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A review of desiccant utilization for soybean variety development pipelines

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A REVIEW OF DESICCANT UTILIZATION FOR SOYBEAN VARIETY DEVELOPMENT PIPELINES

by

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DEDICATION

Special dedication to the memory of my late father, Kenneth V. Gravatt Jr., I will never forget what you taught me, and am sorry that you were not able to see me graduate.

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Thank you to my Gravatt family for supporting me throughout my academic endeavors and providing me with the life-long lessons of growing up on a family farm. I am certain that I will still be asked to help clean out grain bins the next time I am home.

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CHAPTER 1

INTRODUCTION

For soybean variety development pipelines, reduced time needed to produce viable seed can result in faster development and sales of commercial varieties. The utilization of winter nurseries serves this purpose by increasing the number of generations grown per year while developing genotypes, but may involve the use of nurseries that are located in tropical environments for which the genotypes are not adapted (Figure 1). Growing soybeans genotypes that are adapted to specific daylengths (Hartwig, 1970) in continuous nurseries located in the tropics may result in genotypes that do not mature naturally in time to be planted in subsequent nurseries. This is often solved by applying chemical desiccants to physiologically mature plants.

Application of desiccants to some soybean genotypes to shorten the time between physiological and harvest maturity can have undesirable consequences. Unless conditions are ideal, many soybean genotypes produce poor quality seed (TeKrony et al., 1980). It is known that desiccant applications can harm seed quality, but the extent to which these applications can affect a variety development pipeline has not been addressed. Poor seed germination is likely to affect results from small plot field trials because poor quality seed will result in poor performance of genotypes for non-genetic reasons. While a yield reduction of only a few grams may seem insignificant, accurate estimates of yield are necessary to make decisions about genotypes to cull in a pipeline where differences among lines should be due to genotypic differences, not germination differences from non-genetic sources of variability. Also, smaller and inconsistent seed sizes and shapes may result from desiccant application, which will adversely affect outcomes that rely on precision planters for small plot research. Smaller seed sizes are prevalent from desiccant applications between the phenological stages of R5.5 and R7.1 (Zanatta et al., 2018).

