INVESTIGATION OF PEANUT RESPONSE TO FLUMIOXAZIN APPLICATIONS, POLYMER SEED TREATMENT, AND VARIOUS STORAGE REGIMES

By

NICHOLAS LANE HURDLE

(Under the Direction of Timothy L. Grey)

ABSTRACT

Georgia produces over 50% of the U.S. peanut supply annually. Planting quality seed is crucial to establishing an adequate plant population. Proper seed storage conditions contribute to the quality of seed growers plant. Numerous storage regimes studied for their effect on peanut germination and CO^2 emission. After storage, peanut have a fungicidal seed treatment applied to prevent early season disease pressure. Seed treatments have been adapted to include a polymer technology from the pharmaceutical industry. The fungicide will be applied in a liquid form followed by a dust formulated polymer for quick drying and aesthetic properties. There is a gap of information for polymer technology use in peanut production. Both peanut and numerous weed species will begin to germinate and emerge at similar times. Control of these weed species is critical as they can reduce water, nutrient, and sunlight available to the peanut crop. Injury has been noted in previous research under irrigated conditions, leaving non-irrigated growers without information.

INDEX WORDS: Weed management, peanut, flumioxazin, seed treatment, storage, peanut germination

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DEDICATION

To Shanley Steven Hurdle

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Arising from the central region of South America, peanut (*Arachis hypogaea* L.) quickly became a major crop in the southeastern United States (Putnam et al. 1991). Arriving to the U.S. by way of trade routes in the 1770's, peanut gained popularity by being used as livestock feed, oil, and for human consumption over 30 years later (Anonymous a 2019; Valentine 2019). George W. Carver assisted in the spread of peanut by developing over 300 products, while also promoting it as a rotational crop to cotton production, as it will replenish nitrogen and aid in soil erosion prevention (Anonymous b 2020).

As with any crop, weed control is essential to ensure peanut receive the proper amount of sunlight, water, and nutrients (Wilcut et al. 1994). The critical period of weed control describes independent time periods of crop-weed competition: 1. critical timing of weed removal is the maximum amount of time the crop can sustain early season weed interference before unacceptable yield loss occurs and 2. the minimum amount of time the crop needs to be maintained weed free after planting to prevent yield loss is the critical weed free period (Everman et al. 2008). One method of weed control is by applying preemergent (PRE) and postemergent (POST) herbicides (Wilcut et al. 1994; Grichar and Dotray 2011).

Flumioxazin (2-[7-fluoro-3,4-dihydro-3-oxo-4-(2-propynyl)-2H-1,4-benzoxazin-6-yl]-4,5,6,7-tetrahydro-1H-isoindole-1,3(2H)-dione) has been studied by Valent U.S.A Corporation since its development in 1989, for use in numerous crops such as soybean (*Glycine max* L.) Merr., wheat (*Triticum aestivum* L.), cotton (*Gossypium hirsutum* L.), and peanut (Shaner 2014).

Flumioxazin has been classified as a Weed Science Society of America (WSSA) Group 14, or protoporphyrinogen IX oxidase inhibitor (PPO) (Shaner 2014). This group of herbicides affects plant development by preventing the protoporphyrinogen IX oxidase from oxidizing protoporphyrinogen IX (PPGIX) into protoporphyrin IX (PPIX) (Sherwani 2015). PPGIX will then accumulate and absorb sunlight within chlorophyll, causing the formation of triplet state PPIX that will leak from the cell. These triplet state PPIX begin to react with O_2, forming singlet state O^-, followed by lipid peroxidation, cell membrane disruption, and eventual plant death.

Flumioxazin has been noted to cause injury when applied to peanut in adverse weather conditions (Johnson et al. 2006). The investigators reported injury up to 59% with flumioxazin applied at 105 g ai ha^{-1} 10 DAP. This injury was noted as stunting, necrotic lesions, and discolored petioles sustained up to 39% through the midseason in the second season of the study. Studies were performed investigating flumioxazin's effect when directly applied to seed across multiple temperatures. It was reported that temperature had a greater effect on germination and radicle development than flumioxazin (Hurdle et al. 2020). The physiological response of peanut to flumioxazin was also investigated. The investigators noted that physiological parameters (electron transport, yield of PSII, net photosynthesis, and stomatal conductance to CO_2) were affected by flumioxazin applications but varied across the entire growing season. The injury was transient and did not affect yield (Hurdle et al. 2020). Johnson et al., 2006, and Hurdle et al., 2020, conducted experiments under irrigated conditions, spurring similar studies to be performed under non-irrigated conditions.

As flumioxazin is applied PRE, water from rainfall or overhead irrigation is needed for herbicide activation. Activation is when the herbicide is carried down by water into the rhizosphere where the weed seed are located (Nagy 2008). This may cause some growers to plan

an application shortly prior to a rainfall event to reduce irrigation costs. In doing this, growers may not complete the full application due to rainfall, leaving leftover solution. Flumioxazin has been noted to be sensitive to water solution pH (Kwon et al. 2004). These investigators noted that as water pH increased, the rate of flumioxazin hydrolysis increased. At a pH of 5, the half-life of flumioxazin is 16.4 hours and only 0.3 hours at a pH of 9. Flumioxazin was noted to degrade into two products after hydrolysis. Product I was noted to be formed by cleavage of the imide linkage and product II by cleavage of the amide linkage of the benzoxazinone ring. Degradation product I was detected in all pH solutions whereas product II was found only in pH 5 solution. The conclusion of the study indicated that flumioxazin should be degraded in surface water. This raises the question if the grower should use the left-over solution or should a new herbicide solution be made to complete the application.

As the end of harvest draws to a close, the grower must now decide whether to sell their crop or save a portion as seed stock. One factor a grower must consider is the way their saved seed will be stored. Many options of seed storage exist, such as a warehouse or in bulk bags, but environmental conditions (relative humidity, temperature, and microflora) the stored seeds are subjected to can affect the physiological characteristics in the form of deterioration (McDonald 2004). Seed deterioration is a problem that all storage facilities face. No facility can completely halt the process, but attempts can be made to control the rate at which the seeds deteriorate (McDonald 2004). Shelled peanut seeds may be stored in large bulk bags and stacked on one another or may be piled within the facility exposed to the environment. Storage facilities may incorporate several techniques to decrease the rate at which the stored seeds deteriorate such as the type of warehouse constructed, and the use of aeration and ventilation. Storage facility construction specifications and techniques may be found in the American Peanut Shellers

Association's publication titled: Handling and Storage of Farmer Stock Peanuts (Smith Jr. 2015). Though the facility itself may affect the deterioration and longevity of peanut seeds, the aeration and ventilation system directly affect the moisture content and seed quality by controlling the temperature and air flow surrounding the seeds.

Aeration is used to allow air to flow within the peanut pile and remove the heat and excess moisture as the air is exhausted to outside of the facility. A specific aeration scenario that potentially may cause problems is when air that is forced through the piled peanut is not forced out of the facility and is allowed to contact the ceiling. This may cause condensation to form on the ceiling allowing water to freely drip onto the stored peanuts. The high temperature and moisture can potentially cause the stored peanuts to be contaminated with aflatoxins produced by *Aspergillus flavus* or *Aspergillus parasiticus* (Rudolf 2015). These two species of fungi have been known to cause liver cancer, with the B1 strain being the most carcinogenic (Rudolf 2015). The maximum level of aflatoxin for any peanut use is 20 parts per billion (ppb) according to the FDA Mycotoxin Regulatory Guidance produced by the National Grain and Feed Association in 2011. Two techniques of ventilation practices include natural and mechanical. Natural ventilation uses the natural air flow through inlets to remove the excess heat and moisture. Mechanical includes the use of fans to force air into the inlets, rather than depending on natural wind. Specific measurements and requirements can be found in the American Peanut Shellers Association: Handling and Storage of Farmer Stock Peanuts publication.

Harrington (1972) created two guidelines for seed deterioration: 1. Every 1% reduction in seed moisture content doubles the life of the seed and 2. Each 5 C reduction in seed temperature doubles the life of the seed. Harrington also identified a few exceptions to these guidelines: Rule 1 only applies when the moisture content is between 5% and 14% and Rule 2 is applicable only

above 0° C as many biological reactions do not function properly, and temperatures below that level do not result in better seed preservation (McDonald 2004).

A study conducted by Butts et al. (2006) intended to identify proper aeration techniques for farmer stock peanuts that will be stored in warehouses with and without headspace ventilation. Warehouse #1 was structured with 3 fans drawing air down into the peanuts at a rate of 0.31 $m^3 min^{-1}$ and warehouse #2 was fitted with 3 fans forcing air through the bottom of the peanuts along with a damper fitted onto each duct to provide a constant flow of 1.27 $m^3 min^{-1}$. Warehouse #3 was fitted nearly the same as 1 and 2, except only one duct was used forcing air up through the middle of the piled peanut at 0.43 $m^3 min^{-1}$ with the headspace allowing for the entire headspace air volume to be changed every 2 minutes. Warehouse #4 was fitted with only headspace ventilation at the recommended rate. The investigators noted that of the 4 warehouses, warehouse #4, only headspace ventilation, had a significant increase in aflatoxin production. The removal of moisture and temperature control throughout the peanuts was credited to the lower production of aflatoxin in the remaining 3 warehouses. This research confirmed that aeration systems reduced the aflatoxin production in stored peanuts.

Runner and virginia type peanut have a longer life cycle compared to spanish and valencia. This characteristic exposes these peanut seed types to late season rains, potentially allowing the fruit to germinate within the hull (Xu et al. 2020). Spanish and valencia peanut can experience vivipary should the harvest also be delayed. After harvest, peanut will undergo a dormancy period. Minimal research has been performed to determine peanut CO_2 respiration while in storage under various climatic conditions. Little to no research has been conducted to determine the length of storage in which peanut will overcome this dormancy. Germination and

respiration studies will be performed to determine the length of dormancy of peanut under various temperature and storage regimes.

Optimal peanut germination is a crucial part of a successful growing season. Storage conditions have been previously reported to influence germination and vigor the following growing season after being stored (Weaver et al. 2021). Sub-optimal germination often leads to replanting which increases input costs to the grower including fuel, pesticide use, fertilizers, potential delays in maturity and harvest, and potential lower yields. The process of germination begins when the seed imbibes water, with soybean (*Glycine max* L.) Merr. requiring 50% of the seed dry weight to begin (Bryant 2021). As the seed imbibes water, the water potential within the seed decreases, therefore decreasing the water uptake rate causing it to plateau. Following imbibition, respiration occurs through glycolysis, the pentose phosphate pathway, and the Krebs cycle to produce pyruvate and adenosine triphosphate (ATP), nicotinamide adenine dinucleotide phosphate (NADPH), and additional ATP, respectively. The initial glycolysis process may result in little ATP production due to inefficiency, this lack in ATP production is compensated by oxidative phosphorylation utilizing the electron transport chain. As time post-imbibition increases, mitochondrial efficiency increases as well. In peanut specifically, this efficiency is due to the production of new mitochondria (Bewley et al. 2013). As new proteins are formed, genes are upregulated or downregulated depending upon their function in the germination process. The final stage of germination is the protrusion of the radicle through the seed coat. The radicle must overcome resistance of the seed coat and other plant tissues. This can be assisted through enzyme activity weakening plant tissue surrounding the radicle. Cell elongation and division is the final step of germination in which the radicle will begin to expand and lengthen beyond the seed coat.

Seed treatments have become an essential part of seed technology in that they provide the seed with additional nutrients, pesticides for protection, and assist the grower in planting (Kaufman 1991). Currently, peanut seed treatments are formulated as a wettable powder. Prior to mixing with water, the powder poses an inhalation risk to the applicator warranting a safer formulation for application. Liquid fungicide seed treatments are being developed as a safer formulation, while helping reduce early season disease pressure. A dry polymer coating adapted from the pharmaceutical industry is added after the liquid fungicide to enhance drying, handling, and allows for easier planting by increasing the flowability in the planter. Numerous studies have been performed investigating the effects of polymer seed coating on germination and other phenotypical measurements, but peanut has minimal data regarding polymer seed treatments. Investigators performed a study to determine the effects of storage conditions on polymer coated tomato seed (Jacob et al. 2016). It was reported that untreated seed gained more moisture from the air in paper bags compared to treated seed in paper bags under ambient conditions. Germination for the untreated seed in the paper bags was also reduced by 14% after 12 mo. of storage, while the treated seed had only 6% germination reduction. Under low temperature and low humidity storage conditions, germination, seed moisture, and vigor were not different in the paper bags or in sealed aluminum pouches. Data has not been collected regarding the phenotypical response of peanut seed with polymer treatments. These studies will broaden the knowledge of polymer seed treatments and introduce data on the effects on peanut seed under multiple storage conditions.

Objectives

Flumioxazin efficacy, injury, and water sensitivity will be evaluated on peanut under non-irrigated conditions at multiple locations. Research will be performed to: 1. Determine

peanut response to flumioxazin applications at multiple timings after planting. 2. Determine efficacy of flumioxazin when applied at multiple rates. 3. Determine flumioxazin efficacy under non-irrigated conditions. Flumioxazin hydrolysis in water with different pH levels will be studied to determine: 1. Response of flumioxazin in solution with water of different pH levels. 2. Efficacy of flumioxazin in solution with water of different pH levels on common row crop weed species.

Peanut storage studies will be performed to: 1. Determine CO_2 respiration of Georgia-06G seed under various storage conditions. 2. Determine germination rate of peanut cultivars under storage conditions, at numerous storage timings. 3. Determine seed germination effects (via thermal gradient table testing) of treating peanut with polymer seed coatings, dry fungicide treatment, or not treatment, all under variable storage conditions.

Materials and Methods

1. *Flumioxazin applied at multiple rates and timings* – Field trials in Plains and Tifton, GA were conducted under non-irrigated conditions for the duration of the study. Plots were 1.8 m by 9.1 m with 4 replications per treatment set in a split plot design with main plot of application timing and subplot of flumioxazin rate. Peanut cultivar GA 16-HO was planted in single rows at both locations with application timings of 0, 3, 5, 7, 10, 14 days after planting. Flumioxazin rates consisted of 0, 0.25, 0.5, and 1.0x of the 107 g ai ha^{-1} label rate. Data collected included stand counts, plant width, percent injury compared to the NTC, percent weed control, and yield. Data was properly analyzed and graphed using SigmaPlot 14.
2. *Flumioxazin hydrolysis in water with different pH levels:* Water samples were collected from the Southwest Research and Education Center (SWREC) in Plains, GA, UGA

Ponder farm in Ty Ty, GA, and a farm irrigation well from Piperton, TN. The labelled flumioxazin rate was added to a 2L bottle containing water from each location. Solutions included flumioxazin added to the 2L bottle, 14, 10, 7, 5, 3, 1 day prior to application, including 12 and 0 hours prior. Samples were taken at regular intervals prior to application and immediately frozen then analyzed using a high-performance liquid chromatography, coupled with a singlequad mass spectrometer (HPLC/MS) for respective herbicide concentrations. The solutions were applied to 1.8 m by 9.1 m plots at the UGA Ponder Farm and SWREC. Data collected included stand counts, plant width, weed control, and yield. A greenhouse study following the same protocol was conducted in tandem with the field study.

3. *Peanut cultivar CO_2 respiration under various storage conditions:* Shelled peanut cultivar Georgia-06G seed were placed in 22.7 kg bags in stacks of 40 bags/pallet under various storage conditions. Seed were treated with Rancona VPD dust seed treatment. three pallet replications were placed in each storage conditions including cold storage, an insulated warehouse, and an uninsulated warehouse. Two sensors with capabilities to measure CO_2, relative humidity, and temperature were placed in 2 bags at each location. Measurements were taken multiple times per day until seed were removed from storage. CO_2 data was analyzed utilizing PROC GLM and PROC CORR in SAS Studio. Seed samples for germination were taken every 14 days from a bag, then processed using AOSA guidelines for germination testing and analyzed using PROC NLIN in SAS to obtain maximum germination, slope of the regression line, and growing degree days (GDD) for 80% germination. Seed were planted with growth data collected and final yield.

4. *Peanut response to seed treatment:* Shelled, polymer treated and non-treated cultivar GA-09B seed were stored in 22.7 kg bags under multiple environments including an office space, greenhouse, and outdoor shelter. Seed were removed from each location and placed in Petri dishes. The filled Petri dishes were placed on a thermalgradient table with temperatures ranging from 15C to 35C. Ten mL of distilled water were added at trial initiation and maintained to ensure proper moisture levels. Seed remained on the table for 168 hr. Germination counts began 72 hr after and continued 24 hr thereafter until 168 hr after trial initiation. Data was analyzed using PROC NLIN in SAS determining maximum germination, slope of the regression line, and GDD's needed to obtain 80% germination and graphed in SigmaPlot. The seed remained in each storage condition for a total of 70 d, upon which the study was terminated.

External Project(s) Overview

1. *Citrus response to indaziflam*- Indaziflam has been granted labels in a variety of perennial crops such as Bermudagrass, turf grass species, and citrus. This experiment focused on comparing indaziflam to other residual herbicides for efficacy, length of control, and trunk growth. Applications were made in the November and April with ratings taken throughout the year. There were 3 replications per treatment with 5 trees per replication.
2. *Blueberry and pecan response to indaziflam*- The same procedures from the citrus study were applied to blueberry and pecan crops. Several treatments were altered to accommodate blueberry and pecan growth habits compared to citrus.
3. *Peanut seed viability*- Peanut storage conditions have been reported to significantly influence seed germination after planting. This project will adapt the seed viability

equation (Ellis and Roberts 1980) to peanut cultivars and assist in determining the length of time peanut can be stored before germination is lost. Seed will be stored in a stable storage condition with samples taken at regular intervals and germinated. The collected data will be utilized to determine equation constants and develop an equation for shellers to utilize in order to determine which order seed lots should be sold.

References

Anonymous a (2019) History of peanuts & peanut butter. National Peanut Board. Accessed June 7th, 2019 https://www.nationalpeanutboard.org/peanut-info/history-peanuts-peanut-butter.htm.

Anonymous b (2020) The incredible accomplishments & inventions of George Washington Carver. The Peanut Institute. Accessed August 17th, 2020. https://peanut-institute.com/the-incredible-accomplishments-inventions-of-george-washington-carver/

Bewley, J.D., K.J. Bradford, H.W.M Hilhorst, H. Nonogaki. 2013. Seeds: Physiology of development, germination, and dormancy. 3rd ed. Springer. Pgs 133-181.

Bryant, C. 2021. Soybean production in Georgia. University of Georgia Extension

Butts, C.L., J.W. Dornerm S.L. Brown, F. H. Arthur (2006) Aerating Farmer Stock Peanut Storage in the Southeastern U.S. American Society of Agricultural and Biological Engineers. Transactions of the ASABE Vol. 49(2): 457-465.

Ellis, R.H. and E.H. Roberts. 1980. Improved equations for the prediction of seed longevity. Annals of Botany. 45(1): 13-30.

Everman, W.J., S.B. Clewis, W.E. Thomas, I.C. Burke, J.W. Wilcut (2008) Critical period of weed interference in peanut. Weed Tech. 22:63-67.

Grichar, W.J. and P.A. Dotray (2011) Weed control and peanut tolerance with ethalfluralin-based herbicide systems. Int. J. of Agron. Accessed August 18th, 2020. doi:10.1155/2012/597434

Harrington, J.F. (1972). Seed storage and longevity. In Kozlowski. T.T. (Ed.), Seed Biology Volume 3:145-240.

Hurdle, N.L., T.L. Grey, C. Pilon, W.S. Monfort, E.P. Prostko (2020) Peanut seed germination and radicle development response to direct exposure of flumioxazin across multiple temperatures. Peanut Sci. 47:89-93.

Hurdle, N.L., T.L. Grey, C. Pilon, W.S. Monfort, D.G. Shilling. 2020. Interaction of seedling germination, planting date, and flumioxazin on peanut physiology under irrigated conditions. American Journal of Plant Sciences. 11: 2012-2030.

Jacob S.R., M.B. Arun Kumar, E. Varghese, S.N. Sinha (2016) Hydrophilic polymer film coat as a micro-container of individual seed facilitates safe storage of tomato seeds. Scientia Horticulturae. 204:116-122.

Johnson III, W.C., E.P. Prostko, Mullinix jr., B.G. (2006) Phototoxicity of delayed applications of flumioxazin on Peanut (*Arachis hypogaea*). Weed Tech. 20:157-163.

Kaufman, G. (1991) Seed coating: A tool for stand establishment; a stimulus to seed quality. Hort. Tech. 1:98-102.

McDonald, M. (2004). Orthodox Seed Deterioration and Its Repair. Handbook of Seed Physiology 9:273-304.

Nagy, P. (2008) Efficacy of acetochlor as affected by different rainfall conditions. Cereal Research Communications. 36:2019-2022.

Putnam, D.H., E.S. Oplinger, T.M. Teynor, E.A. Oelke, K.A. Kelling, J.D. Doll (1991) Peanut. University of Wisconsin-Extension. Accessed July 7th, 2019. http://corn.agronomy.wisc.edu/Crops/Peanut.aspx

Rudolf, K., K. Nährer, J. L. Richard, I. Rodrigues, R. Schuhmacher, A. B. Slate, T. B. Whitaker (2015) Guide to Mycotoxins: Featuring Mycotoxin Risk Management in Animal Production. Romer Labs.

Shaner, D. (2014) WSSA Herbicide Handbook 10th Ed. Weed Science Society of America.

Sherwani, SI, Arif IA, Khan HA (2015) Modes of action of different classes of herbicides. Price, A, Kelton, J, Sarunaite, L, eds. Herbicides Physiology of Action and Safety. IntechOpen. DOI: 10.5772/61779.

