricultural landscape and improve its commercial potential.

5. Conclusions

Seed coating as a practice has demonstrable impacts on crop growth, particularly when used in pesticide and fertilizer application. The continual investigation into novel applications for this technology holds the potential to help address many of the challenges that crop production continues to face, among these early establishment of plants under drought conditions. The results of this study show inconsistent responses of Kernza to both SAPs and AMF under drought conditions when applied via seed coating. However, the replicable methodology this study developed for coating Kernza seeds provides a basis for testing alternative seed treatments. Seed coat treatments testing beneficial rhizobacteria associated with grasslands may facilitate Kernza establishment and growth more effectively than either SAPs or AMF inoculants associated with agricultural fields. Alternatively, coating seedlings with plant-derived enzymes that facilitate mineralization and uptake of organic N and P in the soil could also aid seedling establishment and growth. These enzyme-based seed treatments have demonstrated efficacy in annual wheat seedlings [75], but have yet to be tested on Kernza. The ability to incorporate seed coat technology with breeding advances has great potential to advance Kernza's expansion on the agricultural landscape and improve its commercial potential.

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Abbreviations

The following abbreviations are used in this manuscript:

SAP Superabsorbent polymer
AMF Arbuscular mycorrhizal fungi

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