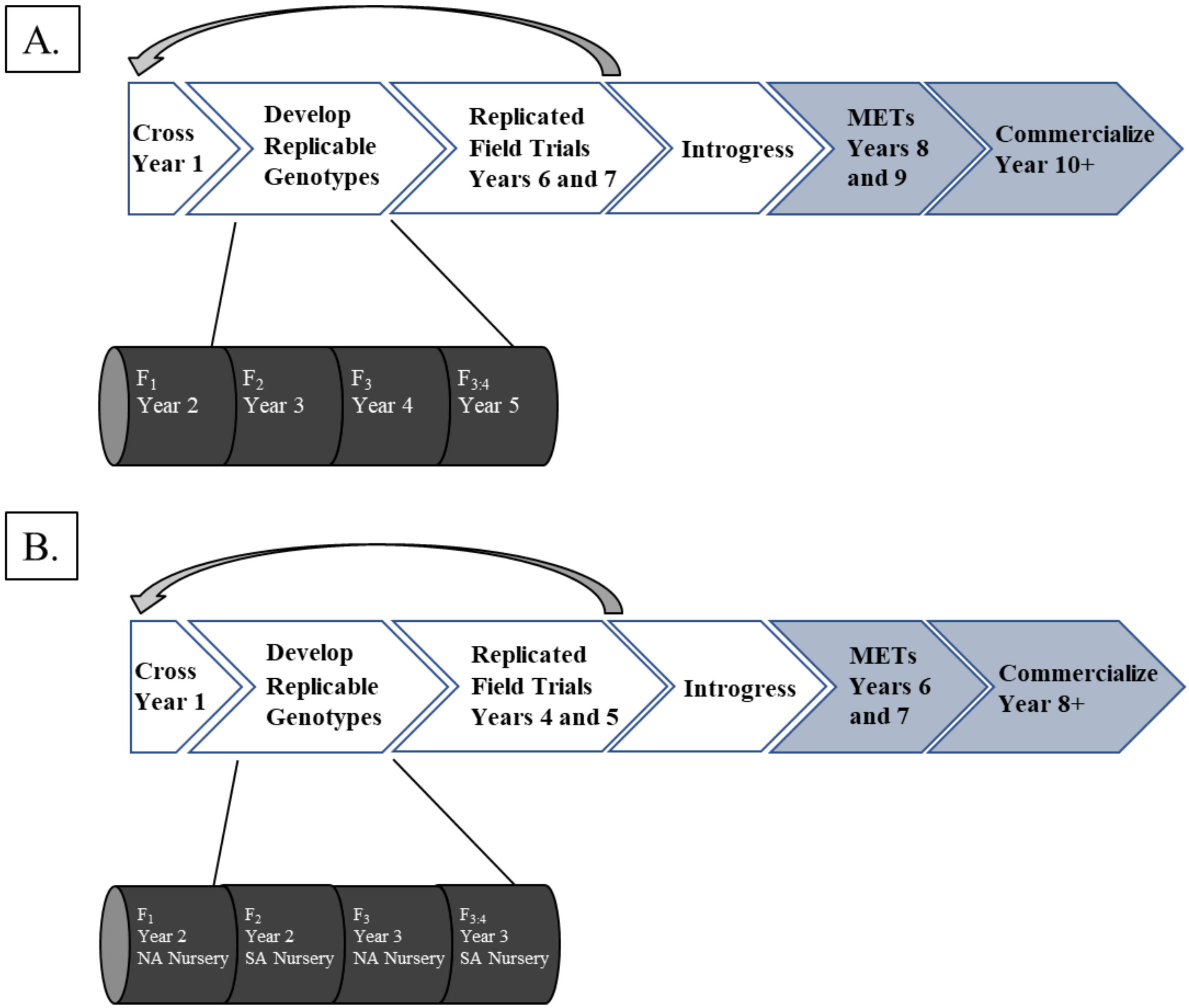

Figure 1. Comparison of soybean development pipelines without (A) and with (B) winter nursery use for developing replicable genotypes (lines), which can be used to introgress desired traits as parents for new crosses and/or enter METs. Based on estimated years, B will begin commercial varieties production 2 years earlier than A.

NA: North America, SA: South America, MET: Multi-Environment Trials

The objective of this creative component is to evaluate desiccant studies in soybeans for the purpose of minimizing the impact of desiccants on seed production for field trials. First, I will report literature on appropriate stages of growth and development for application of desiccants (herein referred to as application windows) to minimize the impact on seed germination, size, and seedling vigor. This inspection of application windows will consist of evaluations of the three most common desiccant chemical products: glyphosate, glufosinate, and paraquat. While assessing application windows would be of great use to minimize detrimental effects, appropriate application windows are not always possible in the context of day-to-day operations of seed nurseries. Consequently, a second objective of evaluating the potential for seed quality reduction in event of inappropriately timed applications is to identify 'next best' options for desiccant choice and application timing. Finally, use of desiccants in breeding pipelines will be recommended based upon generation time, seed quality, and scalability for the variety development pipeline.

CHAPTER 2

APPLICATION TIMING AND EFFECT ON SEED QUALITY

Application windows for soybeans in a production field are based on the morphological stage of the crop, measured by the Vegetative (V) and Reproductive (R) stages of growth and development (Fehr et al., 1971). For seed production, R stages R7 and R8 are indicators of seed physiological and harvest maturities respectively. Many studies and agronomic publications use V and R stages to represent developmental phases during the growing season, but multiple studies will provide the seed moisture content (SMC) instead of R stages. For example, a publication from Iowa State University Extension states that an average translation of SMC to R stage is as follows: R7 is approximately 60% SMC and R8 is approximately 13% SMC (Martinez-Feria et al., 2017). Martinez-Feria et al. (2017) calculated that the average dry down period was 12.5 days across all of their trials, at a rate of 3.2% SMC per day that varied based on coincident weather more than genotypic differences. The model used and lack of fit were not reported for these calculations.