Smith Jr. J., J. Cook, J. Trice, C. Butts (2015) Handling and Storage of Farmer Stock Peanuts. American Peanut Shellers Association.

Sundaraj, S., R. Srinivasan, A.K. Culbreath, D.G. Riley, H.R. Pappu (2014) Host plant resistance against *Tomato spotted wilt virus* in peanut (*Arachis hypogaea*) and its impact on susceptibility to the virus, virus population genetics, and vector feeding behavior and survival. Phytopathology. 104(2) 202-210.

Valentine, H. (2019) Remembering our Past and How it Affected Our Present and Future. Peanut Sci. in Press.

Weaver, C.C., W.S. Monfort, T.L. Grey, C. Pilon, R.S. Tubbs. 2021. Effect of post shelling storage environments on seed germination and vigor of peanut. Agronomy Journal. DOI: https://doi.org/10.1002/agj2.20795

Wilcut, J.W., A.C. York, G.R. Wehtje (1994) The control and interaction of weeds in peanut (*Arachis hypogaea*). Rev. Weed Sci 6:177-205.

Xu, P., G. Tang, W. Cui, G. Chen, C.L. Ma, J. Zhu, P. Li, L. Shan, Z. Liu, S. Wan (2020) Transcriptional differences in peanut (*Arachis hypogaea* L.) seeds at the freshly harvested, after-ripening and newly germinated seed stages: insights into the regulatory networks of seed dormancy release and germination. PLOS ONE 15: e0219413. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6941926/#pone.0219413.ref014

CHAPTER 2

RESPONSE OF DRYLAND PEANUT TO MULTIPLE RATE DELAYED FLUMIOXAZIN APPLICATIONS[1]

[1] Hurdle NL, Grey TL, Monfort WS, Chamberlin KD, Brenneman TB, Moore JM. 2022. To be submitted to *Peanut Science*

Abstract

Flumioxazin is crucial for peanut weed management across the United States with over 75% of growers applying it to control troublesome weed species. For maximum peanut yield, it is essential that weed control is maintained during weeks three through eight after planting. Peanut injury due to flumioxazin PRE applied has been noted under unfavorable moisture or weather conditions, but also due to delays in application as growers plant hundreds of hectares on their farms. Research in Georgia investigated the response of dryland peanut to flumioxazin PRE applied from 0 to 107 g ai ha^{-1} at 0 to 14 d after planting for cultivar GA-16HO. Trends at two locations during the 2020 through 2022 growing seasons indicated that as rate and time after planting of application increased, injury also increased. Over 50% injury was noted in Tifton and 24% in Plains during the 2021 growing season. Peanut pod yield decreased while flumioxazin rate increased and timing of application after planting was delayed in Tifton, but no differences were noted in Plains. The recorded injury coincided with large amounts of rainfall at both locations. It was also noted that peanut may be most sensitive to flumioxazin application injury between days seven and ten after planting.

Introduction

Peanut (*Arachis hypogaea* L.) has become an important source of oil and protein over time. South America is the origin of the peanut, though the name has been changed numerous times (Hammons et al. 2016). The Incan civilization referred to peanut as *ynchic* and was changed by the Spanish conquistadors to *mani*, which is still used in many Spanish speaking countries such as Cuba (Hammons et al. 2016). As exploration and religious missionary expeditions increased, the names of peanut also began to increase and included *mandi, manobi, manduiss, mandubi, amendois,* and *tlalcacuatl* (German, French, Spanish, Spanish, Portuguese,

Nahuatl), respectively. Wild-types *Arachis ipaensis* (Krapov. And W.C. Gregory) and *Arachis duranensis* (Krapov. And W.C. Gregory) are the ancestral parents of *Arachis hypogaea* (L.) which is the commercially grown peanut and contains two subspecies: *Arachis hypogaea sp. hypogaea* (L.) and *Arahcis hypogaea. sp. fastigiata* (L.) (Valentine 2019). The runner and virginia-type peanut belong to the *sp. hypogaea* and the spanish and valencia type peanut belong to the *sp. fastigiata.* The difference between the subspecies is the presence (*sp.* fastigiata) or absence (*sp.* hypogaea) of flowers along the main stem (Moretzsohn et al. 2004). The introductory time and place in the United States has never been fully identified for peanut due to lack of written records. The commonly accepted introduction was through Portuguese and Spanish traders en route to Africa. The traders would transport cargo to the United States where peanut was used as a food supply due to the non-perishing properties. The American Civil War played a major role in the distribution and popularity of peanut as soldiers needed easily transportable food that was high in protein and nutrients. The United States Department of Agriculture reported an annual increase in peanut production of 200 to 300% between 1865 to 1870, with 37 states planting peanut in 1889 (Peterson 1931). This distribution led to peanut primarily being grown in the Southeast, the East coast, and the Southwest (Valentine 2019; Peterson 1931; Prasad et al. 2010). Each peanut growing region has a dominant market-type produced. Georgia growers predominately produce runner-type peanut while New Mexico growers only produce valencia peanut. Peanut producers in Texas and Oklahoma grow runners, spanish, and virginia types, with valencias also grown in Texas.

Many biotic and abiotic factors have the potential to severely hinder peanut growth and development resulting in decreased quality or yield. Drought or excessive rainfall, disease, weeds, insect pressure, and damaging winds during the season are a few examples of stresses

peanut can encounter during a growing season. One factor that must be adequately controlled is the weed species population. Weeds can harbor disease and insects as well as compete with peanut for space, water, nutrients, and sunlight (Royal et al. 1997; Wilcut 1994). Everman et al. (2008) indicated that peanut yields decreased as competition time with broadleaf or grass weed species increased. The investigators indicated that it was weed species specific as to how much yield could be lost if not controlled by a certain time in the growing season. Data indicated yield was affected if control was not maintained by 8 to 10 weeks after planting for broadleaf weed species and 5 to 8 weeks for grass weed species. Weed control is primarily accomplished through chemical applications due to their availability, ease of use, and effectiveness. Herbicides can be applied before (PRE) or after (POST) crop emergence to provide season long weed control.

An effective and widely used PRE herbicide in peanut production is flumioxazin (2-[7-fluoro-3,4-dihydro3-oxo-4-(2-propynyl)-2H-1,4-benzoxazin-6-yl]- 4,5,6,7-tetrahydro-1H-isoindole-1,3(2H)-dione). Flumioxazin was applied by growers across the entire Southeaster peanut growing region with 74%, 64%, 62%, and 58% of Georgia, North Carolina, Florida/Alabama, and South Carolina hectares being treated in 2018, respectively (NASS 2019). Flumioxazin at 107 g ai/ha provides residual control of broadleaf species including pigweeds (*Amaranthus sp.*), Florida beggarweed (*Desmodium tortuosum* Sw.) DC., and kochia (*kochia scoparia* L.) Schrad., and suppression of grass species barnyardgrass (*Echinochloa crus-galli* L.) Beauv., large crabgrass (*Digitaria sanguinalis* L.), and Italian ryegrass (*Lolium perenne* L. spp. *multiflorum* Lam.) Husnot (Anonymous 2016). The mechanism of action of flumioxazin (PPO, WSSA 2017) will affect the plant chlorophyll and heme production by preventing proper function of the protoporphyrinogen oxidase (PPG oxidase) (Shaner 2014). Flumioxazin will bind to the PPG oxidase and prevent the conversion of protoporphyrinogen IX into protoporphyrin IX

causing an overflow of protoporphyrinogen IX to leak from the chloroplast into the cell cytoplasm. Once in the cytoplasm, the protoporphyrinogen IX will be converted into protoporphyrin IX and begin to accumulate light energy. As this occurs, the protoporphyrin IX will begin to develop triplet and singlet oxygen species that will interact and degrade lipids and proteins, leading to leaky membranes and allow rapid desiccation of cells. Flumioxazin can be absorbed either through foliage or roots but has limited foliar translocation due to rapid onset of necrosis on treated foliage (Shaner 2014). Hurdle et al. (2020a) reported that peanut seed germination was affected more by cool temperatures than direct exposure to flumioxazin due to rapid root metabolism. Low concentrations of two metabolites have been identified as 3,4,5,6-tetrahydrophtalmic acid and 1-hydroxy-trans-1,2-cyclohexanedicarboxylic acid at the labelled rate (Dotson 2001). It has been noted that injury can be caused by overhead irrigation or rainfall by splash from water droplets carrying flumioxazin onto green plant matter (Price et al. 2004a). The registration label states that flumioxazin should not be applied more than 2 days after planting due to potential injury (Anonymous 2016). As growers are now planting large hectarages (~ 81 ha/farm) timely PRE herbicide applications can be challenging. Thus, flumioxazin applications can be delayed as growers expand peanut production on their respective farms.

Flumioxazin has been extensively researched under irrigated field conditions with respect to weed control and peanut response (Basinger et al. 2021; Hurdle et al. 2020b; Johnson et al. 2006; Price et al. 2004b), but this leaves peanut growers with little information about the response of dryland peanut to flumioxazin. Therefore, research was conducted to evaluate peanut physiological response under dryland conditions to flumioxazin rate and timing of application.

Materials and Methods

Dryland peanut field experiments were conducted in Georgia at Tifton (31.49 N, -83.52 W) and the Southwest Research and Education Center in Plains (32.03 N, -84.37W) from 2020 through 2022. Soil type in Tifton consisted of Tifton loamy sand (Fine-loamy, kaolinitic, thermic Plinthic Kandiudults) with 7% clay, 84% sand, 9% silt, and 0.8% OM in 2020 and 8% clay, 83% sand, 9% silt, and 0.7% OM in 2021. Plains soil properties in 2020 consisted of a Greenville sandy loam (Clayey, kaolinitic, thermic Rhodic Kandiudults) with 13% clay, 67% sand, 20% silt, and 0.8% OM in 2020 and 20% clay, 63 sand, 17% silt, and 0.6% OM in 2021.

Experimental design was a randomized complete block in a split-plot arrangement with 4 replications. Plots measured 1.9 m by 9.1 m with main plots being herbicide application timings at 0, 3, 5, 7, 10, and 14 days after planting (DAP) and sub-plots of flumioxazin at 0, 27, 54, and 107 g ai ha^{-1} which translate into a 0, 0.25, 0.5, and 1.0x rate (Anonymous 2016). Herbicide treatments were applied using TeeJet TTI 11002 nozzles at 187 L/ha and 207 kPa. Herbicides were activated by natural rainfall (Table 1) and not supplemented by overhead irrigation. Cultivar GA-16HO (Branch 2017) were planted on May 18th, 2020, June 11th, 2021, and May 25th, 2022, in a single row manner for 18 seed/m in Tifton at a depth of 3.8 cm (Prostko 2022). Phorate (diethoxy-(ethylsulfanylmethylsulfanyl)-sulfanylidene-λ^5-phosphane) (Anonymous 2020) was applied at 454 g ai/ha along with a *Bradyrhizobium* sp. *Arachis* inoculant (Anonymous 2019) at a product rate of 141 L/ha. All plots were treated with diclosulam (*N*-(2,6-dichlorophenyl)-5-ethoxy-7-fluoro-[1,2,4]triazolo[1,5-c]pyrimidine-2-sulfonamide) at 27 g ai/ha and pendimethalin (3,4-dimethyl-2,6-dinitro-*N*-pentan-3-ylaniline) at 906 g ai/ha. For Plains, GA-16HO was planted in single-rows to achieve a population of 18 seed/m on June 2nd, 2020, May 18th, 2021, and May 10th, 2022. All plots received a blanket application of diclosulam and pendimethalin at the same rates as the Tifton location. Acephate (N-

[methoxy(methylsulfanyl)phosphoryl]) was applied to peanut in Plains at 819 g ai/ha. Planting occurred when rainfall was predicted to occur immediately following DAP. All plots were maintained weed-free and under University of Georgia agronomic recommendations (Monfort et al. 2021; Prostko et al. 2021) for dryland peanut after the conclusion of data collection.

Data collected included visual (chlorosis and necrosis) percent injury compared to the non-treated control (NTC) of the entire plot on a 0 to 100% scale (0% indicating no injury and 100% representing plant death), plant width, plant population, percent weed control (0% indicating no control and 100% as total weed control) and yield (Blanchett et al. 2017; Chaudhari et al. 2018; Leon 2016; Buchanan et al. 1970). Data for weed control was not collected in Tifton during the 2020 growing season. Plant widths were measured on plants randomly selected within the entire plot from leaf tip to leaf tip of the outermost fully expanded leaves (Hurdle et al. 2020b), and plant population was randomly selected from 1 m of row per plot (Hagan et al. 2015). Visual plant injury and plant width data collection occurred at 10, 14, 18, 22, 25, 29, 32 and 37 DAP in Tifton and 14, 17, and 23 DAP in Plains in 2020, while plant stand counts were collected on the first three measurement timings. These same data were collected 13, 19, and 31 DAP in Tifton and 20, 23, 29, and 36 DAP in Plains for the 2021 season. Visual plant injury (chlorosis and necrosis) and plant widths were collected 12, 19, 22, and 28 DAP in Tifton and 17, 23, 30, 35, and 43 DAP in Plains and stand counts collected only during the first three collections. Data were combined to include the first three ratings of each respective rate and application timing. ANOVA was performed using PROC GLIMMIX in SAS Studio 3.8 (SAS Institute Inc. Cary, NC) for data analysis. When appropriate, herbicide rate, application timing, and rate by timing interactions were further analyzed with means separated by Tukey's HSD set at $\alpha = 0.05$. (Stephenson IV et al. 2019; Besançon et al. 2016).

Results and Discussion

Initial analysis indicated that year and location were significant preventing data combination across year or location. Therefore, data are presented by location and year. Data utilized for analysis are the first three collections after each respective application timing to maintain consistency across all treatments for percent injury compared to the NTC, plant width, plant population, while yield consisted of only one measurement.

Plains

2020

Injury compared to the non-treated control indicated numerous differences with injury increasing as application time after planting increased, regardless of rate (Table 2). Flumioxazin applied at 107 g ai/ha 14 DAP resulted in the greatest amount of injury resulting in less than 7% injury. Visual injury was low due to reduced rainfall compared to the Tifton location and previous research. Injury noted included overall plant stunting, necrotic lesions, and discolored petioles which was also reported in Johnson III et al. (2006), Stephenson IV. et al. (2018), and Jursík et al. (2011).

The greatest amount of weed control was achieved when flumioxazin was applied at 54 or 107 g ai/ha closer to peanut planting (Table 3). Greater than 71% control was observed when applied at the full rate at planting or 7 DAP. The least amount of weed control was provided by flumioxazin applied at all rates 14 DAP.

Prominent weed species included yellow nutsedge (*Cyperus esculentus* L.), morningglory (*Ipomoea* sp.), sicklepod (*Senna obtusifolia* L.), pigweed (*Amaranthus* sp.), and Florida beggarweed (*Desmodium tortuosum* Sw.) DC. These species are controlled by flumioxazin except for yellow nutsedge, which was controlled by hand weeding and late POST applications.

Applications of 54 or 107 g ai/ha applied near planting had reduced control, with 107 g ai/ha achieving 74% control. Less than 6% control was achieved when applied 14 DAP.

Plain's peanut widths noted few differences as plants treated with 27 g ai/ha of flumioxazin 10 DAP sustained the greatest amount of stunting with plants measuring only 8 cm in diameter (Table 4). These plants were different than those treated with 0 flumioxazin at 14 DAP, 54 g ai/ha applied at planting, and 27 g ai/ha when applied at 0, 7, or 14 DAP. No yield differences were indicated for the Plains location in 2020.

2021

Maximum damage occurred on peanut treated with 54 g ai/ha applied 10 DAP (Table 2) when compared to the NTC. The overall trend indicated that as the rate of flumioxazin increased and application time increased, injury increased. The lowest injury sustained was 4% for peanut treated with 27 g ai/ha when applied at planting as compared to the NTC.

Peanut at Plains treated with any rate of flumioxazin at any application timing provided greater control than the NTC (Table 3). Control ranged from 64% to 96% with the greatest amount occurring in peanut treated with 107 g ai/ha at seven DAP, and the least amount at 54 g ai/ha applied five DAP. The rate that provided the greatest amount of control only caused 10% peanut injury while the treatment with the least was 11% injury. No differences were reported for either plant width or yield for peanut in Plains.

2022

Plants treated with 107 g ai/ha applied 14 DAP noted the greatest amount of injury at 28% (Table 2). The next greatest amount of injury occurred on plants treated 10 DAP at the full rate with 20%. As in 2021, the trend noted that as rate and application time after planting increased, injury also increased. All herbicide treatments achieved >76% weed control, with

maximum of 98% occurring at the full rate applied 10 DAP (Table 3). Greater than 90% control was achieved by all treatments except the 27 g ai/ha applied 0, 3, and 7 DAP along with the 54 g ai/ha applied 3 DAP and the NTC's.

Differences were noted for plant widths in 2022 (Table 4). Widths ranged between 10 cm to 14 cm over the three ratings. Plants treated with no flumioxazin or 27 g ai/ha noted the greatest plant widths, regardless of application time. As rate increased, plant width decreased with the smallest plants being treated with the full rate of flumioxazin applied 10, 14, and 7 DAP, respectively.

Tifton

2020

Peanut planted in Tifton also noted similar differences as in Plains, with injury increasing as rate and application time after planting increased (Table 2). Plants treated with either 54 or 107 g ai/ha at 10 or 14 DAP sustained the greatest amount of injury ranging from 20 to 40%. An additional trend indicated the earlier application paired with a lower application rate resulted in less stunting than higher application rates applied further from planting. No differences in plant population were indicated in 2020.

A similar trend to percent injury in which the higher rates applied further from planting caused a yield decrease in Tifton was reported. The lowest yielding plot was treated with 107 g ai/ha 14 DAP yielding only 3,468 kg/ha on average. This was different than peanut treated with 0, 27, or 107 g ai/ha applied at planting as well as 27 g ai/ha when treated at 10 DAP. The highest yielding plots all achieved 4,604 kg/ha on average.

2021

Percent injury was greater than in Plains (Table 2). The maximum injury noted was 52% on peanut treated with 107 g ai ha^{-1} of flumioxazin applied 10 DAP. This treatment was different from all others except peanut treated with 107 or 54 g ai ha^{-1} at seven or 10 DAP, respectively. The trend was similar to the trend in Plains with plants treated with less flumioxazin closer to planting sustaining less injury.

Similar rates of control ranging from 59 to 93% were achieved with the full rate of 107 g ai/ha applied at seven DAP, but the least control was achieved by 27 g ai/ha when applied seven DAP, excluding the NTC (Table 3). As in Plains, all treatments with flumioxazin applied at any rate and time had greater weed control than the NTC.

Plant widths varied in Tifton from 9.6 cm to 16.4 cm width differences (Table 4). Overall, the trend noted plants treated with less flumioxazin closer to planting sustained the least amount of stunting. Plant population differences in Tifton determined that all treatments were similar except plants treated with 0 or 27 g ai/ha (14 plants/m row) than peanut treated with 107 g ai/ha applied at seven or 10 DAP (11 plants/m row). Plants treated with 107 g ai/ha at 14 DAP were not different from any treatment and contained 13 plants/m row.

Yield in Tifton noted rate by application timing interactions. Peanut treated with 27 g ai/ha at 3, 5, and 14 along with those that remained untreated at the 10 DAP application timing yielded higher than the full rate of flumioxazin applied seven DAP. The highest yield was 5,262 kg/ha while the lowest yield noted as only 3,827 kg/ha.

2022

Peanut sustained up to 40% injury in Tifton during the 2022 growing season (Table 2). Plants treated with any rate at either 10 or 14 DAP noted injury of 15 to 40%. All other treatments caused <10% injury. All treatments provided >79% weed control, with the trend

indicating greater control as application time increased after planting. Numerous differences were indicated for plant widths (Table 4). Widths ranged between 14 cm and 19 cm over the three ratings. The trend noted that as rate increased, plant width decreased regardless of application timing. Plants treated with 107 g ai/ha at 7 and 10 DAP noted the smallest plants at <15 cm.

Flumioxazin has been noted to cause injury in the form of necrotic lesions at points of contact on leaves and overall plant stunting. Injury can be exacerbated in unfavorable conditions such as cool, wet soils which can be observed shortly after application. Though peanut is generally planted in May, new cultivars can be planted earlier when these unfavorable conditions may occur. The label states that flumioxazin should not be applied more than two DAP to avoid injury, but some growers may attempt to apply flumioxazin outside of this application window. This study indicated that applications at the full or half rate after peanut has emerged may reduce plant width and yield, while causing greater visual injury. Contrary to this, weed control increased as rate increased when applied at seven DAP, but would directly expose emerged or nearly emerged peanut to flumioxazin. Growers should observe weather reports and apply flumioxazin when conditions are optimal and according to label instructions to avoid injury.

References

Anonymous. 2016. Valor SX herbicide label. Accessed: February 18th, 2022. http://www.cdms.net/ldat/ld3LL006.pdf

Anonymous. 2017. Strongarm label. Accessed: February 18, 2022. http://www.cdms.net/ldat/ld3M2001.pdf

Anonymous. 2019. Optimize label. Accessed: February 18th, 2022. http://www.cdms.net/ldat/ldBLF002.pdf

Anonymous a. 2020. Thimet EZ Load label. Accessed: February 18th, 2022. http://www.cdms.net/ldat/ldB7F005.pdf

Anonymous b. 2020. Prowl H2O label. Accessed: February 18, 2022. http://www.cdms.net/ldat/ld6CT004.pdf

Basinger, N. T., T. M. Randell, E. P. Prostko. 2021. Peanut response to flumioxazin and *S*-metolachlor under high moisture conditions. Peanut Science. 2021.