After reviewing the average rate of dry down between R7 and R8 between multiple soybean varieties with little variability, we can extrapolate between studies that measure application windows for either SMC and R stages. Table 1 below uses data from Martinez-Feria et al. and my calculations to estimate the SMC of each tenth of an R stage between R7 and R8, and will be referenced to compare studies and timings.

Table 1. Reproductive Stage to Approximate Seed Moisture Content (SMC) Comparison

Reproductive Stage	Approximate Seed Moisture Content
R7	60.0%
R7.1	55.3%
R7.2	50.6%
R7.3	45.9%
R7.4	41.2%
R7.5	36.5%
R7.6	31.8%
R7.7	27.1%
R7.8	22.4%
R7.9	17.7%
R8	13.0%

Most studies found that applications prior to R7 resulted in significant reductions of yield and seed quality for all genotypes. This is probably due to the plant still accumulating dry matter in the pods during R6 (Martinez-Feria et al., 2017). Zanatta (2018) reported results from Paraquat applied at five different times in a randomized complete block field trial with four replicates performed in Cabeceiras, Brazil. Their results demonstrated that that paraquat applied at the R5.5 and R6.0 stages compromised the productivity of the soybean plants, with diminishing rates of damage after plants reached R7. The study did not specify the specific year or dates. However, Brazil's growing season for soybeans generally ranges from October planting through the end of March harvest.

Details for results like this may depend on genotype differences, as a study applying Diquat to two genotypes showed a difference in the acceptable application windows. Cultivar 'March' displayed reduced germination to any applications over 30% SMC (R7.6), while cultivar 'Northern Conquest' could be desiccated at 40% SMC (R7.4) and could be harvested six to seven days earlier than the control without reduction in germination in Canterbury, New Zealand (Rahman et al., 2004). The exact year and time of year were not reported. Genetic variation in response to desiccation is supported in a study by Boudreaux and Griffin (2011), a two-year experiment during 2006 and 2007 from April through August, conducted in Baton Rouge, LA. Indeterminate and determinate growth habit soybean cultivars were sprayed with desiccant applications at 60% (R7), 50% (R7.2), 40% (R7.4), 30% (R7.6), and 20% (R7.8) SMC. They reported that indeterminate Maturity Group 4 (MG4) cultivars reduced seed yield by 15.4% on average when desiccated at 60% SMC, while applications to the MG4 cultivars at the 50% SMC timing did not affect yield or seed weight, and cultivars were harvested 14 to 15 days earlier than the control in the first and second years of the trial. Determinate MG5 and MG6 cultivars in the same study showed detrimental effects when exposed to 60% SMC application, with yield reductions of 22% and 18.1% respectively, and also to a 50% SMC application, with yield reductions of 15.6% and 4% respectively. Continued reduction in yield at 50% SMC may have occurred in determinate soybean lines because they continue to produce nodes on branches throughout the growing season and may have more underdeveloped pods exposed to the desiccant than indeterminate soybeans, however this is a hypothesis that still needs to be tested. Determinate cultivars in the Boudreaux and Griffin (2011) study did not display reductions in yield when treated at the 40% SMC stage, and were harvested 8-9 days early for MG5 and 10-14 days early for MG6 when compared to the control.

These studies have all resulted in harvesting the material in question a week or two early without major quality impacts. Breeders should consider if that amount of ‘gained’ time is worth the application costs of desiccants. Logistics requirements for variety development pipelines operating in multiple hemispheres may require desiccant utilization in order to meet shipping deadlines, regardless if the timing of the application is most favorable for optimal seed quality. Missing a shipping deadline may result in an interruption of workflows and force planting crews to return to an already planted location to plant seed returned later than the planned planting dates, or cause a genotype to miss a testing generation and result in delayed commercialization.

Weather conditions during harvest windows can also be a major concern if the seed in question is intended to be used as a source of seed for a yield trial. A nursery return delayed by weather could delay field testing for a year. In a 1980 study on weather and the impact on soybean seed quality, seed vigor declined rapidly between four and 39 days after harvest maturity, which is equivalent to the R8 stage or 13% SMC (TeKrony et al., 1980). Harvesting a seed source a week or two earlier within that time frame can result in salvageable seed to use in yield trials for the following year. A recent example in which a harvest season where a week may have made a difference is Central Iowa's 2018 harvest that ranked the 3rd wettest. The area recorded 367.8 millimeters of precipitation, 164.6 millimeters above the average (Naig, 2019). This wetter than normal harvest season would be dangerously close to the observation that in 4 out of 5 observed environments, germination was less than 50% for both tested cultivars one month after harvest maturity and remaining in the field (TeKrony et al., 1980).

After understanding the potential benefits to using desiccants, a breeder would determine whether or not their development pipeline would need to use them to meet deadlines during growing seasons. If the variety pipeline includes a nursery that is not located in an environment sampled from the same set of environments that are targeted for sales, a desiccant would only be of use in the instance of a cold, wet year where the lines may be maturing slowly. In the case of winter nurseries or off-target growing environments, breeders must ask if it is acceptable to cause a genotype-dependent response to desiccant usage and result in differing germination rates per genotype. This genotype-dependent response could be used as a selection criterion if a program depends on desiccants, especially if germination tests are performed on seed sources prior to being planted the following season. Rahman et al. (2004) found the most restrictive SMC timing, with 30% SMC (R7.6) being the latest application window with no detriments to productivity and seed in response to desiccants across genotypes. If variation across genotypes is intended to be used as a selection criterion, 50% SMC (R7.2) seems to sit in the middle of observed cultivars either displaying no detrimental effects or resulting in reduced productivity when applied with a desiccant. Soybean breeders should note that these are approximate values and may not accurately represent potential interactions with their specific germplasm.

CHAPTER 3

REVIEW of CHEMICAL DESICCANTS

Glyphosate

Originally introduced under the commercial name 'Roundup' in 1974 by Monsanto, glyphosate became the dominant herbicide worldwide after the introduction of Herbicide-Tolerant (HT) soybeans in 1996 (Benbrook, 2016). Glyphosate is a popular choice as a desiccant due to its low cost and status as a safer-to-handle chemical, only featuring the CAUTION warning word on the herbicide label (Benbrook, 2016). Glyphosate is a broad-spectrum herbicide and the only member in the Site of Action Group 9, known as 5-enolpyruvyl-shikimate-3-phosphate Synthase (EPSPS) Inhibitors, a sub-group of the Amino Acid Synthesis Inhibitor family of herbicides (Duke and Powles, 2008). This broad-spectrum activity, in conjunction with its ability to translocate throughout a plant, made glyphosate a popular and effective choice for soybean producers throughout the late 1990s and early 2000s. By 2012 about 56% of all glyphosate applied globally was applied to HT crops.

While glyphosate functions well as a desiccant due to its ability to translocate, this translocation also inhibits how well the chemical can function as a desiccant for *seed production*. Application of paraquat as a desiccant was not observed to affect yield or seed weight when applied two weeks before harvest, but glyphosate was found to significantly reduce germination and seedling vigor in soybeans, thus 'glyphosate is not recommended as a desiccant' (Whigham and Stoller, 1979). We can approximate (Table 1) that if harvest was deemed to be at 13% SMC then the two weeks prior to harvest soybeans will be at the R7 stage of development. This is noteworthy, as all chemicals used in Whigham and Stoller's study displayed significant decreases in yield and seed quality when applied 3 and 4 weeks prior to harvest, once again supporting diminishing detrimental effects of desiccants applied after R7. Cerkauskas et al. (1982) found similar results in their studies conducted at Urbana, Illinois in 1977 and 1978, Vicosa, Brazil in 1978, and Florestal, Brazil in 1978. Glyphosate applications between R5.5, R6, and R7 all displayed significant decreases in seed germination across all lines evaluated at all locations (Cerkauskas et al., 1982). Ratnayake and Shaw (1992) performed evaluations not only on seed germination and yield, but also seedling development with glyphosate as a tested desiccant. In their study, glyphosate reduced seed germination when applied at growth stages of R5, R6, and R7, as well as increased the rate of abnormal seedlings. Also, glyphosate was also the only chemical evaluated in the study to reduce shoot weight of 1-month-old soybean plants when applied at the R5 growth stage of the parent (Ratnayake and Shaw, 1992).