Besançon, T.E., R. Riar, R.W. Heiniger, R. Weisz, W.J. Everman. 2016. Rate and timing effects of growth regulating herbicides applications on grain sorghum (*Sorghum bicolor*) growth and yield. Advances in Agriculture. Vol. 2016. Article ID: 9302507.

Blanchett, B.H., T.L. Grey, E.P. Prostko, W.K. Vencill, T.M. Webster. 2017. The effect of 2,4-dicholorphenoxyacetic acid (2,4-D) on peanut when applied during vegetative growth stages. Peanut Science. 44(1): 53-59.

Branch, W.D. 2017. Registration of 'Georgia-16HO'. J Plant Registrations. 11(3): 231-234.

Buchanan, G.A., R. Dickens, E.R. Burns, R.M. McCormick. 1970. Performance of herbicides for weed control in peanuts. Auburn University. Bulletin 399. Accessed: February 18th, 2022. https://aurora.auburn.edu/bitstream/handle/11200/764/1581BULL.pdf?sequence=1&isAllowed=y.

Chaudhari, S., D.L. Jordan, T.L. Grey, E.P. Prostko, K.M. Jennings. 2018. Weed control and peanut (*Arachis Hypogaea* L.) response to acetochlor alone and in combination with various herbicides. Peanut Science. 45(1): 45-55.

Dotson, D. 2001. Tolerance petitions for the use of flumioxazin on peanuts, soybeans, and sugarcane: evaluation of residue chemistry and analytical methodology. United States Environmental Protection Agency. File 129034_0011000_031201_D259493_R02299.

Everman, W. J., I. C. Burke, S. B. Clewis, W. E. Thomas, J. W. Wilcut. 2008. Critical period of grass vs. broadleaf weed interference in peanut. Weed. Tech. 22 (1):68-73.

Hagan, A.K., H.L. Campbell, K.L. Bowen. 2015. Seeding rate and planting date impacts stand density, diseases, and yield of irrigated peanuts. Plant Health Research. 16(2): 63-70.

Hammons, R.O., D. Herman, H.T. Stalker. 2016. Origin and early history of the peanut *in* Peanut: Genetics, processing, and utilization. Eds H.T. Stalker and R. Wilson. London: AOCS Press, Elsevier; Pgs 1-26.

Hurdle, N. L., T. L. Grey, C. Pilon, W. S. Monfort, E. P. Prostko. 2020a. Peanut seed germination and radicle development response to direct exposure of flumioxazin across multiple temperatures. Peanut Science. 47(2):89-93.

Hurdle, N.L., T. L. Grey, C. Pilon, W. S. Monfort, D. G. Shilling. 2020b. Interaction of seedling germination, planting date, and flumioxazin on peanut physiology under irrigated conditions. Amer. J. Plant Phys. 11:2012-2030.

Johnson III, W. C., E. P. Prostko, B. G. Mullinix Jr. 2006. Phytotoxicity of delayed applications of flumioxazin on peanut (*Arachis hypogaea*). Weed Tech. 20:157-163.

Jursík, M., J. Andr, J. Holec, J. Soukup. 2011. Efficacy and selectivity of post-emergent application of flumioxazin and oxyfluorfen in sunflower. Plant Soil Environ. 57(11): 532-539.

Leon, R.G., M.J. Mulvaney, B.L. Tillman. 2016. Peanut cultivars differing in growth habit and canopy architecture respond similarly to weed interference. Peanut Science. 43:133-140.

Monfort, W.S., R.S. Tubbs, S. Virk, G. Harris, W.M. Porter. 2021. UGA peanut production agronomic quick reference guide. University of Georgia Extension. AP-118.

Moretzsohn, M.C., M.S. Hopkins, S.E. Mitchell, S. Kresovich, J.F.M. Valls, M.E. Ferreira. 2004. Genetic diversity of peanut (*Arachis hypogaea* L.) and its wild relatives based on the analysis of hypervariable regions of the genome. BMC Plant Biology. 4:11. DOI: 10.1186/1471-2229-4-11

NASS. 2019. National Agricultural Statistics Service. United States Department of Agriculture. https://quickstats.nass.usda.gov/results/87B25844-C174-381F-B38A-BAF745DF27A4#1B1E4692-C7C2-3AAB-B86D-6A31E3F2A287. Accessed 12 Oct. 2021.

NASS. 2021. National Agricultural Statistics Service. United States Department of Agriculture. 2020 State Agriculture Overview: Georgia.

Prasad, P. V. V., V. G. Kakani, H. D. Upadhyaya. 2010. Growth and production of groundnuts. *In:* Soils, plant growth and crop production- Vol. II. Ed. W. H. Verheye. Pgs. 135-167.

Peterson, A. G. Peanuts: prices, production, and foreign trade since the Civil War. *Economic Geography*. 7(1): 59-68.

Price, A. J., J. W. Wilcut, J. R. Cranmer. 2004a. Flumioxazin preplant or post-directed application timing followed by irrigation at emergence or after post-directed spray treatment does not influence cotton yield. Weed Tech. 18:310-314.

Price, A. J., J. W. Wilcut, J. R. Cranmer. 2004b. Physiological behavior of root-absorbed flumioxazin in peanut, ivyleaf morningglory (*Ipomoea hederacea*), and sicklepod (*Senna obtusifolia*). Weed Sci. 52:718-724.

Prostko, E.P. 2022. Georgia pest management handbook- 2022 commercial edition vol.1. UGA extension special bulletin 28 p. 219.

Prostko, E.P., M. Abney, R. Kemerait. 2021. UGA peanut production pest management quick reference guide. University of Georgia Extension. AP-118.

Royal, S.S., B. J. Brecke, F. M. Shokes, D. L. Colvin. 1997. Influence of broadleaf weeds on chlorothalonil deposition, foliar disease incidence, and peanut (*Arachis hypogaea*) yield. Weed Tech. 11(1): 51-58.

Shaner, D. L. 2014. Herbicide Handbook 10th ed. Weed Science Society of America. Lawrence, KS.

Stephenson IV, D.O., T.A. Spivey, M.A. Deliberto Jr., D.C. Blouin, B.C. Woolam, T.B. Buck. 2018. Effects of low-dose flumioxazin and metribuzin postemergence applications on soybean. Weed Tech. 33(1): 87-94.

Stephenson IV, D.O., T.A. Spivey, M.A. Deliberto Jr., D.C. Blouin, B.C. Woolam, T.B. Buck. 2019. Cotton (*Gossypium hirsutum* L.) injury, growth, and yield following low-dose flumioxazin postemergence applications. Journal of cotton science. 23:218-224.

Valentine, H. 2019. Remembering our past and how it affected our present and future. Peanut Sci. 46 (1A): 78-81.

Wilcut, J. W., A. C. York, G. R. Wehtje. 1994. The control and interaction of weeds in peanut (*Arachis hypogaea*). Rev. Weed Sci. 6:177-205.

WSSA. 2017. Weed Science Society of America- herbicide site of action (SOA) classification list. Accessed February 18th, 2022. https://wssa.net/wp-content/uploads/WSSA-Herbicide-SOA-20170403.pdf

Table 2.1. Temperature and rainfall[a] for Plains and Tifton, GA in 2020, 2021, and 2022.

		Maximum Temperature (C[b])		Minimum Temperature (C[b])		Rainfall (cm[c])	
Year	Month	Plains	Tifton	Plains	Tifton	Plains	Tifton
2020	May	NA	29.4	NA	19.9	NA	6.6
	June	30.9	30.8	20.2	20.8	7.3	12.9
	July	32.8	33.7	21.8	22.3	9.7	4.7
	Aug.	32.7	33.3	21.8	22.5	16.3	11.6
	Sept.	28.4	29.4	19	20.1	16.8	13.3
	Oct.	26.6	28.3	14.8	17.3	7.3	1.4
	Season	30.3	30.8	19.5	20.5	57.4	50.5
2021	May	30.2	NA	16.3	NA	0.3	NA
	June	30.6	31.1	20.1	21.4	20.1	11.8
	July	31.4	31.7	21.4	22	14.4	20.7
	Aug.	31.6	31.9	21.8	22.5	18.6	14.9
	Sept.	29.3	30.4	18.2	19.2	14.3	9
	Oct.	27.1	27.8	16.6	16.9	10.6	5.1
	Season	30	30.6	19.1	20.4	78.3	61.5
2022	May	29.9	31.6	17.4	19.3	11.2	0.2
	June	33.8	34.2	21.3	21.8	5.1	9.9
	July	32.3	32.9	22.3	22.3	19.8	14.2
	Aug.	31.4	32.2	21.4	21.9	13.2	20.7
	Sept.	29.5	29.5	18.3	18.6	8.1	6.3
	Oct.	NA	27.1	NA	12.3	NA	2.4
	Season	31.4	31.3	20.1	19.4	11.5	9.0

[a]Temperature and rainfall data obtained from georgiaweather.net.
[b]Average of daily values for time period listed.
[c]Sum of daily values for each time period listed.
NA = data not available as peanut were not planted or inverted.

Table 2.2. Effects of multiple application rates and timings of flumioxazin on percent injury compared to the non-treated control in peanut at Plains and Tifton from 2020 to 2022.

		Plains			Tifton		
Herbicide Rate	Application Timing	2020[c]	2021	2022	2020	2021	2022
g ai ha^{-1}	DAP[b]	----------%----------					
0[a]	0	0 c	0 d	0 f	0 d	0 e	0 f
0.25	0	0 c	4.2 cd	0.4 f	0.1 d	0.8 e	0 f
0.5	0	0 c	5.3 cd	0.8 f	0 d	0 e	0.4 f
1	0	0.8 bc	7.0 cd	1.3 f	0.1 d	3.3 e	0.8 f
0	3	0 c	0 d	0 f	0 d	0 e	0 f
0.25	3	0 c	6.3 cd	0 f	0.4 cd	0 e	0 f
0.5	3	0.8 bc	5.0 cd	0 f	0.7 cd	5.4 e	0 f
1	3	0 c	5.5 cd	2.9 ef	0.6 cd	30.4 bcd	0.8 f
0	5	0 c	0 d	0 f	0 d	0 e	0 f
0.25	5	1.3 bc	9.9 a-d	1.25 f	0.4 cd	3.3 e	0 f
0.5	5	0.4 bc	11.1 a-d	0.8 f	1.3 cd	6.3 e	2.5 f
1	5	0 c	11.0 a-d	4.6 def	1.3 cd	31.3 bcd	2.9 f
0	7	0 c	0 d	0 f	0 d	0 e	0 f
0.25	7	0.8 bc	4.3 cd	3.8 def	1.5 cd	16.3 cde	1.7 f
0.5	7	0 c	7.3 bcd	4.2 def	2.4 cd	29.6 cd	2.9 f
1	7	1.7 bc	9.8 a-d	10.8 cde	3.5 cd	50.9 ab	9.2 e
0	10	0 c	0 d	0 f	0 d	0 e	0 f
0.25	10	0 c	19.3 abc	1.7 f	11.5 bc	12.1 de	14.6 de
0.5	10	3.8 abc	25 a	6.3 def	20.1 b	36.3 abc	24.2 c
1	10	2.9 abc	22.8 abc	19.6 b	40.3 a	52.1 a	39.6 a
0	14	0 c	0 d	0 f	0 d	0 e	0 f
0.25	14	3.3 abc	7.5 bcd	11.7 bcd	7.6 cd	10.8 de	13.8 e
0.5	14	5.0 ab	15.6 a-d	14.6 bc	22.1 b	17.5 cde	20 cd
1	14	6.7 a	24.3 ab	27.9 a	36.5 a	20.4 cde	32.9 b

[a]Rate reflects the percentage of the full labelled application rate. 0= 0 g ai ha^{-1}, 0.25= 27 g ai ha^{-1}, 0.5= 54 g ai ha^{-1}, 1= 107 g ai ha^{-1}.

[b]Application time indicates days after planting the herbicide application was made.

[c]Values followed by the same letter within the same column are not significantly different at the 5% probability level. Data were subjected to PROC GLIMMIX in SAS Studio 3.8 with means separated by Tukey's HSD. Data were separated by year and location.

Visual injury consisted of total chlorosis and necrosis of the entire plot compared to the NTC.

Table 2.3. Effects of multiple application rates and timings of flumioxazin on weed control compared to the non-treated control in peanut at Plains and Tifton from 2020 to 2022.

		Plains			Tifton	
Herbicide Rate	Application Timing	2020	2021	2022	2021	2022
g ai ha^{-1}	DAP	----------	----------	%----------	----------	----------
0[a]	0	0 f	0 b	0 d	0 b	0 b
0.25	0	20.8 c-f	66.7 a	85.5 abc	76.6 a	90.4 a
0.5	0	40.8 a-e	74.2 a	89.5 abc	86.4 a	96.4 a
1	0	71.3 ab	74.6 a	94.7 abc	80.5 a	94.3 a
0	3	0 f	0 b	0 d	0 b	0 b
0.25	3	38.3 a-e	82.2 a	76.9 bc	59.7 a	92.8 a
0.5	3	43.3 a-d	74.5 a	75.7 c	91.2 a	97.1 a
1	3	47.5 abc	74.1 a	93.0 abc	87.9 a	79.0 a
0	5	0 f	0 b	0 d	0 b	0 b
0.25	5	34.2 b-f	70.2 a	93.8 abc	82.1 a	79.7 a
0.5	5	45.8 abc	63.9 a	96.7 a	68.7 a	82.4 a
1	5	48.3 abc	76.3 a	97.3 a	88.1 a	96.2 a
0	7	0 f	0 b	0 d	0 b	0 b
0.25	7	43.3 a-d	82.6 a	88.5 abc	58.7 a	96.7 a
0.5	7	60 ab	88.8 a	90.9 abc	83.1 a	97.8 a
1	7	74.4 a	96.0 a	95.9 ab	93.0 a	95.0 a
0	10	0 f	0 b	0 d	0 b	0 b
0.25	10	16.7 c-f	85.2 a	94.8 abc	75.5 a	97.1 a
0.5	10	18.3 c-f	78.5 a	94.9 abc	73.3 a	94.6 a
1	10	37.1 a-f	92.5 a	97.8 a	89.7 a	96.6 a
0	14	0 f	0 b	0 d	0 b	0 b
0.25	14	5.8 def	64.2 a	96.4 ab	92.8 a	98.8 a
0.5	14	3.3 ef	74.6 a	96.0 ab	72.9 a	97.3 a
1	14	5.8 def	76.8 a	97.7 a	78.3 a	98.0 a

[a]Rate reflects the percentage of the full labelled application rate. 0= 0 g ai ha^{-1}, 0.25= 27 g ai ha^{-1}, 0.5= 54 g ai ha^{-1}, 1= 107 g ai ha^{-1}.
[b]Application time indicates days after planting the herbicide application was made.
[c]Values followed by the same letter within the same column are not significantly different at the 5% probability level. Data were subjected to PROC GLIMMIX in SAS Studio 3.8 with means separated by Tukey's HSD. Data were separated by year and location.

Table 2.4. Effects of multiple application rates and timings of flumioxazin on peanut plant diameters at Plains and Tifton from 2020 to 2022.

		Plains			Tifton		
Herbicide Rate	Application Timing	2020	2021	2022	2020	2021	2022
g ai ha^{-1}	DAP	----------%----------					
0[a]	0	10.1 ab	12.9 a	12.7 abc	13.4 abc	15.6 abc	16.4 b-f
0.25	0	10.1 a	12.3 a	13 ab	13.3 abc	16.4 a	16.7 b-f
0.5	0	10.2 a	12.1 a	12.4 bcd	13.6 ab	14.9 a-e	16.7 b-f
1	0	9.5 ab	12.3 a	11.2 b-e	13.2 abc	13.8 a-f	17.4 a-e
0	3	9.4 ab	12.7 a	12.6 bc	13.1 abc	15.8 ab	17.5 a-e
0.25	3	9.9 ab	11.9 a	12.9 abc	14.1 a	15.9 ab	16.8 a-f
0.5	3	10.0 ab	12.4 a	12.9 abc	13.3 abc	14.2 a-e	17.7 a-d
1	3	9.8 ab	12.4 a	11.9 bcd	12.6 abc	11.6 c-g	16.1 c-g
0	5	9.9 ab	12.1 a	12.5 bc	12.5 a-d	15.2 a-d	15.6 c-g
0.25	5	9.9 ab	12.5 a	12.5 bcd	13.3 abc	15.2 a-d	16.6 b-f
0.5	5	9.8 ab	11.7 a	12.2 bcd	12.5 a-d	15.5 a-d	16.3 c-g
1	5	10.0 ab	11.6 a	11.7 bcd	12.3 a-d	10.9 efg	16.4 b-f
0	7	10.0 ab	12.7 a	12.9 abc	13.4 abc	15.8 ab	17.6 a-d
0.25	7	10.1 a	13.1 a	12.3 bcd	13.1 abc	13.5 a-g	15.9 c-g
0.5	7	9.9 ab	11.4 a	11.4 b-e	12.6 abc	11.5 d-g	16.9 a-f
1	7	9.5 ab	12.7 a	10.7 de	11.4 b-e	9.6 g	14.8 fg
0	10	9.3 ab	12.5 a	12.5 bcd	13.6 ab	16.2 ab	17.8 abc
0.25	10	8.3 b	11.9 a	12.2 bcd	11.0 cde	13.4 a-g	15.4 d-g
0.5	10	9.5 ab	10.6 a	11.2 cde	9.9 de	12.3 b-g	15.2 efg
1	10	10.0 ab	11.6 a	9.7 e	8.9 e	10.1 fg	14.0 g
0	14	10.3 a	12.6 a	14.4 a	13.6 abc	16.2 ab	19.1 a
0.25	14	10.1 ab	12.3 a	12.6 abc	12.3 a-d	14.2 a-e	18.7 ab
0.5	14	9.8 ab	12.1 a	12.0 bcd	11.3 b-e	12.5 a-g	17.3 a-e
1	14	9.7 ab	12.2 a	9.8 e	11.3 b-e	12.5 a-g	16.0 c-g

[a]Rate reflects the percentage of the full labelled application rate. 0= 0 g ai ha^{-1}, 0.25= 27 g ai ha^{-1}, 0.5= 54 g ai ha^{-1}, 1= 107 g ai ha^{-1}.
[b]Application time indicates days after planting the herbicide application was made.
[c]Values followed by the same letter within the same column are not significantly different at the 5% probability level. Data were subjected to PROC GLIMMIX in SAS Studio 3.8 with means separated by Tukey's HSD. Data were separated by year and location.

CHAPTER 3

GERMINATION RESPONSE OF TREATED PEANUT (*Arachis Hypogaea L.)* TO OPTIMAL AND SUBOPTIMAL STORAGE CONDITIONS

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Abstract

Crop establishment is a crucial component of a successful growing season. The conditions in which inshell and shelled peanut seed are stored can influence the overall germination and vigor. Once shelled, peanuts are treated with fungicides to protect vulnerable seedlings from disease after planting. Seed treatment technology has adapted a polymer type seed coating that is infused with liquid fungicides to completely coat the seed. Information regarding the effects of this polymer seed coating on peanut germination has not been investigated. An experiment was performed on multiple peanut cultivars to investigate the germination response of treated peanut stored under various conditions. Stored seed were treated with the standard dust formulation, a polymer seed treatment, or left untreated and stored in a controlled or ambient environment storage location. The controlled storage area was maintained at stable temperature and humidity levels, while the ambient storage varied in temperature and humidity for several weeks. Seed were placed on a thermal gradient table for germination testing. Data were analyzed using non-linear regression to determine the growing degree days to reach 80% germination. As time passed in the ambient storage, germination for all seed decreased. Germinating seed treated with the polymer seed treatment decreased by 23% within six weeks of storage, while the remaining seed continued to germinate up to 45%. Seed stored in the controlled storage continued to germinate throughout the study but decreased as time progressed. Overall, polymer treated seed stored in an uncontrolled environment decreased in vigor to unacceptable levels. Additional research is needed to determine the potential for cultivar by polymer interactions and testing new polymer seed treatment technology as it is released.

Introduction

Adequate germination of seed and emergence of peanut (*Arachis hypogaea* L.) is a crucial process in stand establishment. Before germination can occur, the seed must break

dormancy by following a series of species-specific processes induced by environmental and physiological cues (Bewley et al. 2013). Abscisic acid (ABA) is a plant hormone that plays a major role in regulating seed dormancy. ABA synthesis first starts in the 2C-methyl-D-erythritol-4-phosphate (MEP) pathway of cell plastids followed by carotenoid biosynthesis of violaxanthin which will be broken down into a 15C molecule (xanthoxin) which will be exported out of the plastid and oxidized into abscisic aldehyde and finally synthesize abscisic acid by the addition of a carboxyl group by abscisic acid oxidase (Finkelstein 2013). ABA begins to accumulate in seeds during the maturation stage of development and rapidly declines as water is imbibed (Nambara et al. 2010). Germination is initiated with the triphasic uptake of water beginning with a rapid phase, followed by a steady phase, and ending with a second rapid uptake and is completed with radicle protrusion from the seed coat (Bewely 1997). This rapid uptake can account for up to 91% of the water content of the peanut sprout and up to 73% in the cotyledons (Li et al. 2014). ABA levels will begin to decrease within the seed as gibberellin, another plant hormone that plays a significant role in germination, levels increase. Like ABA, gibberellins are synthesized in plastids utilizing the MEP pathway to convert geranylgeranyl diphosphate to *ent*-Kaurene via the *ent*-copalyl diphosphate synthase and *ent*-kaurene synthase. The *ent*-Kaurene is then oxidized into *ent*-kaurenoic acid via a cytochrome P450 to be converted once more by a cytochrome P450 to GA_{12} which is not an active form of gibberellic acid. The GA_{12} must be converted into an active form by oxidations at the C20 and C3 positions by $GA20_{ox}$ and $GA3_{ox}$, respectively (Shinjiro 2008). The concentrations of these plant hormones during imbibition will determine if the seed germinates or stays dormant and has been noted to function in an antagonistic manner (Liu and Hou 2018; Ye and Zhang 2012; Bewely 1997).