Published research on glyphosate as a desiccant after the mid-1990s declined for two possible reasons. First, previous studies had shown that glyphosate would function as a desiccant, but not for producing viable and healthy seed. Second, the advent of HT crops in 1996 resulted in breeding pipelines that rapidly filled with glyphosate-tolerance across the breeding populations. Just 11 years after HT soybeans were introduced, 91% of the United States' soybean acres were planted to transgenic crops in 2007 (Bonny, 2008). In addition, countries that are newer to soybean production such as Argentina and Brazil planted 100% and 93% of their respective acres were planted with HT soybeans in 2014 (Benbrook, 2016). Use of glyphosate as a desiccant in a breeding pipeline that contains mostly glyphosate-tolerant lines is not an effective strategy, and that does not leave opportunity for the chemical as a desiccant based upon use rates of the HT technology in the largest soybean producing countries in the world. However, a smaller conventional or non-glyphosate HT varietal development pipeline may be able to utilize a desiccant if they are willing to apply after R7, and are aware that detrimental effects to seed may still occur. It should be noted that glyphosate is often the cheapest option of the three chemicals.

Glufosinate

The sole member of the herbicide Site of Action Group 10 and the Nitrogen Metabolism Inhibitor chemical family, glufosinate is a glutamine synthesis inhibitor commonly sold under the name of Liberty. This chemical is a broad-spectrum herbicide that does not translocate well within a plant, and is moderately dangerous to handle and apply featuring the WARNING signal word on the herbicide label. A HT trait for glufosinate was introduced to the commercial market in 2009 by Bayer CropScience.

Delgado et al., (2015) evaluated the effects of glufosinate as a desiccant on soybean vigor and reserve mobilization when applied at R7.1 on two cultivars, 'Benso1RR' and 'NA 5909 RG', relative to a non-desiccated control of the same varieties. Results showed 'Benso1RR' retained high seedling vigor for both the control and glufosinate treatments while losing 5% germination rates, but 'NA 5099 RG' displayed low seedling vigor and a 13% reduction in germination rates between glufosinate treatment and the control. Additionally, it was observed that desiccated seeds exhibited slower radicle protrusion than their control counterparts with 40% and 25% less protrusion in 24 hours of soaking for 'NA 5909 RG' and 'Benso1RR' respectively. After 48 total hours of soaking the desiccated seeds reached 'almost the same percentage of root protrusion compared to their controls'. Ratnayake and Shaw (1992) also included glufosinate in their 1992 study, observing that glufosinate reduced yield and normal seedling percentages only when applied at growth stages R5 and R6.

Utilization of glufosinate as a desiccant is more appealing than glyphosate due to reduced impact on germination. It should be noted that a breeding pipeline with a large glufosinate-tolerant HT program will not be able to desiccate broadly across all cultivars, for reasons similar to how glyphosate may be restricted in use in the respective HT variety development pipeline.

Paraquat

Paraquat is a non-selective contact herbicide that was first sold under the trade name Gramoxone by Imperial Chemical Industries in 1962. Paraquat is a Photosystem I Electron Diverter under the Cell Membrane Disrupter chemical family, along with protoporphyrinogen oxidase (PPO) Inhibitors. As the most dangerous chemical of the three reviewed in this paper, Paraquat is a Restricted Use Pesticide, and is marked with the DANGER/POISON signal words on the pesticide label.

Cerkauskas et al. (1982) applied paraquat between R5.5 and R6 as well as at R7, and observed significantly reduced seed weight and germination at all three of their testing locations, as well as increased disease incidence of *Fusarium* and *Phomopsis* spp. at the Florestal testing location for both cultivars UFV_1 and UFV_2 when compared to their controls. Paraquat's deleterious effects on yield were observed by Whigham and Stoller (1979) when applied three and four weeks prior to harvest date. Yields were not significantly reduced when applied two weeks before harvest while also proving to be the most effective desiccant for accelerating harvest date in the same study (Whigham and Stoller, 1979). As noted in the glyphosate section, two weeks before harvest is analogous to R7 assuming harvest date is determined by cultivars reaching 13% SMC. Despite reduction in yield and seed weight, paraquat was not found to affect seed germination or seedling vigor (Whigham and Stoller, 1979). From Boudreaux and Griffin (2011) paraquat desiccation did not result in yield or seed weight reductions when applied at 50% SMC or lower (R7.2 or later) for MG 4 cultivars, or at 40% SMC or lower (R7.4 or later) for MG5 and MG6 cultivars. MG4 cultivars were also able to be harvested 14-15 days before the control treatment, MG5 cultivars eight to nine days before the control treatment, and MG6 cultivars 10-14 days before the control treatment.

Paraquat's effect of reduced seed sizes may be a consideration for breeding programs relying on more precision-based planting technologies that need a more consistent and average seed size to smoothly operate processing, treating, and planting equipment. Zanatta et al. (2018) conducted an in-depth observation of paraquat's influence on seed sizes as well as the impact on productivity and seed weight. Results of productivity and mass of one thousand seeds under the guideline of not applying a desiccant before R7 to avoid negative impact found that the highest retention of seeds in a single sieve size was the 6.0 mm sieve when applications were made at R5.5 and R6. Negative impacts to frequencies of germination and abnormal seedlings were also recorded at harvest, as well as 40, 80, 120, and 160 days into seed storage with frequencies of abnormal seedlings increasing slightly as duration of storage increased (Zanatta et al., 2018). In these findings, Paraquat did not differ across all reproductive stage applications for both frequencies of abnormal seedling nor germination rate (Zanatta et al., 2018). However, after 40 days of storage seed quality begins to dip slightly for all growth stages of application, but slightly more so for the R5.5 and R6.0 stages, which may be a major consideration for nursery operations that will be holding seed over a winter or skipping a growing season.

Comparing paraquat to the other chemicals, Whigham and Stoller (1979) found it to be the most effective desiccant in their study for accelerating harvest date. However, desiccation effectiveness can be associated with reductions in seed size and weight, while maintaining yield over glyphosate and glufosinate. Growth stage of application with paraquat appears to influence seed size more than influences on seed production. The largest problem with paraquat for a breeding program to consider would be safety of handling the chemical, due to its Restricted Use Pesticide status and DANGER/POISON signal words.

CHAPTER 4

TIMING of APPLICATION COMBINED WITH CHOICE of CHEMICAL

Studies cited in Chapter 2 were fairly clear that it is not advisable to apply desiccants to a soybean crop before R7 (Martinez-Feria et al., 2017, Zanatta et al., 2018, Specht, 2019). However, damage to seed productivity or germination quality can still occur after R7, with negative impacts in one cultivar observed in an application as late as a 40% SMC (R7.4) (Rahman et al., 2004). Waiting to apply the desiccant until there are no repercussions is not a feasible strategy, as the benefits to early harvest would be minimized and reduce the benefits of the desiccant. For genotype variation in response, applying desiccant to each individual line as they reach 40% SMC is not a reasonable alternative to ease and low costs broadcast application. When determining an optimal and secondary application window, applications at 50% SMC or 30% SMC appear to provide a reasonable compromise. Leaving the 30% SMC application window available allows for some flexibility in the operations of a nursery, especially if fall weather is cooler or wetter than expected.