After germination, cotyledons and immature true leaves will emerge from the soil seven to 10 days after planting (Beasley et al 1995). As these seedlings emerge, numerous early season diseases may inhibit proper growth and development, or cause death and reduce the plant population. Diseases such as Aspergillus Crown Rot caused by *Aspergillus niger*, Cynlindrocladium Black Rot caused by *Cylindrocladium parasiticum,* and Rhizoctonia Crown Rot caused by *Rhizoctonia sp.* can threaten young peanut seedlings. Growers can reduce infections by planting high quality seed, at the optimal planting conditions with optimal planting methods and utilizing premium seed treatments.

After peanut seed has been stored overwinter, processing facilities will begin to shell and treat seeds. Seed treatments provide a layer of defense to protect germinating seed and emerging seedlings from numerous diseases. Seed treatments are available in multiple formulations, but the most common in peanut production is a wettable power. The treatment is typically applied at approximately 114 g per 45 kg of seed. Due to the dust nature of the wettable powder treatments, airborne particles pose a significant inhalation risk to applicators. Some commercially available seed treatments require at least 24 hours to pass until a person can reenter an area where seed were treated (Anonymous A 2020, Anonymous B 2015). This risk does not cease once seed are treated but continues until planting as growers are at risk when filling hopper boxes on planters. Seed treatment developers have adopted technology from the pharmaceutical industry to create polymer seed treatments and reduce the inhalation risk. The polymer seed treatment is formulated as a dust, same as the current seed treatments, but there are no active ingredients with fungicidal properties. The polymer seed treatment will be combined with a liquid fungicide seed treatment to aid in drying and binding to the seed. The polymer seed treatment also assists in seed flowability through the planter and enhances seed aesthetics by creating a shell-like outer

layer (Anonymous C 2015). Currently, only a few polymer seed treatments are registered for use in peanut, while others are seeking approval with the addition of microbes. Limited data is available for the effects of polymer seed treatments on peanut germination. These studies will begin to bridge the gap and establish methods in which to test polymer seed treatments on peanut as they are developed.

Materials and Methods

2021

Thermal Gradient Table Experiment 1

Peanut cultivar Georgia-07W seed (Branch and Brenneman 2008) were stored and shelled following standard peanut storage and shelling procedures. Once shelled, seed were treated with carboxin plus metalaxyl plus ipconazole [Rancona VPL (UPL Corporation Limited Group Company, King of Prussia, PA)] as a custom, ready to use liquid pesticide blend created for use by Kannar Earth Science, Ltd. (Kannar Earth Science, Ltd. Buford, GA). Once applied, SlipShine 6300, (Kannar Earth Science, Ltd. Buford, GA) a polymer seed finisher (Anonymous C 2015), was applied to assist in fungicide drying and enhance seed aesthetics and flowability within the planter for a combination of Rancona VPL plus SlipShine 6300 seed treatment (P1). Treated peanut seed were placed into 22.7 kg bags and received by the study investigators. Seed were divided further into three equal groups based on storage location treatments. Storage locations consisted of an area with exposure to natural weather conditions (protected from rainfall) (O), an area with fluctuating humidity and temperature (V), and an area with controlled temperature and humidity (C). Divided seed were placed into paper bags and relocated into the respective storage locations. Treated peanut seed remained in these conditions for a total of 10

weeks. Seed samples were taken every week and subjected to germination testing on a thermal gradient table.

A total of 720 seed, in three replications of 240 seed, from each storage location were removed and placed in a 100 mm by 25 mm deep dish Petri dish (Fisher Scientific, Hampton, NH) with 95 mm diameter blotter paper (Anchor Paper Co., St. Paul, MN). Seed were moistened by 10 mL of distilled water when placed on the table and supplemented as needed. The thermal gradient table (Grey et al. 2011) was developed using aluminum blocks measuring 2.4 m by 0.9 m and 7.6 cm in height. A 1.0 cm hole was drilled into both sides of the table to allow fluid from a warming or cooling unit (Anova Model 40, Anova Industries Inc., Stafford, TX) to flow on the respective side. The fluid was composed of a 1:10 ethylene glycol to water mixture set to flow at 3.8 L per minute and remained independent for each unit. The units were set to achieve a temperature gradient of 12 C to 33 across the length of the table. A total of 216 cells in which a dish can be placed are available arranged in nine rows by 24 columns. Thermocouples consisting of duplex insulated PRT-24 wire (Omega Engineering, Stamford, CT) were placed under the table surface through drilled holes measuring 8 mm wide and 7 cm deep to be within 5 mm of the table surface and placed every 10 cm under each cell. Temperatures for each thermocouple were recorded every 30 minutes using a Graphtec data logger (MicroDAQ.com Ltd. Contoocook, NH) for the duration of the study to ensure proper temperature was maintained. Recorded temperature data was utilized to determine growing degree day (GDD) accumulation using the equation

$$t_n = \sum_{i=1}^{n} \left[\frac{Ti_{max} + Ti_{min}}{2} - T_b \right] \quad [1]$$

where t_n is the sum of GDD for *n* days, Ti_{max} and Ti_{min} are the daily maximum and minimum temperatures of day *i* (Ketring and Wheless 1989), and T_b is the base temperature for peanut which was set to 15 C (Ketring et al. 1982).

The study design consisted of a randomized complete block as a split plot, with the polymer seed treatment as the whole plot, and storage condition as the sub plot. Storage location was considered as a fixed effect and replication was considered a random effect. Data was analyzed using SAS Studio 5.2 (SAS Institute Inc., Cary, NC). Nonlinear regression analysis was utilized to evaluate the parameter estimates to determine if the response could be described using the three-parameter logistic growth curve utilized by Freund and Littell (1991):

$$y = \frac{b0}{[(1+((1-P)/P))*e)\,(-b1*(x-XP))\,]} \quad [2]$$

where b0 is the upper asymptote, b1 is the relative slope, XP is the value of x when y is at P percent of the upper asymptote, P was set at 80 for this model. Multiple vigor indices were computed from this equation including growing degree days at b0, growing degree days at 80% germination, and germination at *XP*. A separate curve was fit for each storage location with the 95% confidence limits of each parameter to determine differences between them. Temperature was averaged of the 72 cells (three replications of 24) for the 168 hours seed were on the table to determine the temperature effect on germination using the logistic growth equation [2]. This determined the minimum temperature for germination to occur within a 168-hour period. Data were graphed in SigmaPlot 14 (Systat Software, Inc., Chicago, IL)

Thermal Gradient Table Experiment 2

After the completion of experiment one, a second experiment was performed in a similar manner. Seed were treated with Rancona VPD (R), Rancona VPL plus SlipShine 6300 (P1), Rancona VPL plus SlipShine 2050 (P2) (Kannar Earth Science Ltd., Buford, GA) (Anonymous

E), and a non-treated control (NTC). The storage location with exposure to the natural weather conditions from experiment one was eliminated due to thermal gradient table size, but the fluctuating condition and stable condition locations remained the same. Seed remained in these conditions for a total of six weeks at which point all available seed were used. Seed treated with R and Rancona VPL and SlipShine 6300 were stored in a controlled environment until placed in the respective storage location. SlipShine 2050 contains a similar formulation to SlipShine 6300 with the addition of 2 microbial species: *Bacillus amyloliquefaciens* and *Bacillus methylotrophicus*. Data were analyzed in the same manner as experiment one.

Thermal Gradient Table Experiment 3

An additional experiment was performed to investigate the germination response of seed peanut treated with a polymer seed treatment developed by Syngenta (Syngenta Crop Protection, LLC, Greensboro, NC). Cultivar Georgia-07W peanut seed were treated with Dynasty PD (D), (Anonymous E), a liquid Dynasty PD formulation and polymer (P3), and non-treated control. Seed were stored in the same conditions as experiment two for six weeks when all available seed were utilized. Data were analyzed in the same manner as experiment one and two.

Field Experiment 1

A non-irrigated peanut field trial was conducted in 2021 and 2022 on a University of Georgia farm located at 31.489 N and 83.517 W in Tifton, GA. Soil properties consisted of 100% Tifton loamy sand (Fine-loamy, kaolinitic, thermic Plinthic Kandiudults) with 84.1%, 9.4%, and 6.5% of sand, silt, and clay, respectively. Experimental design consisted of a randomized complete block design with three replications. All seed from germination experiments (1, 2, and 3) were planted for field observations, except non-treated seed. Seed were planted in single rows to achieve a population of 19 seed m^{-1}. All seed received a blanket PRE

application of pendimethalin at 1067 g ai ha^{-1} and diclosulam at 27 g ai ha^{-1} and maintained under University of Georgia recommendations for non-irrigated production and weed-free. Peanut in 2021 were planted on June 11th and inverted on November 2nd. Data collected included population, plant width, and yield. Plant population was measured by counting each emerged plant within one meter of row selected randomly. Plant widths were determined by selecting three random plants within each plot and measuring leaf tip to opposite leaf tip of the same plant. Data were analyzed using SAS Studio 5.2 utilizing the PROC GLIMMIX function for analysis of variance (ANOVA). Significant effects for means were separated using Tukey-Kramer HSD set at an alpha of 0.05. Data was analyzed by year.

2022

Thermal Gradient Table Experiment 4

The germination experiments were repeated in 2022 with newly developed polymers that were provided without tradenames or components. Treatment names were created by the investigators and included R1, SA, S1, S2, and R2. Seed were subjected to the same germination testing for six weeks and analysis procedures as previously discussed. These seed were stored under ambient room temperature and humidity for the duration of the six week experiment.

Field Experiment 2

Seed from the UPL plus Syngenta Experiment 1 were planted for field growth and development observations. Seed were planted and maintained in the same field as the 2021 experiment being on the opposite end. Peanut were planted and maintained under the same University of Georgia agronomic recommendations as the 2021 experiment. Peanut were planted on May 25th, with data collection including plant population, plant widths, and yield. Data analysis followed the same methods as the 2021 experiment.

Results and Discussion

Germination

Thermal Gradient Table Experiment 1

Initial germination data was recorded for polymer treated seed prior to storage treatments to determine a baseline germination rate. The overall trend indicated a slight decrease overtime in maximum germination rate for P1 treated seed in all three storage locations. Initial germination rate was recorded at 96% and only 37 GDD's at *XP*. CP1 stored seed maintained nearly the same germination rate after 10 weeks of storage as the initial germination rate decreasing by only 3%. GDD's at *XP* decreased by 16% requiring only 31 GDD's. OP1 stored seed germination rate also decreased over the 10 weeks, but only by 4%. GDD's at *XP* decreased substantially by 21% to 29. Seed that had the greatest changes in germination and GDD's at *XP* were VP1. Germination decreased by 10% to a maximum germination rate of 88%. While still acceptable, storing seed in these conditions will potentially cause seed to germinate below acceptable levels. GDD's needed for 80% of the upper asymptote increased by 56% requiring 58 GDD's. This would cause seed to remain in the soil longer without germinating increasing the chance of pathogen infection or seed rot.

Vigor indices were also calculated and noted a similar trend as the germination calculations. A trend was indicated for the number of GDD's required to reach maximum germination as GDD's increased, then decreased, and increased alternatively each week for the 10 weeks before ending at 216 GDD's. This was nearly the same amount of GDD's needed for seed before any storage treatments were applied. GDD's required to reach 80% germination decreased as storage time increased (up to five weeks) followed by an increase to only 4 GDD's below the initial seed. CP1 had germination at *XP* (80% of upper asymptote) decrease over time

to 75% germination. GDD's needed to reach maximum germination decreased until five weeks in the natural weather condition storage at which GDD's increased to 188. This amount of GDD's was still lower than the initial seed required. Additionally, these seed noted a 7 GDD decrease to reach 80% germination compared to the initial seed. Germination at *XP* only noted a 2% decrease after 10 weeks in storage for OP1 seed. VP1 had the greatest changes with respect to vigor indices. GDD's required to reach maximum germination increased by 119 GDD's after 10 weeks in storage. The number of GDD's to reach 80% germination nearly doubled by requiring 75 GDD's compared to only 40 GDD's for the initial seed. Finally, germination at *XP* only decreased by 7% to 70% germination compared to initial seed at 77% germination at *XP*.

Thermal Gradient Table Experiment 2

Seed in experiment two noted a greater decrease in all parameters compared to seed in experiment one. The controlled stored NTC seed (CNTC) seed had an initial maximum germination rate of 88% and decreased by 12% over six weeks of storage. VNTC (variably stored NTC) seed decreased by 25% in the same storage period. This resulted in a maximum germination rate of 63%. CR (controlled storage with Rancona) and VR (variable storage with Rancona) seed noted a decrease in germination. The greatest loss was in the VR stored seed in which germination was reduced by 37% over six weeks which was nearly 3x more than CR at 13% reduction. CP1 (controlled storage with polymer 1) and CP2 (controlled storage with polymer 2) seed lost no more than 8% germination. The greatest reduction of all seed treatments and environments occurred to VP1 (variable storage with polymer 1) and VP2 (variable storage with polymer 2). VP1 seed had a 14% reduction in germination after four weeks in storage. At six weeks of storage, the germination data no longer fit the model, indicating a significant

decrease in overall germination. VP2 also did not fit the model after six weeks in storage but noted a 22% reduction in germination only 2 weeks in storage.

Vigor indices also noted similar trends as the germination data. Initial NTC seed needed 233 GDD's to reach maximum germination, 48 GDD's to achieve 80% germination, and 71% germination at *XP*. After six weeks, CNTC seed required only 79 GDD's to reach maximum germination, but this was due to a low germination rate. Germination at *XP* was determined to only be 61%. VNTC were noted to require 251 GDD's for maximum germination, but also had a low germination rate with germination at *XP* being only 51%. Neither seed reached 80% germination resulting in no germination at 80% vigor index. Initial R seed required 302 GDD's to achieve maximum germination with only 64% at *XP* at the trial beginning. These seed did not reach 80% germination for the duration of the study in either storage location except for week two in the C storage and required 136 GDD's. CR seed had a 21 GDD reduction to achieve maximum germination and a 10% reduction in germination at *XP*. P1 peanut seed required 366 GDD's initially to achieve maximum germination and germinated only 66% at *XP*. Deviating from the other seed, these seed initially achieved 80% and required 102 GDD's to achieve it. The CP1 seed decreased in GDD's for maximum germination up to four weeks, but increased up to 281 GDD's after six weeks in storage, 85 fewer GDD's than the initial P1 seed. Germination at *XP* decreased by 6% after six weeks of storage and did not reach 80% germination after the initial germination. VP1 seed did not fit the analysis model indicating poor germination after six of storage. Initial data collected for seed treated with P2 peanut seed indicated a requirement of 213 GDD's for maximum germination and 56% germination at *XP*. GDD's for 80% germination was not determined due to seed not achieving 80% germination initially. CP2 required 99 GDD's

for maximum germination and only achieved only 50% germination at *XP* while VP2 seed did not fit the model due to poor germination.

Thermal Gradient Table Experiment 3

Data for week four of the controlled environment and week six of the ambient seed sample timings were not included in the analysis due to equipment failure during germination testing.

Initial experiment data indicated NTC seed achieved 89% maximum germination and needed 44 GDD's to reach 80% of the upper asymptote, D (Dynasty) treated seed achieved 80% germination and required 54 GDD's for 80% of the upper asymptote, and the P3 (polymer 3) treated seed achieved 69% germination while requiring 57 GDD's for 80% of the upper asymptote. After six weeks in either storage condition, all seed germinated less than 80%. The greatest decrease occurred in the VNTC seed with a 32% reduction to only 55%. The greatest increase in GDD's required for 80% of the upper asymptote was in CP3 seed increasing by 42 GDD's.

Vigor indices also indicated a decrease in seed vigor as storage time increased. CNTC seed indicated a decrease in maximum germination by 158 GDD's at six weeks. A decrease in germination at *XP* was noted as 17% for the CNTC in the same amount of time. D seed initially required 363 GDD's for maximum germination and achieved 64% germination at *XP*. After four weeks VD (variable storage dynasty treated) required an additional 144 GDD's to achieve maximum germination with a reduction in germination at *XP* to 62%. After six weeks, CD (controlled storage Dynasty treated seed) required 168 fewer GDD's to achieve maximum germination and maintained 64% germination. P3 seed required 321 GDD's for maximum germination and germinated at 55% at trial initiation. Seed required 126 more GDD's for

maximum germination and had a 3% reduction in germination after four weeks V storage. CP3 seed required fewer GDD's for maximum germination than the initial seed (206), but also noted a reduction in germination at *XP* to 48%.

Field Experiment 1

The University of Georgia recommends peanut planting rate should be high enough to reduce the impact of Tomato Spotted Wilt Virus (Family: Bunyaviridae Genus: *Tospovirus*) and reflect expected germination. The suggested planting rate under these considerations is implemented as 19 seed m^{-1}. Plant population and plant width data were recorded 13, 19, 31 days after planting (DAP). As the season progressed, germination increased therefore increasing plant population. Differences were not noted for any measurement of plant population. Germination increased from 9 to 11 seed/m 13 DAP to 11 to 15 seed/m 31 DAP. Generally, regardless of seed treatment, seed stored in the controlled setting germinated more than seed stored in the uncontrolled setting. P1 and P2 seed germinated rapidly regardless of storage conditions, but also reached maximum germination rapidly with minimal additional seed germinating after 19 DAP. VP2 and VR seed consistently germinated the lowest across all measurements. D and P3 treated seed stored in either condition generally did not germinate as rapid as P1, P2, or R treated seeds. This was not noted across the entire experiment as germination for D and P3 seed germinated the greatest at the termination of the population data recording. All seed had an average germination of at least 61% after 31 DAP. All D and P3 seed germinated at least 77% in from either storage condition, while P1, P2, and R treated seed germinated between 60% and 74% for all treated seed regardless of storage environment.

No trend was noted for plant widths, but differences were indicated at 19 DAP in which CP1 and CP3 had greater widths than VR seed. All polymer treated seed, regardless of storage had greater widths than all dry seed treatments, except CP3.

Peanut were inverted 144 DAP and remained on the soil surface for 13 days after inversion. Moisture content at harvest was 8.8%. Due to seed being different cultivars, results will be discussed for each respective cultivar.

CP3 and VP3 seed treated yielded greater than the CD or PD treated seed. CP3 yield was different than the CD seed. No differences were noted for any of the P1, P2, and R seed treatments stored in any condition. P1 and P2 seeds stored in the uncontrolled setting yielded higher than any other treatment.

2022

Thermal Gradient Table Experiment 4

The R1 treated seed achieved 72% germination at experiment initiation and required 27 GDD's to reach 80% germination of the upper asymptote. After six weeks of constant ambient storage, these seed achieved only 63% germination and required 51 GDD's to obtain 80% germination of the upper asymptote. SA treated seed germinated poorly to begin the experiment at only 55% and need 13 GDD's to reach 80% germination of the upper asymptote. Germinated seed after six weeks of storage did not fit the model and achieved 87% germination, but required 93 GDD's for 80% germination of the upper asymptote. The 95% confidence intervals after six weeks ranged between 60 to 114 for the maximum germination, with 51 to 136 GDD's for 80% germination of the upper asymptote. The S1 treated seed had a maximum germination rate of 69% at the beginning of the trial and required 40 GDD's for 80% germination of the upper asymptote. These seed remained stabled with respect to germination over the six weeks by

germinating at 71% and requiring 57 GDD's for 80% germination of the upper asymptote. S2 treated seed were able to germinate a maximum of 71% requiring 48 GDD's to reach 80% germination of the upper asymptote. Seed with this polymer treatment only decreased germination by 8% over six weeks (63%) and required nearly the same GDD's (47). The R2 treated seed maintained the highest germination rates and GDD values of all treatments in this experiment. Seed with this polymer seed treatment achieved 95% germination and required only 15 GDD's to reach 80% of the upper asymptote with germination decreasing by only 4% and GDD's increasing by 7.

Vigor indices were also determined for these germinated seed (Table 4). The R1 treated seed initially were able to be described by the analysis model, but after several weeks of storage was no longer able to fit the model. The germination at *XP* at experiment initiation was noted to be at 61% and rose to 91% after four weeks of storage, subsequently rapidly falling to only 54% after five weeks. It was only after one week of storage that the R1 treated seed no longer fit the model. The SA seed treatment provided only 47% germination at *XP* at initiation and 45% after four weeks in storage, but remaining storage timings did not fit the model. The GDD's needed to reach the maximum asymptote were determined to be 171 and 176 for initiation and four weeks of storage, respectively. The S1 treated seed also did not fit the model for multiple weeks after storage germination. These seed at initiation noted 58%, 60%, and 63% germination at *XP* at initiation and after one and four weeks of storage, respectively. These seed required 265, 171, and 246 GDD's to reach maximum germination at initiation and one or four weeks of storage, respectively. The S2 treated seed decreased over time with respect to the germination at *XP*. Initially these seed noted 60% germination at *XP* and decreased to 53% after 5 weeks of storage. The GDD's needed for maximum germination also decreased, but reach a maximum of 446 after

only two weeks of storage. The R2 treated seed is the only seed treatment to fit the model for the duration of the experiment. The seed maintained an adequate level of germination at *XP*, falling only 4% from 81% to 77% after five weeks in storage. The GDD's needed to reach maximum germination also remained constant only increasing by 10 GDD's over the five weeks storage period. This index decreased during the first two weeks in storage and subsequently rose after 3 weeks of storage. The GDD's needed for 80% germination also stayed low compared to the remaining treatments beginning at 14 GDD's and ended needing only 26. It is worth noting that the majority of these seed never reach 80% germination, therefore the $germ_{80}$ index was not able to be determined, except in all weeks of the R2 treated seed.