Deciding on which chemical to use for desiccation depends on germplasm HT traits, preservation of germination rates and seed quality, and safety for workers. The most obvious determining factor for a desiccant chemical in consideration is compatibility with the breeding program’s HT allele introgression. A glyphosate-tolerant program will not be able to use glyphosate to desiccate lines developed in the continuous nurseries, and the same applies to glufosinate-tolerant material and applying glufosinate as a desiccant. This factor alone diminishes the potential to use glyphosate wide-spread as a desiccant, as Argentina, Brazil, and the United States produced 82% of the global soybean harvest in 2014 and planted a large majority of their acres in that year to HT soybean varieties (Benbrook, 2016). Unfortunately, glyphosate is not favored by the consideration of retaining high germination rates and seed quality. Two studies indicated that using glyphosate as a desiccant strongly decreased germination rate, as well as maintained a potential to result in higher rates of abnormal seedlings (Whigham and Stoller, 1979, Cerkauskas et al., 1982, Ratnayake and Shaw, 1992). Based upon those observations, the quote ‘glyphosate is not recommended as a desiccant’ appears accurate as long as the goal for the produced seed is to be regrown (Whigham and Stoller, 1979). These two considerations are enough to remove glyphosate from consideration as a consistently-used desiccant at any application timing.

Azevedo et al. (2015) evaluated glufosinate and paraquat, with glufosinate applied at R7.1 and paraquat applied at R7.2, and found that glufosinate performed worse under both production and germination rate categories. These results showed glufosinate application resulting in 14% less productivity than paraquat, and also reduced frequencies of green seeds by 1%. When comparing the results for germination rates between glufosinate and paraquat, glufosinate reduced germination by 3.75%. The differences in production and germination rate could be explained by the 0.1 R stage application timing difference, however a second study comparing the chemicals glufosinate, reglone, safluenacil, and paraquat (listed as Gramoxone) found similar results. (Zuffo et al., 2019) Here, all chemicals were applied at the R7.1 stage with harvest times and observations for germination rate and moisture damage at R8, R8 plus 7 days (R8+7), and R8 plus 14 days (R8+14). In their study, paraquat maintains a slight advantage over glufosinate when harvested at R8 in germination rate, but has an 18% advantage when harvested at R8+7. Moisture damage rates appear to follow the same pattern, with paraquat maintaining higher seed quality by almost 10% at R8 and almost 8% at R8+7. The R8+14 harvest date displayed a slight advantage to using glufosinate over paraquat. However, they noted that the lowest germination means overall for the study were observed at R8 and R8+14 due to 50 mm of rainfall being recorded on those dates, and only 20 mm recorded at R8+7. Paraquat's high moisture damage rate at R8+14 is also explained by Zuffo et al. (2019) as 'reinforcing the relationship between harvest delay and damage by moisture'. Last, their results were consistent with Delgado et al., (2015) in finding that glufosinate resulted in the lowest germination rates regardless of soybean harvest time. These studies clearly display that paraquat is preferable to glufosinate as a desiccant when germination rate and seed quality are determining factors.

For a recommended chemical and application timing combination, paraquat applied at 50% SMC would enable early harvest of a soybean crop by 14-15 days potentially, while also maintaining seed quality over glufosinate (Boudreaux and Griffin, 2011, Azevedo et al., 2018, Zuffo et al., 2019). Safety when handling paraquat would be the primary concern; handlers and applicators should familiarize themselves with proper personal protective equipment (PPE) procedures and read the chemical label to understand how to safely use their product of choice.

CHAPTER 5

SPEED BREEDING AND THE USE OF DESICCANTS

Genetic gain is the main objective of breeding programs for commodity row crops and could be increased by increasing the number of generations per year (Figure 1). Desiccants are primarily a tool used in field-based winter nurseries for the purpose of completing another generational cycle. Nagatoshi and Fujita (2019) recently published a study on utilization of growth chambers for the purpose of accelerating soybean breeding. They utilized carbon dioxide (CO_2) supplementation, prolonged photoperiod, harvest and replanting of immature seeds in a growth chamber to allow up to five generations of their tested cultivar per year instead of one or two currently possible in field and greenhouses (Figure 2), shortening generation time of their test cultivar ‘Enrei’ from 103-132 field days to 70 growth chamber days. In addition to shortening cycle times, CO_2 supplementation was found to increase average seed numbers per plant from 26.6 +/- 2.5 to 53.4 +/- 9.1, essentially doubled with significantly increased average seed weight as well. Immature pods were harvested at 37 days after flowering, as their colors started to change from green to yellow (approximately R6), and air dried for eight days which allowed the seeds to mature. These seeds were then sown, and one week later had a germination rate of 100%.