Field Experiment 2

Initial plant population counts were taken 13 DAP with counts ranging from seven to 11 plants. A difference was noted as the R2 treated seed had a higher population compared to SA treated seed. Population counts were recorded again 19 DAP with the same differences as the first rating, but counts ranging from nine to 14. Population counts three and four indicated no differences, with counts ranging from 14 to 18 and 17 to 23, respectively. The R2 treated seed was noted to have the highest population over all ratings, coinciding with the germination table data. Plant width data was also collected at the same time as plant population. Widths at the first rating were between seven and 10 cm with the R2 treated seed being wider compared to SA treated seed with no other differences indicated. No differences were noted for the second width rating with plants ranging between 11 and 14 cm wide. The third width rating indicated that R2 treated seed were wider compared to S1 treated seed, with widths being between 13 and 16 cm. The fourth rating indicated no differences with plants ranging between 17 and 20 cm.

Polymer seed treatments can be composed of several ingredients such as mica minerals, proteins, microbes, and inorganic compounds. One inorganic compound in some polymers is titanium dioxide. Titanium dioxide (TiO_2) is a component of numerous items humans use daily such as toothpaste, confectionary items, plastics as well as paints and cosmetics (Skocaj et al. 2011). TiO_2 can be processed into a powder and classified as a nanoparticle if the particle has a dimension of 100 nm or less (Skocaj et al. 2011). The TiO_2 assists in the drying process and enhances the seed aesthetic properties. It's concentration within the seed treatment is not static and can vary greatly depending on other treatment components. Titanium dioxide nanoparticles have been noted to have beneficial and detrimental effects on plants (Singh et al. 2021). Minimal research has been performed on peanut regarding the effects of TiO_2 on peanut, but Rui et al. (2018) indicated that TiO_2 and other metal-based nanoparticles affect yield and nutritional quality, but minimal to no information is available regarding peanut germination response to TiO_2 nanoparticles. Hatami et al. 2014 investigated the response of multiple crops germination to TiO_2 and reported an increase in germination for all tested crops. Contradictory to that study, tobacco (*Nicotiana tabacum* L.) and peppermint (*Mentha piperita* L.) responded negatively to TiO_2 nanoparticle treatments including a toxic limit in peppermint (Ghosh et al. 2010; Samadi et al. 2014). It was also noted that corn (*Zea mays* L.) germination responded negatively to titanium dioxide nanoparticle treatments but was able to recover over time (Castiglione et al. 2010). This study will begin to accumulate preliminary data regarding the effects of TiO_2 on peanut germination.

Proper seed storage after harvest has been indicated to influence germination. Seed stored in well-regulated conditions generally germinated more than seed stored in unregulated or fluctuating conditions. Harrington (1972) developed guidelines regarding seed deterioration: 1.

For every 1% reduction in seed moisture content, the life of the seed doubles and 2. every 5˚C reduction in seed temperature will double the life of the seed. These only apply for seed with moisture content between 5% and 14% and seed above 0˚C, respectively (McDonald 2004). The temperature of the controlled storage location was stable at 21˚C, the outside location varied daily, and the uncontrolled storage location varied between 21˚ and 49˚C with high humidity. Seed deteriorated quickly under the high temperature and humidity environment. Seed treatments may also have influenced germination due to the presence of TiO_2 nanoparticles. Additional research is warranted to gather further information regarding effects of storage environment and polymer seed treatments on peanut germination. Germination studies should be performed on multiple peanut cultivars to determine if any cultivar by storage environment or cultivar by seed treatment interactions exist.

Literature Citations

Anonymous A. 2020. Rancona V PD seed treatment label. UPL NA Inc. King of Prussia, PA 19406.

Anonymous B. 2015. Dynasty PD seed treatment label. Syngenta Crop Protections, LLC. Greensboro, NC 27419.

Anonymous C. 2015. SlipShine 6300 dry seed finisher label. Kannar Earth Science, Ltd. Buford, GA 30518.

Anonymous D. 2015. SlipShine 2050 dry seed finisher label. Kannar Earth Science, Ltd. Buford, GA 30518.

Anonymous E. 2015. Dynasty PD label. Syngenta Crop Protection, LLC. Greensboro, NC 27419.

Beasley, J., S.L. Brown, G.B. Padgett, S.M. Brown. 1995. Peanut scout handbook. Eds. S. L. Brown. Cooperative Extension Service, The University of Georgia, College of Agricultural and Environmental Science.

Bewely, J.D. 1997. Seed germination and dormancy. The Plant Cell. 9:1055-1066.

Bewely, J.D., K. J. Bradford, H.W.M. Hilhorst, H. Nonogaki. 2013. Seeds: Physiology of development, germination, and dormancy. 3rd edition. DOI: 10.1007/978-1-4614-4693-4_6.

Branch, W.D. and T. Brenneman. 2008. Registration of 'Georgia-07W' peanut. Journal of Plant Registrations. 2(2). DOI: 10.3198/jpr2007.12.0682crc

Castiglione, M.R., L. Giorgetti, C. Geri, R. Cremonini. The effects of nano-TiO_2 on seed germination, development and mitosis of root tip cells of *Vicia narbonensis* L. and *Zea mays* L. J. Nanoparticle Research. 13:2443-2449.

Finkelstein, R. 2013. The Arabidopsis book. The American Society of Plant Biologists. DOI: 10.1199/tab.0166. https://doi.org/10.1199/tab.0166

Freund, R.J. and R. Littell. 1991. SAS system for regression. 2nd ed. SAS Institute, Cary NC.

Ghosh, M., M. Bandyopadhyay, A. Mukherjee. 2010. Genotoxicity of titanium dioxide (TiO_2) nanoparticles at two trophic levels plant and human lymphocytes. Chemosphere. 81(10): 1253-1262.

Grey, T., J. Beasley, T. Webster, C. Chen. 2011. Peanut seed vigor evaluation using a thermal gradient. International Journal of Agronomy. DOI: 10.1155/2011/22341.

Hatami, M., M. Ghorbanpour, H. Salehiarjomand. 2014. Nano-anatase TiO_2 modulates the germination behavior between seedling vigority of some commercially important medicinal and aromatic plants. J. Biol. Envrion. Sci. 8(22): 53-59.

Ketring, D.L., R.H. Brown, G.A. Sullivan, B.B. Johnson. 1982. Peanut Physiology. *In*: Pattee H.E., Young C.T. eds. *Peanut Science and Technology*, American Peanut Research and Education Society, Inc. Stillwater, OK.

Ketring, D.L. and T.G. Wheless. 1989. Thermal time requirements for phonological development of peanut. Agron. J. 81:910-917.

Li, Y-C., H. Qian, X-L. Sun, L. Cui, H-Y. Wang., C. Du, X-H. Xia. 2014. The effects of germination on chemical composition of peanut seed. Food Science and Technology Research. 20(4): 883-889.

Liu, X. and X. Hou. 2018. Antagonistic regulation of ABA and GA in metabolism and signaling pathways. Front. Plant Sci. DOI: 10.3389/fpls.2018.00251

Nambara, E., M. Okamoto, K. Tatematsu, R. Yano, M. Seo, Y. Kamiya. 2010. Abscisic acid and the control of seed dormancy and germination. Seed Science Research. 20(2): 55-67.

Rui, M., C. Ma, J.C. White, Y. Hao, Y. Wang, X. Tang, J. Yang, F. Jiang, A. Ali, Y. Rui, W. Cao, G. Chen, B. Xing. 2018. Metal oxide nanoparticles alter peanut (*Arachis hypogaea* L.) physiological response and reduce nutritional quality: a life cycle study. Environmental Science: Nano. 2018(5): 2088-2102.

Singh, R. K. Patel, I. Tripathi. 2021. Interaction of titanium dioxide nanoparticles with plants in agro-ecosystems. n: Singh P., Singh R., Verma P., Bhadouria R., Kumar A., Kaushik M. (eds) Plant-Microbes-Engineered Nano-particles (PM-ENPs) Nexus in Agro-Ecosystems. Advances in Science, Technology & Innovation (IEREK Interdisciplinary Series for Sustainable Development). Springer, Cham. https://doi.org/10.1007/978-3-030-66956-0_4

Samadi, N., S. Yahyaabadi, Z. Rezayatmand. Effect of TiO_2 and TiO_2 nanoparticle on germination, root and shoot length and photosynthetic pigments of *Mentha piperita.* Int. J. Plant Soil Sci. 3(4): 408-418.

Shinjiro, Y. 2008. Gibberellin metabolism and its regulation. Annual Review of Plant Biology. 59:225-251.

Skocaj, M., M. Filipiic, J. Petkovic, S. Novak. 2011. Titanium dioxide in our everyday life: is it safe? Radiol. Oncol. 45(4): 227-247.

Tubbs, R.S., W.S. Monfort, S. Virk, G. Harris, W.M. Porter. 2021. UGA Peanut Production Guide: The Agronomic Quick Reference Guide. University of Georgia Extension. Publication AP-118.

Ye, N. and J. Zhang. 2012. Antagonism between abscisic acid and gibberellins is partially mediated by ascorbic acid during seed germination in rice. Plant Signal Behav. 7(5): 563-565.

Table 3.1. Seed germination, logistic growth parameter estimates, standard error, and vigor indices using a thermal gradient table of runner-type peanut cultivar GA-07W with a polymer seed treatment under controlled storage conditions for 10 weeks[a].

		Parameter estimates[b,c]							Vigor indices		
Storage Location	Weeks in Storage	Germination[d]	*b0*	SE[2]	*b1*	SE	*XP*	SE	Germination @ *XP*	GDD @ *b0*	$Germ_{80}$
		——————%——————					——GDD——		——%——	——GDD———	
	Initial	70.1 ab	95.8	(2.6)	0.08	(0.06)	37.2	(3.5)	76.6	215	40
Controlled	1	75.0 ab	96.7	(2.1)	0.08	(0.06)	32.1	(3.0)	77.4	223	34
	2	78.9 a	96.4	(1.5)	0.09	(0.01)	23.2	(2.1)	77.1	188	25
	3	76.7 a	96.7	(1.5)	0.09	(0.01)	28.2	(2.1)	77.4	205	30
	4	78.2a	94.8	(1.6)	0.12	(0.02)	18.4	(2.1)	75.8	151	21
	5	77.2 a	95.6	(1.6)	0.10	(0.01)	21.8	(2.2)	76.4	182	25
	6	75.8 ab	95.3	(1.9)	0.14	(0.02)	19.9	(2.1)	76.3	132	22
	7	73.1 ab	96.3	(2.4)	0.11	(0.02)	30.0	(2.9)	77.0	176	32
	8	71.8 ab	96.1	(2.1)	0.13	(0.02)	32.4	(2.3)	76.9	154	34
	9	74.7 ab	96.7	(2.0)	0.11	(0.01)	27.8	(2.4)	77.4	174	30
	10	71.1 ab	93.5	(2.3)	0.08	(0.01)	31.2	(3.3)	74.5	216	36
Outside	1	74.0 ab	95.1	(2.7)	0.07	(0.01)	32.2	(4.1)	76.1	239	36
	2	78.5 a	96.0	(1.4)	0.08	(0.01)	24.8	(2.1)	76.8	210	28
	3	78.1 a	96.0	(1.5)	0.10	(0.01)	22.5	(2.1)	76.8	178	25
	4	76.3 a	93.0	(1.7)	0.12	(0.02)	18.2	(2.2)	74.4	151	22
	5	75.8 ab	94.4	(1.9)	0.10	(0.01)	22.5	(2.4)	75.5	170	26
	6	72.8 ab	92.1	(1.9)	0.11	(0.02)	21.9	(2.4)	73.7	157	26
	7	73.2 ab	95.1	(1.8)	0.10	(0.01)	27.4	(2.2)	76.1	183	30
	8	69.2 ab	93.6	(1.8)	0.10	(0.01)	33.8	(2.2)	74.9	176	38
	9	73.6 ab	95.7	(1.9)	0.09	(0.01)	31.2	(2.6)	76.6	206	34
	10	71.0 ab	93.6	(2.2)	0.10	(0.01)	29.0	(2.8)	74.9	188	33
Variable	1	70.4 ab	90.4	(2.1)	0.11	(0.02)	26.5	(2.8)	72.4	163	32
	2	76.9 a	92.2	(2.5)	0.08	(0.01)	23.7	(4.1)	73.8	225	30
	3	72.1 ab	91.9	(2.0)	0.08	(0.01)	29.2	(3.0)	73.5	212	35

4	71.4 ab	92.9	(2.7)	0.07	(0.01)	32.6	(4.4)	74.3	261	39
5	73.9 ab	91.4	(1.9)	0.12	(0.02)	18.8	(2.4)	73.1	144	23
6	63.9 ab	84.7	(2.6)	0.08	(0.01)	31.7	(4.2)	67.8	230	51
7	66.8 ab	93.4	(2.6)	0.07	(0.01)	39.3	(3.9)	74.7	260	45
8	70.4 ab	89.2	(2.2)	0.10	(0.02)	26.8	(3.0)	71.4	177	34
9	60.7 ab	88.8	(3.1)	0.07	(0.01)	46.5	(4.6)	71.1	264	58
10	55.4 b	87.8	(4.5)	0.06	(0.01)	57.9	(7.2)	70.3	334	75

[a]Abbreviations: SE, standard error, $Germ_{80}$, cumulative growing degree day value at 80% germination, GDD, growing degree day.

[b]Parameter estimates calculated by nonlinear regression equation, $y = \frac{b0}{\left[(1+((1-P)/P))*e^{(-b1*(x-XP))}\right]}$ for seed germination with respect to time based on GDD accumulation:

b0 is the height of the horizontal asymptote at a very large X, *XP* is expected value of *x* when *y* is at *P* percent of the upper asymptote. *P* used in this model was 0.8. Three indices of vigor are GDD at *b0*, GDD at 80% germination ($Germ_{80}$), and germination at parameter *XP*. Number in () represents the SE. (n=720 seed)

[c]Values for each parameter within a column for each cultivar followed by the same letter are not significantly different at the 5% probability level. General linear model procedures were used with mean separation using 95% asymptotic confidence intervals.

[d]Values for each storage location and weeks in storage germination followed by the same letter are not significantly different at the 5% probability level using GLIMMIX procedure in SAS 9.4.

Germination is the average cumulative germination of the 3 reps per location per week. All cells (24*3=72 cells averaged per week per location)

Table 3.2. Seed germination, logistic growth parameter estimates, standard error, and vigor indices using a thermal gradient table of runner-type peanut cultivar GA-09B under controlled storage conditions for 6 weeks[a].

Storage Location	Seed Treatment	Weeks in Storage	Germination	Parameter estimates[b,c]						Vigor indices		
				b0	SE[2]	*b1*	SE	*XP*	SE	Germination @ *XP*	GDD @ *b0*	$Germ_{80}$
			%					GDD		%	GDD	
Initial[*]	NTC[**]	0	65.2 a	88.2	(3.1)	0.08	(0.01)	36.2	(4.6)	70.6	233	48
	R	0	49.8 abcde	79.5	(4.5)	0.06	(0.01)	56.6	(7.5)	63.6	302	NA
	P1	0	51.9 abcde	82.6	(6.1)	0.05	(0.01)	60.2	(11.1)	66.1	366	102
	P2	0	46.7 abcde	69.8	(3.7)	0.09	(0.03)	44.0	(6.2)	55.9	213	NA
Variable	NTC	2	57.5 abc	68.8	(3.0)	0.27	(0.14)	10.9	(3.6)	55.1	67	NA
	R	2	35.8 cdefg	64.7	(11.6)	0.04	(0.01)	80.8	(27.6)	51.7	491	NA
	P1	2	38.5 cdefg	78.0	(16.3)	0.03	(0.01)	98.1	(32.7)	62.4	587	NA
	P2	2	32.5 efg	47.6	(4.8)	0.07	(0.03)	45.6	(13.2)	38.1	254	NA
	NTC	4	56.0 abcd	75.3	(4.0)	0.08	(0.03)	33.1	(7.0)	60.2	215	NA
	R	4	35.6 cdefg	55.9	(5.6)	0.06	(0.02)	56.8	(13.4)	44.7	309	NA
	P1	4	42.3 bcdefg	69.0	(4.5)	0.07	(0.02)	57.2	(8.1)	55.2	278	NA
	P2	4	36.0 cdefg	99.2	(36.5)	0.03	(0.01)	135.0	(51.3)	79.4	747	137
	NTC	6	45.1 abcdef	63.4	(4.3)	0.07	(0.02)	38.7	(9.3)	50.7	251	NA
	R	6	23.5 fg	42.8	(6.1)	0.04	(0.02)	75.4	(20.8)	34.2	418	NA
	P1	6	34.0 defg	105	(45.9)	0.03	(0.01)	139.3	(57.2)	83.6	639	132
	P2	6	22.9 g	67.5	(19.2)	0.04	(0.01)	117.8	(32.0)	54.0	523	NA
Controlled	NTC	2	62.1 ab	86.3	(3.9)	0.07	(0.01)	43.4	(6.4)	69.1	279	61
	R	2	45.2 abcdef	81.5	(5.9)	0.04	(0.01)	76.7	(10.3)	65.2	426	136
	P1	2	51.3 abcde	74.5	(4.2)	0.06	(0.01)	47.0	(7.8)	59.6	283	NA
	P2	2	50.8 abcde	69.9	(3.1)	0.10	(0.03)	36.1	(5.2)	55.9	191	NA
	NTC	4	64.4 ab	77.3	(2.5)	0.15	(0.04)	15.6	(3.4)	61.9	120	NA
	R	4	48.1 abcde	73.0	(5.0)	0.05	(0.01)	54.0	(10.0)	58.4	330	NA
	P1	4	54.6 abcde	75.7	(3.3)	0.11	(0.03)	35.3	(4.9)	60.6	178	NA
	P2	4	52.1 abcde	76.8	(3.9)	0.07	(0.02)	45.2	(6.5)	61.4	251	NA
	NTC	6	64.4 ab	76.5	(3.0)	0.23	(0.10)	12.5	(3.4)	61.2	79	NA

R	6	45.4 abcdef	66.8	(4.1)	0.06	(0.02)	47.1	(8.3)	53.4	281	NA
P1	6	46.7 abcde	74.8	(3.9)	0.07	(0.01)	52.9	(6.8)	59.8	281	NA
P2	6	43.5 abcdefg	62.8	(3.5)	0.22	(0.11)	31.3	(4.3)	50.3	99	NA

[a]Abbreviations: SE, standard error, $Germ_{80}$, cumulative growing degree day value at 80% germination, GDD, growing degree day, NA, not applicable.

[b]Parameter estimates calculated by nonlinear regression equation, $y = \frac{b0}{\left[(1+((1-P)/P))*e^{(-b1*(x-XP))}\right]}$ for seed germination with respect to time based on GDD accumulation: *b0* is the height of the horizontal asymptote at a very large X, *XP* is expected value of *x* when *y* is at *P* percent of the upper asymptote. *P* used in this model was 0.8. Three indices of vigor are GDD at *b0*, GDD at 80% germination ($Germ_{80}$), and germination at parameter *XP*. Number in () represents the SE. (n=480 seed)

[c]Values for each parameter within a column for each cultivar followed by the same letter are not significantly different at the 5% probability level. General linear model procedures were used with mean separation using 95% asymptotic confidence intervals.

[d]Values for each storage location and weeks in storage germination followed by the same letter are not significantly different at the 5% probability level using GLIMMIX procedure in SAS 9.4.

Germination is the average cumulative germination of the 2 reps per location per week. All cells (24*3=72 cells averaged per week per location)

*Initial = experiment initiation, Variable = stored in greenhouse, Controlled = stored in laboratory

**Abbreviations: NTC = nontreated control, R = Rancona V PD dry seed treatment, P1 = Rancona V PL plus SlipShine 6300, P2 = Rancona V PL plus SlipShine 2050

Table 3.3. Seed germination, logistic growth parameter estimates, standard error, and vigor indices using a thermal gradient table of runner-type peanut cultivar GA-07W under controlled storage conditions for 6 weeks[a].

Storage Location	Seed Treatment	Weeks in Storage	Germination	Parameter estimates[b,c]						Vigor indices		
				b0	SE[2]	*b1*	SE	*XP*	SE	Germination @ *XP*	GDD @ *b0*	$Germ_{80}$
			%					GDD		%	GDD	
Initial*	NTC**	0	65 a	88.5	(3.3)	0.05	(0.01)	43.9	(6.1)	70.8	334	60
	D	0	53.9 abcde	80.0	(4.7)	0.05	(0.01)	54.4	(9.0)	64.0	363	184
	P3	0	43.2 bcdefg	68.8	(4.7)	0.06	(0.01)	57.3	(9.4)	55.1	321	NA
Variable	NTC	2	56.0 abcd	77.3	(3.1)	0.07	(0.01)	37.9	(5.5)	61.9	251	NA
	D	2	56.4 abc	88.1	(5.6)	0.04	(0.01)	63.8	(10.7)	70.5	454	87
	P3	2	39.0 efgh	83.2	(11.1)	0.04	(0.01)	90.2	(17.7)	66.6	479	137
	NTC	4	42.1 cdefg	62.0	(3.8)	0.07	(0.02)	48.3	(8.2)	49.6	269	NA
	D	4	39.4 defgh	77.0	(8.8)	0.04	(0.01)	88.7	(16.7)	61.6	507	NA
	P3	4	26.8 ghi	64.7	(9.8)	0.04	(0.01)	98.6	(18.1)	51.7	447	NA
	NTC	6	32.2 fghi	57.0	(5.5)	0.09	(0.02)	58.3	(7.0)	54.3	176	NA
	D	6	24.3 hi	60.9	(15.8)	0.07	(0.02)	76.3	(16.7)	63.5	195	NA
	P3	6	20.4 i	54.9	(9.2)	0.10	(0.03)	71.3	(8.8)	47.5	206	NA
Controlled	NTC	2	64.2 a	84.8	(3.0)	0.07	(0.01)	34.7	(5.1)	67.9	260	56
	D	2	59.7 ab	85.2	(4.0)	0.06	(0.01)	45.9	(7.2)	68.2	323	71
	P3	2	43.9 bcdef	77.8	(5.5)	0.05	(0.01)	70.8	(9.8)	62.2	395	NA
	NTC	6	51.4 abcde	67.9	(3.2)	0.10	(0.02)	21.7	(4.8)	54.3	176	NA
	D	6	55.4 abcde	79.3	(3.4)	0.09	(0.02)	29.9	(4.0)	63.5	195	NA
	P3	6	41.4 cdefg	59.1	(4.8)	0.09	(0.03)	30.9	(8.0)	47.5	206	NA

[a]Abbreviations: SE, standard error, $Germ_{80}$, cumulative growing degree day value at 80% germination, GDD, growing degree day, NA, not applicable.

[b]Parameter estimates calculated by nonlinear regression equation, $y = \frac{b0}{\left[\left(1+\left((1-P)/P\right)\right)*e^{\left(-b1*(x-XP)\right)}\right]}$ for seed germination with respect to time based on GDD accumulation: *b0* is the height of the horizontal asymptote at a very large X, *XP* is expected value of *x* when *y* is at *P* percent of the upper asymptote. *P* used in this model was 0.8. Three indices of vigor are GDD at *b0*, GDD at 80% germination ($Germ_{80}$), and germination at parameter *XP*. Number in () represents the SE. (n=720 seed)

[c]Values for each parameter within a column for each cultivar followed by the same letter are not significantly different at the 5% probability level. General linear model procedures were used with mean separation using 95% asymptotic confidence intervals.

[d]Values for each storage location and weeks in storage germination followed by the same letter are not significantly different at the 5% probability level using GLIMMIX procedure in SAS 9.4.
Germination is the average cumulative germination of the 3 reps per location per week. All cells (24*3=72 cells averaged per week per location)
*Initial = experiment initiation, Variable = stored in greenhouse, Controlled = stored in laboratory
**Abbreviations: NTC = nontreated control, D = Dynasty PD dry seed treatment, P3 = Liquid Dynasty formulation plus Syngenta proprietary polymer.

Table 3.4. Seed germination, logistic growth parameter estimates, standard error, and vigor indices using a thermal gradient table of runner-type peanut cultivar GA-06G under controlled storage conditions for 6 weeks[a].

Seed Treatment	Weeks in Storage	Parameter estimates[b,c]						Vigor indices			
		Germination	*b0*	SE[2]	*b1*	SE	*XP*	SE	Germination @ *XP*	GDD @ *b0*	$Germ_{80}$
		——%——					——GDD——		——%——	——GDD——	
R1*	0	58.5 b	71.7	(3.2)	0.10	(0.03)	27.1	(5.47)	61	171	NA
SA	0	48.3 b	55.5	(2.7)	0.16	(0.07)	13.0	(5.3)	47	104	NA
S1	0	53.2 b	68.6	(3.8)	0.07	(0.02)	39.8	(7.6)	58	265	NA
S2	0	51.4 b	71.2	(4.6)	0.05	(0.01)	48.0	(9.6)	60	320	NA
R2	0	84.4 a	95.1	(2.1)	0.09	(0.02)	14.8	(3.4)	81	191	14
R1	1	50.7 b	80.7	(12.1)	0.03	(0.01)	78.1	(26.2)	69	564	176
SA	1	51.1 b	92.4	(10.5)	0.04	(0.01)	82.5	(16.8)	79	501	86
S1	1	56.7 b	71.0	(2.8)	0.10	(0.03)	30.1	(4.8)	60	175	NA
S2	1	51.8 b	75.4	(5.1)	0.05	(0.01)	55.3	(10.2)	64	365	NA
R2	1	82.6 a	94.3	(1.6)	0.11	(0.01)	18.2	(2.2)	80	162	18
R1	2	52.1 b	79.7	(11.0)	0.03	(0.01)	69.5	(24.0)	68	521	NA
SA	2	48.8 b	87.7	(13.4)	0.03	(0.01)	89.0	(24.8)	75	586	109
S1	2	49.7 b	92.1	(19.5)	0.03	(0.01)	98.7	(36.0)	78	669	105
S2	2	46.5 b	74.4	(8.5)	0.04	(0.01)	70.2	(17.2)	63	446	NA
R2	2	81.3 a	91.7	(1.6)	0.16	(0.03)	9.9	(2.0)	78	105	11
R1	3	45.4 b	78.0	(7.3)	0.04	(0.01)	74.5	(13.8)	66	461	NA
SA	3	42.1 b	68.3	(6.8)	0.05	(0.01)	65.1	(14.2)	58	383	NA
S1	3	46.1 b	76.0	(7.3)	0.04	(0.01)	67.9	(14.0)	65	413	NA
S2	3	42.9 b	78.5	(7.4)	0.05	(0.01)	72.7	(11.6)	67	373	NA
R2	3	79.4 a	91.1	(2.00	0.12	(0.02)	14.9	(2.9)	77	144	17
R1	4	46.1 b	107	(53.7)	0.03	(0.01)	114.3	(35.3)	91	578	91
SA	4	39.3 b	53.5	(2.9)	0.11	(0.03)	38.4	(5.5)	45	176	NA
S1	4	50.1 b	73.8	(3.5)	0.07	(0.02)	47.7	(5.7)	63	246	NA
S2	4	41.8 b	53.8	(2.9)	0.13	(0.05)	31.3	(5.7)	46	147	NA
R2	4	78.5 a	93.5	(1.8)	0.10	(0.01)	24.6	(2.5)	79	179	25

R1	5	44.7 b	62.9	(4.9)	0.06	(0.02)	51.4	(10.7)	54	305	NA
SA	5	43.5 b	86.9	(13.6)	0.04	(0.01)	93.1	(21.2)	74	514	114
S1	5	48.5 b	70.5	(5.9)	0.05	(0.01)	56.8	(12.1)	60	355	NA
S2	5	46.1 b	62.7	(4.5)	0.06	(0.02)	47.4	(10.3)	53	298	NA
R2	5	77.1 a	90.6	(2.6)	0.08	(0.02)	21.9	(4.1)	77	201	26

[a]Abbreviations: SE, standard error, $Germ_{80}$, cumulative growing degree day value at 80% germination, GDD, growing degree day, NA, not applicable.

[b]Parameter estimates calculated by nonlinear regression equation, $y = \frac{b0}{\left[\left(1+\left((1-P)/P\right)\right)*e^{\left(-b1*(x-XP)\right)}\right]}$ for seed germination with respect to time based on GDD accumulation: *b0* is the height of the horizontal asymptote at a very large X, *XP* is expected value of *x* when *y* is at *P* percent of the upper asymptote. *P* used in this model was 0.8. Three indices of vigor are GDD at *b0*, GDD at 80% germination ($Germ_{80}$), and germination at parameter *XP*. Number in () represents the SE. (n=720 seed)

[c]Values for each parameter within a column for each cultivar followed by the same letter are not significantly different at the 5% probability level. General linear model procedures were used with mean separation using 95% asymptotic confidence intervals.

[d]Values for each storage location and weeks in storage germination followed by the same letter are not significantly different at the 5% probability level using GLIMMIX procedure in SAS 9.4.

Germination is the average cumulative germination of the 3 reps per location per week. All cells (24*3=72 cells averaged per week per location).

*R1 = Rancona V PL, SA = Syngenta seed treatment, S1 = Syngenta seed treatment plus Polymer #1, S2 Syngenta seed Treatment plus Polymer #2, R2 = Dry V PL.

Table 3.5. Field Experiment 2 plant population and plant width measurements.

Treatment	Plant Population[a]				Plant Width[b]			
	Rating 1	Rating 2	Rating 3	Rating 4	Rating 1	Rating 2	Rating 3	Rating 4
R1	8.5 ab[c]	12.0 abc	15.5 a	19.8 a	7.3 ab	12.2 a	14.4 ab	17.8 a
SA	7.3 b	9.3 c	13.8 a	17.3 a	7.1 b	12.3 a	14.9 ab	16.8 a
S1	8.3 ab	10.3 bc	14.3 a	18.8 a	7.8 ab	12.8 a	13.3 b	17.5 a
S2	7.5 ab	10.8 abc	14.0 a	20.3 a	8.9 ab	11.1 a	15.3 ab	17.9 a
R2	10.8 a	14.3 a	18.3 a	22.8 a	9.7 a	13.5 a	15.8 a	19.8 a

[a]Plant population was measured as the number of plants in 1 m of row per plot.

[b]Plant width was measured from leaf tip to leaf tip at the widest portion of an individual peanut plant with three plants measured per plot

[c]Values for each rating of plant population and plant width followed by the same letter within the same column are not significantly different using the GLIMMIX procedure in SAS 9.4 for mean separation using Tukey's HSD at an alpha level of 0.05.

CHAPTER 4

MONITORING OF CO_2 CONCENTRATION AND GERMINATION OF STORED PEANUT UNDER VARIOUS STORAGE CONDITIONS

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Abstract

Peanut has become a significant oil and food source worldwide. After harvest, inshell peanut are stored and undergo a period of dormancy. Storage typically occurs in warehouses with adequate ventilation, but temperatures may vary. While dormant, cellular respiration is minimal but still occurs to maintain seed viability. Carbon dioxide (CO_2) is a byproduct of respiration and is released during glycolysis and the Krebs' cycle. A study was performed to investigate the effect of multiple storage conditions on peanut CO_2 emission. Treated peanut seed were stored in stacked paper bags with sensors capable of monitoring CO_2 concentration, temperature, and humidity within the bag. Seed were monitored in an open-air shelter, ventilated warehouse, and cold room for 24 days in 2021 and 105 days in 2022. A total of six bags were monitored at each storage location with an additional sensor in ambient conditions. The progeny of the 2021 seed were monitored in 2022 with four bags per location. Sensors were placed in the middle and uppermost bags for monitoring. Data indicated that regardless of storage condition, CO_2 emission decreased as temperature or humidity increased, with temperature being more significant than humidity. Germination slightly decreased as storage time increased in all conditions.

Introduction

Peanut (*Arachis hypogaea* L.) has been a crucial source of nutrition since it was first cultivated by the Incan civilization in Peru and includes 25% protein, 46% fat, 4% fiber, and 37% carbohydrates (Alhassan et al. 2017; Arya et al. 2015). Expansion of peanut into the U.S. was first noted via Portuguese and Spanish traders along routes around the world, but the specific location of entry into the U.S. is unknown. The U.S. Civil War played a vital role in the spread of peanut throughout the country as a nutrient dense food that was light and travelled well. Today,

peanut is primarily grown in the Carolina and Virginia region, the Southeastern U.S., and numerous Western states including Oklahoma, Texas, and New Mexico.

Georgia consistently supplies approximately 50% of the total U.S. peanut production each year. Over 620,000 ha of peanut were harvested in the U.S. in 2021 with nearly 304,000 ha harvested in Georgia (NASS 2021). The Southeastern U.S. predominately grows the Runner-type peanut (Jordan et al. 2000). After harvest ends in late Autumn, peanut will be cleaned to remove debris and stored until processing in the spring. Peanut undergo dormancy while in storage in which seed under favorable conditions will not undergo the germination process (Bewley 1997). This dormancy can be the result of a chemical imbalance that is not conducive to germination or may require external stimuli to initiate germination and can last for several month. Once the chemical balance is conducive or external stimulation has occurred, proper germination may begin upon the imbibition of water in a triphasic manner (Manz et al. 2005). Phase one is a rapid uptake of water, followed by phase two in which water uptake plateaus, and phase three consists of a second rapid water uptake as the embryonic axis begins to elongate and protrude through the testa. While remaining dormant, minimal cellular processes occur to maintain seed viability and conserve energy for germination. One such process that is minimized is the conversion of glucose and CO_2 into adenosine triphosphate (ATP) via cellular respiration.

Cellular respiration occurs in cytosol and mitochondria of respiring cells (Saraste 1999). The first component of cellular respiration, glycolysis, occurs in the cell cytosol and is the conversion of glucose into two pyruvate molecules. This conversion requires the use of two adenosine triphosphate (ATP) molecules, but the entire reaction has a net gain of two ATP per glucose. Following glycolysis is the Krebs Cycle (Krebs and Johnson 1937) which occurs in the matrix of the mitochondria. The pyruvate from glycolysis will lose one carbon molecule as it

combines with coenzyme A (CoA) to form acetyl-CoA and release one CO_2 molecule and nicotinamide adenine dinucleotide (NADH). Acetyl-CoA will combine with oxaloacetate (OAA) to form citrate which will undergo multiple reactions that release CO_2 and energy molecules. In total, two ATP, eight NADH, two $FADH_2$ (flavin adenine dinucleotide), and six CO_2 molecules are formed after two turns of the citric acid cycle completing the catabolism of one glucose molecule. CoA is released as citrate is formed and OAA is the final product of the Krebs cycle to restart the cycle. The final reaction of cellular respiration is oxidative phosphorylation in which the majority of ATP is formed. Along the mitochondrial membrane reside complexes that will transport electrons from high energy molecules through an electron transport chain while pumping hydrogen ions into the intermembrane space. The electrons being transported will be used to reduce free oxygen into water. The hydrogen ions of the intermembrane space will be pumped across the membrane via the ATP synthase to form ATP molecules. A total of up to 36 ATP molecules can be formed via oxidative phosphorylation. Though there is a significant production of energy from cellular respiration, negative byproducts are also produced. One of the byproducts are reactive oxygen species (ROS) which are produced as an electron is lost from the electron transport chain forming superoxides via binding to an oxygen molecule (Huang et al. 2019). These superoxides can interact with superoxide dismutase to form hydrogen peroxide which is transported via aquaporins on the cell membrane and have been reported to potentially cause injury (Bienert et al. 2007). The hydrogen peroxide may then split at the double bonded oxygen position during the Fenton reaction to form hydroxyl radical ions (Das and Roychoudhury 2014). Hydrogen peroxide and hydroxyl radical ions have been repeatedly indicated to cause substantial injury or seed death due to phospholipid membrane degradation, protein structure alteration, enzyme deactivation, and DNA alteration if not scavenged

(Ratajczak et al. 2015; Chen et al. 2013; Kibinza et al. 2006; Kong et al. 2015; Goel and Sheoran 2003).

The purpose of this experiment was to investigate the potential of relating CO_2 emission of peanut stored under various condition to seed viability. Specific objectives of this experiment include: 1. Determine CO_2 emission of peanut under multiple storage regimes. 2. Germinate peanut seeds at regular intervals to determine germination over storage regimes. 3. Determine if CO_2 emission can be utilized as a measure of seed quality and viability during storage.

Materials and Methods

CO_2 monitoring

Registered peanut cultivar GA-06G (Branch 2007) seed from the 2020 growing season were harvested in October of 2020 and stored according to Tifton Peanut Company procedures. The inshell seed were stored in a warehouse until shelling on March 17th, 2021. A sample of seed was submitted to the Georgia Department of Agriculture Tifton Seed Laboratory for germination tests with a rate of 89% germination after seven days of standard germination testing and 78% germination after 14 days of cold testing. Seed were treated with Rancona VPD (UPL NA Inc., King of Prussia, PA) at 113.4 g per 45.4 kg on March 26, of 2021 and subsequently bagged and placed onto pallets containing 40 bags per pallet in layers of five bags. Pallets of bagged seed were moved to storage treatment locations on April 9th, 2021. Storage location treatments included a warehouse with proper ventilation and fluctuating temperature (PVF), an inner room within the warehouse with stable temperature, humidity, and minimal air flow (STH), and an overhead (OHS) shelter with exposure to natural weather events but protected from rainfall.

Sensors with capabilities to measure temperature, humidity, and CO_2 were obtained from Paragon Robotics, LLC (N Series sensor with DB75 expansion sensor, Twinsburg, OH). During

the 2021 CO_2 measurement period, sensors were placed in bags on April 14th, 2021 and were removed May 7th, 2021. A small cut was made on the surface of the bag to expose seed and insert sensors and was resealed with tape. Sensors were placed in an uppermost bag and a bag in the third layer from the top. Each sensor location in the stack was replicated three times in individual stacks (3 stacks total/location) and included a sensor near the pallets to record ambient conditions. Data were recorded every five minutes and stored on the sensor and collected every three days. In 2022, the seed progeny of the 2021 planted seed were inverted on September 28th, 2021 and placed in a large bulk bag for in-shell winter storage. The peanut pods were stored in a non-insulated warehouse after cleaning until January and subsequently moved into a garage for additional storage until March 2nd, 2022. The peanut were then shelled, treated, and bagged into 22.7 kg bags, providing only 12 bags total. The bags were divided evenly into four bags/storage location and placed onto a pallet in the respective storage conditions on March 4th, 2022. Due to the reduced number of bags compared to 2021, it was not possible to measure according to sensor location within a stack. The sensors were inserted in the same fashion as 2021, with recordings occurring every 60 minutes. Sensors remained in the bags for a total of 105 days.

Data analysis was performed utilizing PROC GLM to determine the CO_2 response to temperature and humidity for each sensor in SAS Studio 5.2 (SAS Institute, Cary, NC). The interaction of temperature and humidity was unable to be determined due to multicollinearity potentially skewing the coefficients of covariates. Carbon dioxide level was determined for each air temperature and humidity percentage by averaging all readings at each respective air temperature and humidity percentage for a single CO_2 measurement.

Germination

A germination trial was performed concurrently to determine germination of seed overtime from each storage location. Seed were removed at trial initiation to determine germination before exposure to the storage treatments. 400 seed were collected from the uppermost bag from a single stack at each location totaling four replications of 100 seed. 20 seed were placed in Petri dishes (Catalog No. FB0875711, Fisher Scientific, Waltham, MA) lined with blotter paper (85.7 mm diameter blotter paper, Anchor Paper Co., St. Paul, MN) and 10 mL of distilled water added. Petri dishes were placed in germination chambers (SG5 Countertop Controlled Environment Chamber, Hoffman Manufacturing, Inc., Corvallis, OR) under a 16/8 hour cycle at 20˚C/30˚C for 10 days according to Association Official Seed Analysts procedures (AOSA 2018). Water was added to Petri dishes as needed to maintain constant moisture. Germination counts were performed on day five and 10 after being placed in the germination chamber and were considered germinated once the protruding radicle measured five mm or greater and removed. This germination testing procedure was performed after two and four weeks in storage at which point sensors were removed and seed sold in 2021. The 2022 experiment had seed removed every other week after initiation until 14 weeks in storage (8 testings). Data were analyzed using PROC GLIMMIX to determine the germination response of peanut seed to timing, storage condition, and timing by storage condition interactions. Data were subjected to Analysis of Variance (ANOVA) with mean separation according to Tukey's HSD set at $\alpha = 0.05$.

Field

A field experiment was performed to investigate the response of seed under different storage regimes. Seed from each storage regime were planted on May 10th, 2021 at the University of Georgia Ponder farm located in Ty Ty, GA. Soil properties consisted of Tifton

loamy sand (Fine-loamy, kaolinitic, thermic Plinthic Kandiudults) with 84.1%, 9.4%, and 6.5% sand, silt, and clay, respectively. Seed were planted in a single row fashion to achieve a plant population of 19 seed/m row at a depth of 3.8 cm. Phorate was applied for insect control at a rate of 1.12 kg ai ha-1. Each storage regime was replicated four times in 1.8 m by 9.1 m plots. Peanut were maintained under University of Georgia agronomic recommendations. Peanut were harvested on October 13th, 2021. The 2022 seed were planted on May 4th and followed the same production practices as the 2021 seed in an adjacent field from 2021. Data collected included plant population and widths over time, and yield. The 2021 yield data along with the 2022 plant population, width, and yield was analyzed using ANOVA with means separation using Tukey's HSD at $\alpha = 0.05$ when appropriate.

Results and Discussion

CO_2 Monitoring

In addition to the PROC GLM analysis, the Pearson's Correlation Coefficient was also determined to indicate the positive or negative relationship between CO_2 and temperature or humidity utilizing PROC CORR in SAS. Numerous sensor locations indicated significant interactions preventing data combination by stacks or location in 2021 and by location in 2022. Data collected in 2021 was not combined by storage location or location within the stack due to stack and storage location noting differences with respect to the Pearson Correlation Coefficient.

2021

Both temperature and humidity had a significant effect on CO_2 concentration within numerous peanut bags in each storage location and placement in the stack (Table 1). In the STH location, sensors indicated that as temperature increased, the CO_2 concentration decreased in four of the six bags. Two of the three bags of seed on top of the stack had a CO_2 concentration

increase as the temperature increased. The inverse occurred with respect to humidity in which five of the six seed bags noted a decrease in CO_2 as humidity increased, with one bag of seed being excluded due to sensor malfunctions. A similar trend was noted in the PVF location that as temperature increased, the CO_2 concentration increased in four out of six seed bags, with the remaining two decreasing. Two of the three top seed bags noted the increase in CO_2 similar to the cold storage location. Only one bag of seed located in the middle of the stack noted an increase in CO_2 concentration as humidity increased, as the remaining five decreased. Finally, the OHS location noted different trends compared to the other two storage locations. As temperature increased, two out of four seed bags indicated an increase in CO_2 concentration and the remaining two noted a decrease. The two seed bags that increased in CO_2 were also located in the middle of the stack, which was noted in the other two storage locations. With respect to humidity, three out of four seed bags noted an increase in CO_2 as humidity increased.