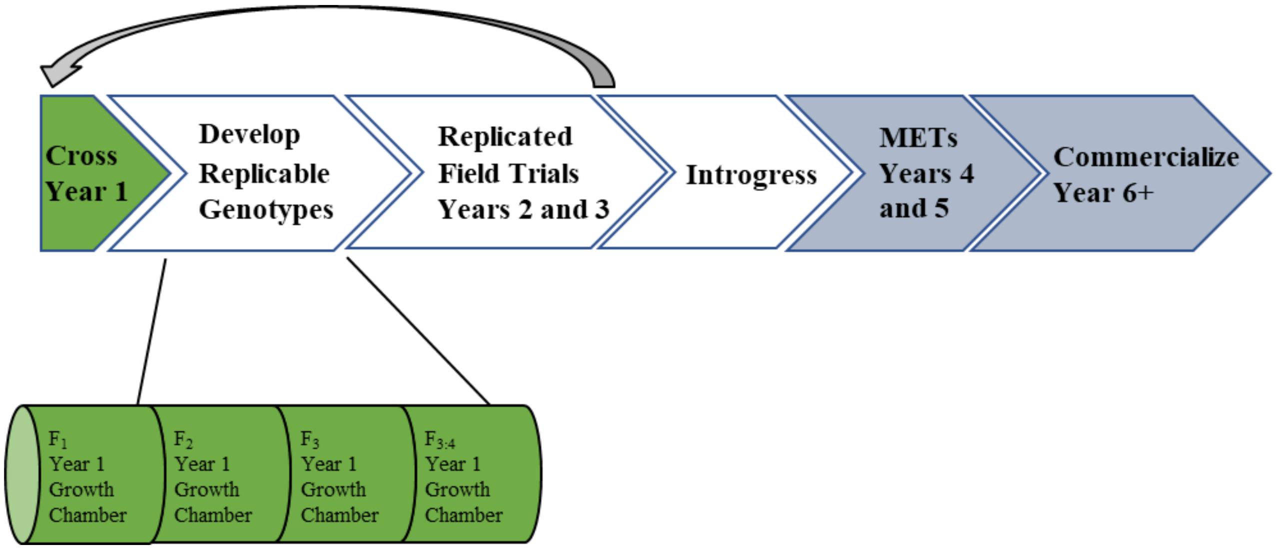

Figure 2. Soybean development pipeline using growth chambers to develop replicable genotypes. Green shading represents a single year grown in a growth chamber. Compared to year estimates in Figure 1, this pipeline requires two fewer years than programs with winter nurseries and four fewer years than programs with no winter nurseries.

While desiccant use will not reduce generation times for soybeans to 70 days such as achieved with growth chambers, nor will they maintain 100% germination or pristine seed quality, they do retain the advantage of scalability. Until techniques like CO_2 saturation or photoperiod extension become feasible on a field level, desiccants remain the primary option for reducing generation time by as much as 14-15 days (Boudreaux and Griffin, 2011). After these techniques become feasible in-scale however, their ability to maintain quality and even increase seed production would result in their rapid adoption in crop breeding pipelines, where seed quality and seed count are priority over raw yield.

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APPENDIX A

ABBREVIATIONS

V(x) – Vegetative stage ‘x’

R(x) – Reproductive stage ‘x’

SMC – Seed Moisture Content

METs – Multi-Environment Trials

HT – Herbicide Tolerant

CO_2 – Carbon dioxide