2022

Temperature had a significant effect on CO_2 emission of all peanut bags stored in all locations except rep three and rep four of the OHS and STH locations, respectively (Table 2). In addition, data indicated a negative correlation between CO_2 emission and temperature, as temperature increased, CO_2 emission decreased. The exceptions to this were seed bags stored in the PVF and the one seed bag under the OHS in which a positive correlation was noted. Overall, the trend indicated that as temperature increased, CO_2 emission decreased.

Humidity did not affect CO_2 emission significantly. The overall trend indicated that as humidity increased, CO_2 concentration either decreased or remained constant as the initial measurement. Instances in which CO_2 concentration increased were noted in two samples. Two from the STH stored seed and one from the PVF stored seed.

These data indicate that temperature and humidity can affect the emission of CO_2 from stored seed. The air flow surrounding the bags may influence to CO_2 accumulation within the bag as the seed stored in the warehouse inner room had a greater concentration of CO_2. It was also noted that as temperature and humidity increased, the amount of CO_2 released by the seed also increased (data not shown) in a diurnal cycle. Previous investigations support that seed should be stored in conditions with low temperature and humidity along with adequate air flow (Ketring 1992; McDonald 2004).

Germination

Germination rate data was analyzed utilizing ANOVA followed by means separation according to Tukey's HSD with an alpha of 0.05. Data were recorded prior to storage treatments to establish a baseline germination rate. Weeks in storage germination data were compared to this initial baseline data and not other weeks in storage data.

After five days, average germination was 50%, with 70% after 10 days. After two weeks in storage, location, time of data collection, and their interactions indicated differences. Seed stored in the PVF location had a higher germination rate compared to OHS stored seed, while the STH stored seed were not different than either location. Cumulative germination was greater after 10 days compared to five for all storage locations. Location by measurement interactions indicated that the PVF and STH stored seed measured after 10 days in the germination chamber were greater than seed stored in the STH and PVF when measured at five days being in the germination chamber. After four weeks in storage, differences were only indicated for measurement timing and the interaction of storage location and measurement timing with 10 days in the chamber allowing greater germination compared to only five days. The interactions

of measurement timing and storage location are indicated in Table 3. No differences for field data were indicated.

Numerous differences were indicated for seed germination in 2022 (Table 2). As in 2021, the 10 DAP germination ratings generally indicated a greater number of germinated seed. Germinated seed ranged between 7.6 and 9.6 for all germination data collections indicating the importance of planting high quality seed to ensure an adequate population, regardless of length and condition of seed storage.

Field

Yields in 2021 range from 22.2 to 22.9 kg/ha, but no differences were noted.

Plant population data was combined across days after planting (DAP) for analysis using Tukey Kramer HSD set at an alpha of 0.05. No differences were indicated for any collection timing, allowing for data combination in which no differences were noted (data not shown). Plant width data also noted no differences for any DAP timing and were combined with no differences noted.

Current seed storage facilities follow strict protocols to ensure adequate peanut seed germination the following growing season. This may include storage regimes of refrigerated cold storage or adequate air flow and stable temperatures. Storage facilities should avoid uncontrollable storage conditions such as locations with high temperature and humidity. These conditions have been known to cause premature seed deterioration and suboptimal germination. Germination samples should be collected periodically, if possible, to ensure seed lots are not losing viability in an excessive manner prior to marketing to growers. Monitoring CO_2 levels of stored peanut may not be a viable method to determine the viability of stored peanut with the instruments utilized in this experiment. Numerous equipment malfunctions may have influenced

the data collected, warranting more precise and reliable CO_2 monitoring equipment to be utilized. The current storage procedures and facilities are adequate in preserving seed viability for at least 14 weeks with only a minor decrease in germination of seed stored in the fluctuating environment. This translated to stable field production with high plant populations and rapid, even growth among all stored seed and no yield loss. The seed growers receive from proper storage facilities should be of high quality enabling them to start the growing season strong.

References

Alhassan, K., J.K. Agbenorhevi, J.Y. Asibuo, G.O. Sampson. Proximate composition and functional properties of some new groundnut accessions. J. Food Security. 5(1): 9-12.

Arya, S. S., A.R. Salve, S. Chauhan. 2015. Peanuts as functional food: a review. J. Food Sci. Technol. 53(1): 31-41.

AOSA. 2018. AOSA rules for testing seeds. Association of Official Seed Analysts. Washington, DC. 2018.

Bewley, J. D. 1997. Seed germination and dormancy. The Plant Cell. 9:1055-1066.

Bienert, G. P., A. L. B. Møller, K. A. Kristiansen, A. Schulz, I. M. Møller, J. K. Schjoerring, T. P. Jahn. Specific aquaporins facilitate the diffusion of hydrogen peroxide across membranes. J. of Biol. Chem. 282: 1183-1192.

Branch, W. D. 2007. Registration of 'Georgia-06G' peanut. Journal of Plant Registrations. 1(2): 120.

Chen, H., D. Osuna, L. Colville, O. Lorenzo, K. Graeber, H. Küster, G. Leubner-Metzger, I. Kranner. 2013. Transcriptome-wide mapping of pea seed ageing reveals a pivotal role for genes related to oxidative stress and programmed cell death. PLoS ONE 8(10): e78471. DOI: 10.1371/journal.pone.0078471.

Das, K. and A. Roychoudhury. 2014. Reactive oxygen species and response of antioxidants as ROS-scavengers during environmental stress in plants. Front. Environ. Sci. 2:53. DOI: 10.3389/fenvs.2014.00053.

Goel, A. and I. S. Sheoran. 2003. Lipid peroxidation and peroxide-scavenging enzymes in cotton seeds under natural ageing. Biol. Plant. 46:429-434.

Huang, H., F. Ullah, D.X. Zhou, M. Yi, and Y. Zhao. 2019. Mechanisms of ROS regulation of plant development and stress responses. Fron. Plant Sci. DOI:10.3389/fpls.2019.00800.

Jordan, D.L., C.W. Swann, J.F. Spears, R.L. Brandenburg, J.E. Bailey, M.R. Tucker. 2000. Comparison of virginia and runner market-type peanut (*Arachis hypogaea*) grown in the Virginia-Carolina production region. Peanut Science. 27:71-77.

Ketring, D.L. 1992. Physiology of Oil Seeds. X. Seed Quality of Peanut Genotypes as Affected by Ambient Storage Temperature. Peanut Science. 19:72-77.

Kibinza, S., D. Vinel, D. Crôme, C. Bailly, F. Corbineau. 2006. Sunflower seed deterioration as related to moisture content during ageing, energy metabolism, and active oxygen species scavenging. Physiologia Plantarum. 128(3):496-506.

Kong, L., H. Huo, P. Mao. 2015. Antioxidant response and related gene expression in aged oat seed. Front. Plant Sci. DOI: 10.3389/fpls.2015.00158.

Krebs, H. A. and W. A. Johnson. 1937. Metabolism of ketonic acids in animal tissue. Biochem J. 31(4): 645-660.

Manz, B., K. Müller, B. Kucera, F. Volke, G. Leubner-Metzger. Water uptake and distribution in germination tobacco seeds investigated in vivo by nuclear magnetic resonance imaging. Plant Physiol. 138(3): 1538-1551.

McDonal, M.B. 2004. Orthodox seed deterioration and its repair. *In:* Benech-Arnold, R.L. and Sánchez, R.A., Eds., Handbook of Seed Physiology, Application to Agriculture, Haworth Press, Inc., New York, 273-298.

Ratajczak, E., A. Malecka, A. Bagniewska-Zadworna, E. Kalemba. 2015. The production, localization and spreading of reactive oxygen species contributes to the low vitality of long-term stored common beech (*Fagus sylvatica* L.) seeds. Plant Physiol. 174(2015): 147-156.

Saraste, M. 1999. Oxidative phosphorylation at fin de siècle. Science. 283(5407): 1488-1493.

Valentine, H. Remembering our past and how it affected our present and future. Peanut Science. 46(1A): 78-81

Table 4.1. Recorded amount of CO_2 in Rancona V PD treated peanut stored under stable, variable, and outside storage conditions over 23 days.

Storage Location[a]	Sensor Location	Temperature[b,c]			Humidity		
		R^2	P-Value	Peason Coefficient	R^2	P-Value	Peason Coefficient
STH	Bag 1 High	0.06	0.3414	-0.25453	0.17	0.2629	-0.4180
	Bag 1 Middle	0.00	0.9577	-0.01934	0.68	0.0436	-0.82428
	Bag 2 High	0.84	<0.0001	-0.91906	0.20	0.2339	-0.44174
	Bag 2 Middle	0.04	0.7160	0.19166	NA	NA	NA
	Bag 3 High	0.00	0.9148	-0.04552	0.52	0.4878	-0.72058
	Bag 3 Middle	0.03	0.6105	0.17321	0.68	0.0116	-0.82565
PVF	Bag 1 High	0.43	0.0005	-0.65798	0.01	0.9072	-0.07293
	Bag 1 Middle	0.22	0.1279	0.46482	0.87	0.0673	-0.93267
	Bag 2 High	0.01	0.5791	-0.11919	0.46	0.0925	-0.68041
	Bag 2 Middle	0.36	0.0406	0.59652	0.36	0.5935	-0.59604
	Bag 3 High	0.37	0.0027	-0.60844	0.56	0.0529	-0.74846
	Bag 3 Middle	NA	NA	NA	NA	NA	NA
OHS	Bag 1 High	NA	NA	NA	NA	NA	NA
	Bag 1 Middle	0.05	0.2520	0.21581	0.10	0.1910	0.31364
	Bag 2 High	0.74	<0.0001	-0.85992	0.41	0.0078	-0.63846
	Bag 2 Middle	0.35	0.0703	0.59376	0.02	0.7746	0.15144
	Bag 3 High	0.64	<0.0001	-0.79826	0.44	0.0719	0.66517
	Bag 3 Middle	0.20	0.0411	0.44924	0.20	0.0411	0.44924

[a]Storage locations included an inner insulated room of a warehouse providing stable air temperature and humidity (STH), a warehouse with proper ventilation and fluctuating air temperature (PVF), and under an overhead shelter providing fluctuating air temperature and humidity (OHS).

[b]Temperature, humidity, and carbon dioxide were measured using an N-series sensor with a DB75 expansion sensor manufactured by Paragon Robotics, LLC (Twinsburg, OH). Measurements were collected every 5 minutes for 23 days. Bags were arranged on the top and middle of an 80 bag palled in 8 levels of 5 bags.

[c]Data analysis was performed utilizing PROC GLM and PROC CORR in SAS Studio 5.2 (SAS Institute, Cary, NC). A single carbon dioxide level was determined by averaging all CO_2 readings at each individual air temperature and humidity percentage.

NA indicates data not available due to sensor malfunction and loss of data.

Table 4.2. Recorded amount of CO_2 in Rancona V PD treated peanut stored under stable, variable, and outside storage conditions over 105 days.

Storage Location[a]	Bag Number	Temperature[b,c]			Humidity		
		R^2	P-Value	Pearson Coefficient	R^2	P-Value	Pearson Coefficient
STH	1	0.19	0.0481	-0.43616	0.57	0.4544	-0.75593
	2	0.42	0.0014	-0.64939	0.67	0.0479	0.81549
	3	0.60	<0.0001	-0.77749	0.52	0.1687	0.72169
	4	0.09	0.1761	-0.29218	0.11	0.5897	-0.32827
PVF	1	0.45	<0.0001	-0.66763	0.01	0.8679	-0.08827
	2	0.35	<0.0001	0.58807	0.00	0.9236	-0.05096
	3	0.30	0.0001	-0.54701	0.67	0.0129	0.81884
	4	0.22	0.0072	0.47275	0.14	0.4159	-0.36858
OHS	1	0.49	<0.0001	-0.70350	0.23	0.1583	-0.482
	2	0.55	<0.0001	0.74472	0.01	0.7910	-0.10351
	3	0.05	0.1384	-0.21760	0.00	0.8091	-0.06337
	4	0.11	0.0217	-0.33407	0.00	1.00	0.00000

[a]Storage locations included an inner insulated room of a warehouse providing stable air temperature and humidity (STH), a warehouse with proper ventilation and fluctuating air temperature (PVF), and under an overhead shelter providing fluctuating air temperature and humidity (OHS).

[b]Temperature, humidity, and carbon dioxide were measured using an N-series sensor with a DB75 expansion sensor manufactured by Paragon Robotics, LLC (Twinsburg, OH). Measurements were collected every 60 minutes for 103 days. Bags were arranged in a single layer on one pallet.

[c]Data analysis was performed utilizing PROC GLM and PROC CORR in SAS Studio 5.2 (SAS Institute, Cary, NC). A single carbon dioxide level was determined by averaging all CO_2 readings at each individual air temperature and humidity percentage.

Table 4.3. Germination of seed stored in 2021 CO_2 monitoring.

Weeks in Storage[a]	Storage Location[b]	Days after Initiation[c]	Germination[d]	
Initial	All Combined	10	70	a
2	STH	10	68	ab
	PVF	10	70	a
	OHS	10	59	abc
4	STH	10	62	ab
	PVF	10	67	a
	OHS	10	60	abc

[a]Initial germination data taken before seed were placed in storage conditions.
[b]Storage locations included an inner insulated room of a warehouse providing stable air temperature and humidity (STH), a warehouse with proper ventilation and fluctuating air temperature (PVF), and under an overhead shelter providing fluctuating air temperature and humidity (OHS).
[c]Days after seed were placed in germination chambers when data was recorded. Data was recorded 5 and 10 days after seed were placed in the germination chamber after seed had been in storage for 2 or 4 weeks.
[d]Data was analyzed using PROC GLIMMIX in SAS Studio 5.2 (SAS Institute, Cary, NC) with means separated using Tukey's HSD set at an alpha of 0.05. Estimates followed by the same letter are not difference from each other.

Table 4.4. Germination of seed stored in 2022 CO_2 monitoring.

Weeks in Storage[a]	Storage Location[b]	Days after Initiation[c]	Germination[d]		Weeks in Storage	Storage Location	Days after Initiation	Germination	
Initial	STH	5	79	cd	2	STH	5	89	ab
	STH	10	88	ab		STH	10	92	a
	PVF	5	75	d		PVF	5	85	abc
	PVF	10	90	ab		PVF	10	92	a
	OHS	5	83	bcd		OHS	5	88	ab
	OHS	10	91	ab		OHS	10	92	a
4	STH	5	86	bcd	6	STH	5	87	abc
	STH	10	93	ab		STH	10	93	a
	PVF	5	84	cde		PVF	5	86	abc
	PVF	10	95	a		PVF	10	92	ab
	OHS	5	84	bcde		OHS	5	85	abc
	OHS	10	93	ab		OHS	10	91	ab
8	STH	5	93	a	10	STH	5	84	abcd
	STH	10	93	a		STH	10	93	a
	PVF	5	91	ab		PVF	5	84	abcd
	PVF	10	94	a		PVF	10	91	ab
	OHS	5	91	ab		OHS	5	85	abc
	OHS	10	96	a		OHS	10	91	ab
12	STH	5	90	ab	14	STH	5	86	abc
	STH	10	94	a		STH	10	91	ab
	PVF	5	90	ab		PVF	5	90	ab
	PVF	10	93	a		PVF	10	93	a
	OHS	5	89	ab		OHS	5	88	abc
	OHS	10	95	a		OHS	10	90	ab

[a]Initial germination data taken before seed were placed in storage conditions.
[b]Storage locations included an inner insulated room of a warehouse providing stable air temperature and humidity (STH), a warehouse with proper ventilation and fluctuating air temperature (PVF), and under an overhead shelter providing fluctuating air temperature and humidity (OHS).
[c]Days after seed were placed in germination chambers when data was recorded. Data was recorded 5 and 10 days after seed were placed in the germination chamber after seed had been in storage between 2 and 14 weeks.
[d]Data was analyzed using PROC GLIMMIX in SAS Studio 5.2 (SAS Institute, Cary, NC) with means separated using Tukey's HSD set at an alpha of 0.05. Estimates followed by the same letter are not difference from each other. Data within each column are not compared to each other. Each week is compared to the initial germination with the estimates including the same letter not being different.

ADDENDUM

SATSUMA ORANGE TOLERANCE TO SPRING AND AUTUMN INDAZIFLAM APPLICATIONS IN GEORGIA

Hurdle NL, Grey TL, Rucker K. 2022.

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Abstract

Citrus is a major crop in the SE US with groves being located primarily in Florida, but adapted cultivars has allowed expansion of commercial production into the Coastal Plains region of Georgia. Indaziflam, a cellulose biosynthesis inhibiting residual herbicide, controls numerous grass and broadleaf weed species. Research conducted in Georgia from 2020 to 2022 determined optimal rate and tree response to indaziflam applications. Biannual treatments applied in April and November in established 'satsuma' citrus groves included indaziflam, glyphosate, flumioxazin, diuron, pendimethalin, simazine, and norflurazon. Data included tree diameter and residual weed control. Indaziflam provided excellent residual weed control in the first year with >80% weed control for summer weed species, and >70% for winter weed species. Greater than 88% weed control was achieved for summer species with indaziflam. All other herbicides provided inadequate residual weed control in the second experiment. Environmental conditions may have enhanced herbicide dissipation. Indaziflam PRE applied in citrus groves can provide growers a reliable herbicide option that has been proven to be safe for trees and season long weed control.

Introduction

The Southeastern United States has a climate that is conducive for optimal growth of a multitude of perennial crops, with high temperatures and rainfall averaging 127 cm per year (Frankson et al. 2017). These include grass species for hay, blueberry, blackberry, tree nut, and peach production. Florida citrus includes oranges, tangerines, and grapefruits that can be sold as fresh or processed goods. These three fruits totaled over 2.98 mt for the 2020 Florida growing season (NASS 2020). Interest by growers in Southern Georgia has increased for citrus production as both regions have similar climatic conditions. This interest may stem from the

growing issue of citrus greening disease as it spreads throughout Florida citrus groves. This disease is spread by the Asian citrus psyllid (*Diaphorina citri* Kuwayama) which feeds on phloem sap and transmits the bacterium *Candidatus* Liberibacter asiaticus which causes the citrus greening disease (Grafton-Cardwell et al. 2018). No resistant citrus varieties currently exist, but investigators from academia and Federal agencies are working to develop resistant cultivars (Buck 2020). Citrus crops are typically harvested during the winter months, while a lack of pest control throughout the summer months can prove detrimental to the number and quality of fruit produced. A critical component of field and perennial crop production is weed management. Due to perennial crops not being removed from the field during harvest, tillage practices are not possible, causing a heavy reliance on herbicides for weed control. Investigators have reported that the Asian citrus psyllid can use weed species as a short-term alternate host if citrus plant species are not available for feeding (George et al. 2020).

Herbicides have become a primary method of weed control due to their effectiveness and ease of use compared to other control methods (Gianessi and Sankula 2003; Alemseged et al. 2001). Early weed management practices were primarily hand pulling and mechanical methods which proved very labor intensive. As agriculture changed from subsistent to commercial, field sizes increased, leading to more and more human labor required. This led to the need of a control method that could be easily applied in large quantities with high efficacy. Substances such as salts, oil, and acids were used as early chemical control methods to clear large sections of land due to their nonselective nature, but required substantial amounts (Green et al. 1987). Research was then performed to develop 2,4-dichlorophenoxy acetic acid (2,4-D) as a selective broadleaf herbicide. Herbicide research advanced over time to develop compounds that were selective for

certain weed species (grass or broadleaf) and optimal application timing for maximum efficacy and crop safety (PRE or POST) (Vats 2015).

A current herbicide used in multiple perennial crop production is indaziflam, N-[(1R,2S)-2,3-dihydro-2,6-dimethyl-1H-inden-1-yl]-6-[(1RS)-1-fluoroethyl]-1,3,5-triazine-2,4-diamine. Indaziflam prevents weed growth and development by disrupting the production of cellulose which is composed of β-1,4-glucan chains that provide structure to plant cell walls (Jarvis 2013). These cellulose chains are formed on the plasma membrane by the hexagonal cellulose synthase complex protein and must contain at least three cellulose synthase A (CESA) proteins per cellulose synthase complex to form the cellulose microfibrils (Davis 2012; Desprez et al. 2007). These microfibrils are layered orthogonally to each other and connect to adjacent microfibrils by cross-linking glycan strands within a pectin network (Alberts et al. 2002). Indaziflam inhibits cellulose production by increasing CESA density along the plasma membrane paired with reducing CESA particle velocity up to 65%, therefore preventing polymerization (Brabham et al. 2014). Several studies have been performed investigating the efficacy and safety of indaziflam in a multitude of perennial crops (Grey et al. 2018; Hurdle et al. 2019; Brabham 2014). Though indaziflam has been indicated to cause minimal injury, certain soil types and agricultural practices may promote injury on trees. Injury was observed in pecan orchards of Arizona and New Mexico potentially due to tillage practices and soil sand content (González-Delgado 2015). Necrotic leaves and various trunk injuries were noted among the affected trees. Southern Georgia soils may be composed of more than 90% sand which may raise concerns for citrus growers.

Though minimal to no injury has been reported after indaziflam usage in perennial crops, the response of citrus crops in Georgia has not been evaluated (Jhala et al. 2013; Jhala and Singh

2012; Blanco et al. 2014; González-Delgado et al. 2015). Research was performed to establish this information for Southern Georgia citrus growers and determine the effects on trunk diameter and residual activity indaziflam has in citrus production.

Materials and Methods

Field studies were conducted in 2-year established (Experiment 1) and newly transplanted (Experiment 2) 'Brown Satsuma' citrus trees in Tift County, Georgia (31˚34'1.63"N, 83˚36' 20.83"W) from 2020 to 2022. Soil samples were collected and analyzed by the Soil, Plant, and Water Laboratory of the University of Georgia (University of Georgia, Athens, GA) and determined to consist of Tifton loamy sand (Fine-loamy, kaolinitic, thermic Plinthic Kandiudults); pH 6.10; and 83.4%, 9.1%, 7.5%, and 0.75% sand, silt, clay, and organic matter, respectively. The crop was maintained using standard citrus agronomic techniques determined by the grower for the duration of the experiments. The trees were deflowered to promote vegetative growth.

The experimental design consisted of a randomized complete block with 10 treatments and two application dates, one in April and November each year (Table 1), with three replications in Experiment 1 and four in Experiment 2. Treatments consisted of glufosinate at 1269 g ai ha^{-1} in combination with either indaziflam at 51 g ai ha^{-1}, flumioxazin at 215 g ai ha^{-1}, diuron at 1774 g ai ha^{-1}, pendimethalin at 2448 g ai ha^{-1}, simazine at 2369 g ai ha^{-1}, norflurazon at 1123 g ai ha^{-1} or mixed with pendimethalin and simazine, with additional treatments being glyphosate alone at 1336 g ae ha^{-1} or mixed with indaziflam and a non-treated control (10 total treatments). A glufosinate burndown application (1269 g ai ha^{-1}) was made to all plots two to four weeks prior to experimental treatment applications, except year one of experiment 1 in which no burndown application was made. All treatments had an addition of 468 ml ha^{-1} of 28%

urea ammonium nitrate. Treatments were applied using a CO_2-pressurized backpack sprayer at 187 L ha^{-1} with 207 kPa of pressure, utilizing TeeJet TTI11002 nozzles (TeeJet Technologies LLC, Springfield, IL). Applications were made to either side of the plot using a 4 nozzle, 1.8 m boom on the vegetation free strip. The lack of translocation within the plant allowed glufosinate to be used if it should contact the crop foliage, localized injury would occur and not terminate the crop compared to glyphosate (Shaner 2014a).

Plot size was 3 m by 9 m containing 5 trees per plot with data being collected from the entire plot for residual activity and each tree for trunk measurements. Trunks were marked with a white paint marker approximately 30 cm above the soil level and above the graft to ensure measurements occurred at the same location on the tree. Trunks were measured (cm) using calipers angled parallel to the row at experiment initiation and termination on the same mark. Percent growth was determined for each tree and then averaged over the five trees per plot, then averaged over the three replications using the percent change equation:

$$C = \left(\frac{x_2 - x_1}{x_1}\right) * 1 \quad [1]$$

where x_1 indicates the trunk diameter before treatment application and x_2 indicates trunk diameter at experiment termination.

Data collected also included percent residual control from application to application and during the winter months. The citrus groves had sufficient weed pressure at the start of each experiment that primarily consisted of wild radish (*Raphanus raphanistrum* L.), cutleaf evening primrose (*Oenothera laciniata* Hill), bermudagrass (*Cynodon dactylon* L.), pink purselane (*Portulaca pilosa* L.), and cutleaf geranium (*Geranium dissectum* L.)

Data Analysis

Data were subjected to ANOVA to determine season (spring or autumn) by year interactions. Visual ratings of percent residual control and caliper measured trunk diameters were analyzed using PROC GLIMMIX in SAS software (version 9.4, SAS Institute Inc., Cary, NC). Replication was considered a random effect for analysis. Means were separated using Tukey's HSD at the $\alpha < 0.05$ level. Trunk diameter data consisted of the combined average of trunks within each plot. Yield data was not collected due to grower not allowing trees to fruit.

Results and Discussion

The ANOVA procedure indicated season by year interactions for each experiment preventing data from being combined. Data is presented by season each year per experiment for percent residual control, while data for tree diameter is presented from experiment initiation to termination.

Experiment One

Tree trunk diameters ranged from 15.8 mm to 20.5 mm at experiment initiation and 48.0 mm to 56.4 mm at termination (Table 2). No differences were indicated at initiation or termination. Change in growth was also determined and indicated greater than 158% growth over the experiment for all treatments. The least amount of growth occurred in the NTC and glufosinate plus norflurazon at only 158% growth. The greatest amount of growth occurred in trees where glufosinate plus diuron were applied, but no differences were indicated. Trees in plots treated with glyphosate or glufosinate plus indaziflam indicated the next greatest amount of growth with 189% and 188%, respectively.

Ratings for percent residual control were recorded after the first glufosinate application made after treatments until the next burndown application (Table 3). Greater than 72% control was achieved by all treatments except for the NTC, glyphosate alone, and glufosinate plus

norflurazon. Glyphosate alone provided little residual control, but was not different than glufosinate plus either pendimethalin, simazine, or norflurazon.

Fall residual control of all treatments decreased ranging from 3 to 39% compared to spring treatment residual control. The glufosinate plus pendimethalin noted the greatest reduction in both residual control and percent control compared to the NTC in Autumn of 2020.

Spring 2021 percent residual control decreased in all treatments except glyphosate or glufosinate plus indaziflam and glufosinate plus norflurazon compared to spring 2020 applications. Decrease in residual control ranged between 17 and 48% for the respective treatments, while glyphosate or glufosinate plus indaziflam increased by 9 or 8%, respectively (Table 3). Glufosinate plus norflurazon residual control increased by 12% compared to the previous spring application. All treatments provided 73% or less residual control except the indaziflam tank-mixtures.

Experiment Two

Experiment two was located within the same field as experiment one and contained the same variety of satsuma but were newly transplanted and not established. Adequate weed pressure was present at the beginning of experiment two and noted to be denser than experiment one. ANOVA indicated differences by season and year preventing data from being combined.

Trunk diameters of trees in experiment two were slightly larger at initiation compared to experiment one, with diameters ranging between 28.2 mm and 30.5 mm with no differences noted (Table 2). Diameters at experiment termination ranged between 39.6 mm and 45.5 mm. Differences were indicated in plots treated with glyphosate plus indaziflam with trees having a greater diameter compared to the NTC. Glyphosate plus indaziflam and glufosinate plus indaziflam were not different from each other, but misapplication of glyphosate may have more

severe consequences than glufosinate due to its systemic properties compared to the contact properties of glufosinate (Duke 2017; Bromilow et al. 1997). Percent growth was also lower compared to experiment one ranging from 38.8% to 58.6%. Trees in plots treated with glyphosate and indaziflam noted the largest amount of growth. The reduced trunk growth may be due to the increased weed pressure lowering available water and nutrients to the trees.

No treatment provided greater than 66% residual control (Table 4). This may have been due to the residual herbicide not contacting the soil surface and remaining on the plant matter due to heavy weed presence, or not receiving proper rainfall for activation (Anonymous 2019). Glufosinate plus flumioxazin provided the greatest amount of residual control at 66%, while the tank mixture of glufosinate plus pendimethalin plus simazine provided only 18%. Flumioxazin has been noted to provide adequate weed control in numerous crops (Niekamp and Johnson 2001; Ramirez et al. 2012; Richardson and Zandstra 2009).

Autumn applied herbicide treatment residual control was 55% or lower for all treatments, except glufosinate or glyphosate plus indaziflam, providing 81% and 75% control, respectively. The unusually warm winter weather experienced following the Autumn applications may have increased the weed pressure, causing the reduced control.

Spring 2022 residual control noted a decrease in all treatments except glyphosate alone, glufosinate plus pendimethalin, and glufosinate plus flumioxazin which either remained the same or slightly increased (Table 4) compared to the Autumn 2021 application. Both indaziflam treatments indicated the greatest amount of residual control, providing 69% control or greater, followed by norflurazon at 64% and diuron at 50% residual control.

Reports have indicated that injury may occur from indaziflam applications under specific environmental conditions. Soils that are predominately sand and maintained under flood

irrigation have been noted to be conducive to crop injury from indaziflam (González-Delgado et al. 2015; Jhala and Singh 2012). South Georgia soils typically have sand content above 90% with pH levels between 5 and 6 as noted in these experiments. Crop root structure may also play a role in the occurrence of injury. Pecan lateral roots are typically within the top 15 to 30 cm whereas citrus roots may be as deep as 91 cm, therefore increasing the depth in which indaziflam must travel to cause injury. The label states that citrus trees must be established for greater than one year or be transplanted from pots for longer than one month (Anonymous 2019). The irrigation practices are predominately micro-emitters or non-irrigated reducing the movement of indaziflam through the soil profile by only allowing small amounts of water be emitted for long periods of time reducing movement into the soil. This is supported by Basinger et al. (2019) in which the investigators reported no indaziflam injury on grape or muscadine growth, yield, or quality grown under similar soil and irrigation conditions as the citrus experiments. Indaziflam has been demonstrated to provide excellent residual weed control in citrus production. Spring and autumn applications of indaziflam were noted to provide up to 88% control of weeds after two years of applications. Indaziflam would be an excellent tool to integrate into South Georgia citrus weed management due to its effectiveness and safeness on sandy soils.

Literature Cited

Alberts, B., A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter. 2002. Molecular Biology of the cell. 4th ed. Garland Science. https://www.ncbi.nlm.nih.gov/books/NBK26928/

Alemseged, Y., R.E. Jones, R.W. Medd. A farmer survey of weed management and herbicide resistance problems of winter crops in Australia. *Plant Protection Quarterly.* 16(1): 21-25.

Anonymous. 2019. Alion® herbicide product label. Research Triangle Park, NC: Bayer CropScience. 66 p.

Basinger, N.T., K.M. Jennings, D.W. Monks, W.E. Mitchem. 2019. Effect of rate and timing of indaziflam on 'Sunbelt' and muscadine grape. Weed Technol 33:380-385.

Blanco, F.M.G., Y.G. Ramos, M.G. Scarso, L.A.C. Jorge. 2015. Determining the selectivity of herbicides and assessing their effect on plant roots- a case study with indaziflam and glyphosate herbicides. *In* Herbicides, Physiology of Action, and Safety. *Eds.* A. Price, J. Kelton, L. Sarunaite. DOI: 10.5772/61721.

Brabham, C., L. Lei, Y. Gu, J. Stork, M. Barrett, S. DeBolt. 2014. Indaziflam herbicidal action: a potent cellulose biosynthesis inhibitor. Plant Physiol. 166(3): 1177-1185.

Bromilow, R.H., Chamberlain, K., Tench, A.J., Williams, R.H. 1997. Phloem translocation of strong acids- glyphosate, substituted phosphonic and sulfonic acids in *Ricinus communis* L. Pestic. Sci. 37: 39-47.

Buck, B. 2020. UF scientists make big stride toward greening-resistant citrus trees. UF IFAS Blog. Accessed: September 3rd, 2022. https://blogs.ifas.ufl.edu/news/2020/10/14/uf-scientists-make-big-stride-toward-greening-resistant-citrus-trees/

Chang, S.Y. and C.H. Liao. 2002. Analysis of glyphosate, glufosinate, and aminomethylphosphonic acid by capillary electrophoresis with indirect fluorescence detection. J. Chromatography A. 959(1-2): 309-315.

Davis, J.K. 2012. Combining polysaccharide biosynthesis and transport in a single enzyme: dual-function cell wall glycan synthases. Front. Plant Sci 3:138.

Desprez, T., M. Juraniex, E.F. Crowell, H. Jouy, Z. Pochylova, F. Parcy, H. Höfte, M. Gonneau, S. Vernhettes. 2007. Organization of cellulose synthase complexes involved in primary

cell wall synthesis in *Arabidopsis thaliana.* Proc. Natl. Acad. Sci. USA 104: 15572-15577.

Duke, S.O. 2017. The history and current status of glyphosate. Pest Management Science. 74(5): 1027-1034.

Frankson, R., K. E. Kunkel, L.E. Stevens, B.C. Stewart, W. Sweet, B. Murphey. 2017. Georgia State Climate Summary. NOAA Technical Report NESDIS 149-GA, 4 pp.

Gianessi, L.P. and S. Sankula. 2003. The value of herbicides in U.S. crop production. National Center for Food and Agricultural Policy. 1 pp.

George, J., R. Kanissery, E-D. Ammar, I. Cabral, L.T. Markle, J.M. Patt, L. L. Stelinski. 2020. Feeding behavior of Asian citrus psyllid [*Diaphorina citri* (Hemiptera: Liviidae)] nymphs and adults on common weeds occurring in cultivated citrus described using electrical penetration graph recordings. Insects. 11(48). 17 pages. DOI: 10.3390/insects11010048.

González-Delgado, A.M., J. Ashigh, M. K. Shukla, R. Perkins. 2015. Mobility of indaziflam influenced by soil properties in a semi-arid area. PLoS One. 10(5): e0126100. DOI: 10.1371/journal.pone.0126100.

Grafton-Carwell, E.E., and M.P. Daugherty. 2018. UC IPM Pest Notes: Asian Citrus Psyllid and Huanglongbing Disease. UC ANR Publication 74155. Oakland, CA. Accessed: September 3rd, 2022. http://ipm.ucanr.edu/PDF/PESTNOTES/pnasiancitruspsyllid.pdf

Green, M.B., G.S. Harley, T.F. West. 1987. Chemicals for crop improvement and pest management. 3rd Ed. Pergamon Press. Oxford. P 207.

Grey, T.L., K. Rucker, L. Wells, X. Luo. 2018. Response of young pecan trees to repeated applications of indaziflam and halosulfuron. Hort Sci. 53(3): 313-317.

Hurdle, N.L., T.L. Grey, McCullough, P.E., D. Shilling, J. Belcher. 2019. Bermudagrass tolerance of indaziflam PRE applications in forage production. Weed Tech. 34:125-128.

Jarvis, M.C. 2013. Cellulose biosynthesis: counting the chains. Plant Physiol 163: 1485-1486.

Jhala, A.J. and M Singh. 2012. Leaching of indaziflam compared with residual herbicides commonly used in Florida citrus. Weed Technol 26:602–607.

Jhala, A.J., A.H.M. Ramirez, M. Singh. 2013. Tank mixing saflufenacil, glufosinate, and indaziflam improved burndown and residual weed control. Weed Techn. 27(2): 422-429.

Morillo, E., T. Undabeytia, A. Cabrera, J. Villaverde, C. Maqueda. 2004. Effect of soil type on adsorption-desorption, mobility, and activity of the herbicide norflurazon. J. Agric. Food Chem. 52:884-890.

National Agricultural Statistics Service (NASS) 2020. United States Department of Agriculture. https://www.nass.usda.gov/Quick_Stats/Ag_Overview/stateOverview.php?state=FLORIDA. Accessed: September 1st, 2021.

Niekamp, J.W. and W.G. Johnson. 2001. Weed management with sulfentrazone and flumioxazin in no-tillage soyabean (*Glycine max*). Crop Protection. 20(3):215-220.

Ramirez, A.H.M., A.J. Jhala, M. Singh. 2012. Efficacy of PRE and POST herbicides for control of citron melon (*Citrullus lanatus* var. *citroides*). Weed Technology. 26:783-788.

Richardson, R.J and B.H. Zandstra. 2009. Weed control in Christmas trees with flumioxazin and other residual herbicides applied alone or in tank mixtures. HortTechnology. 19(1): 181-186.

Shaner DL, ed. 2014a. Pages 240-242 *in* Herbicide Handbook. 10th Edition. Lawrence, KS: Weed Science Society of America.

Shaner DL, ed. 2014b. Pages 266–267 *in* Herbicide Handbook. 10th Edition. Lawrence, KS: Weed Science Society of America.

Vats, S. 2015. Herbicides: History, classification, and genetic manipulation of plants for herbicide resistance. Sustainable Agriculture Reviews. E. Lichtfouse ed. Springer International Publishing. DOI: 10.1007/978-3-319-09132-7_3

Table 5.1. Residual herbicide treatment application dates for experiment one and two[a].

	2020		2021		2022
	Spring	Autumn	Spring	Autumn	Spring
Experiment 1	April 6	Nov. 4	April 5		
Experiment 2			April 5	Nov. 3	March 26

[a]Experiment one and two are located within the same field in Chula, GA. Experiment one are trees that have been established for two years and Experiment two are newly transplanted trees located next to experiment one.

Table 5.2. Citrus tree trunk diameter response to April and November residual herbicide applications.

Treatment	Rate	Experiment One[a,c]						Experiment Two[a,c]					
	g ai ha^{-1}	April 2020[b]		November 2021		% Change		April 2021		November 2022		% Change	
		mm						mm					
NTC[a]		18.7	a	48.0	a	158	a	28.2	a	39.6	b	40.2	a
Glyphosate	1336	19.4	a	50.3	a	160	a	28.8	a	43.0	a	50.6	a
Glyphosate plus Indaziflam	1336 + 51	17.6	a	49.3	a	189	a	29.0	a	45.5	a	58.5	a
Glufosinate plus Indaziflam	1269 + 51	18.2	a	52.2	a	188	a	30.1	a	44.4	ab	48.3	a
Glufosinate plus Flumioxazin	1269 + 215	20.5	a	56.4	a	175	a	30.3	a	43.8	ab	44.5	a
Glufosinate plus Diuron	1269 + 1774	15.8	a	48.9	a	213	a	30.0	a	41.6	ab	39.8	a
Glufosinate plus Pendimethalin	1269 + 2448	18.1	a	48.3	a	169	a	30.5	a	42.3	ab	38.8	a
Glufosinate plus Simazine	1269 + 2369	20.4	a	55.3	a	171	a	30.8	a	43.9	ab	42.5	a
Glufosinate plus Pendimethalin plus Simazine	1269 + 2448 + 2369	18.5	a	51.9	a	181	a	29.2	a	42.4	ab	45.9	a
Glufosinate plus Norflurazon	1269 + 1123	19.6	a	50.0	a	158	a	29.8	a	43.0	ab	44.2	a

[a]ANOVA using the GLIMMIX procedure in SAS, Type 3 Tests of Fixed Effects (SAS Institute, Cary, NC) prevented data combination.
[b]Letters indicate statistical significance at the $\alpha < 0.05$ within each season and year according to Tukey-Kramer HSD.
[c]Experiment One consisted of 2 year old trees and Experiment two consisted of newly transplanted trees within the same field.

Table 5.3. Residual control compared to the NTC ratings after one month of herbicide application combined in two year old trees of Experiment One.

Treatment	Rate	April 2020		November 2020		April 2021	
	g ai ha^{-1}	%					
NTC[a]		0	c	0	e	0	e
Glyphosate	1336	39	b	26	d	22	de
Glyphosate plus Indaziflam	1336 + 51	80	a	88	a	89	a
Glufosinate plus Indaziflam	1269 + 51	80	a	69	ab	88	a
Glufosinate plus Flumioxazin	1269 + 215	92	a	64	b	73	ab
Glufosinate plus Diuron	1269 + 1774	84	a	81	a	56	abc
Glufosinate plus Pendimethalin	1269 + 2448	72	ab	33	cd	47	bcd
Glufosinate plus Simazine	1269 + 2369	76	ab	41	c	28	cde
Glufosinate plus Pendimethalin plus Simazine	1269 + 2448 +2369	91	a	75	ab	63	ab
Glufosinate plus Norflurazon	1269 + 1123	41	b	34	cd	53	bcd

[a]ANOVA using the GLIMMIX procedure in SAS, Type 3 Fixed Effects (SAS Institute, Cary, NC) prevented data combinated.
[b]Letters indicated statistical significance at the $\alpha < 0.05$ within each season and year according to Tukey-Kramer HSD.
Weed species included *Raphanus raphanistrum* L. (RAPRA), *Oenothera laciniata* Hill. (OEOLA), *Cynodon dactylon* L. Pers. (CYNDA), *Portulaca pilosa* L. (PORPI), and *Geranium dissectum* L. (GERDI).

Table 5.4. Residual control compared to the NTC ratings after one month of herbicide application combined in newly transplanted trees of Experiment Two.

Treatment	Rate	April 2020		November 2020		April 2021	
	g ai ha^{-1}			%			
NTC[a]		0	d	0	e	0	f
Glyphosate	1336	30	bcd	16	de	17	ef
Glyphosate plus Indaziflam	1336 + 51	62	ab	81	a	79	a
Glufosinate plus Indaziflam	1269 + 51	55	ab	75	ab	69	ab
Glufosinate plus Flumioxazin	1269 + 215	66	a	39	cd	39	bcde
Glufosinate plus Diuron	1269 + 1774	40	abc	55	abc	50	abcd
Glufosinate plus Pendimethalin	1269 + 2448	28	bcd	34	cde	37	def
Glufosinate plus Simazine	1269 + 2369	43	abc	30	cde	23	bcde
Glufosinate plus Pendimethalin plus Simazine	1269 + 2448 + 2369	18	cd	47	bcd	45	bcde
Glufosinate plus Norflurazon	1269 + 1123	28	bcd	57	abc	64	abc

[a]ANOVA using the GLIMMIX procedure in SAS, Type 3 Fixed Effects (SAS Institute, Cary, NC) prevented data combinated.
[b]Letters indicated statistical significance at the $\alpha < 0.05$ within each season and year according to Tukey-Kramer HSD.
Weed species included *Raphanus raphanistrum* L. (RAPRA), *Oenothera laciniata* Hill. (OEOLA), *Cynodon dactylon* L. Pers. (CYNDA), *Portulaca pilosa* L. (PORPI), and *Geranium dissectum* L. (GERDI).