

COTTON SEED AND SEEDLINGS

NUMBER TEN
THE COTTON FOUNDATION
REFERENCE BOOK SERIES



Edited by
Daryl R. Chastain, Gurpreet Kaur,
K. Raja Reddy, and Derrick M. Oosterhuis

**COTTON SEED
AND SEEDLINGS**

THE COTTON FOUNDATION

Reference Book Series

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The editor and contributors of *COTTON SEEDS AND SEEDLINGS* are indebted to Charlie Yarwood for preparation and page setting of the manuscripts for publication. In addition, grateful acknowledgement is made to all that provided assistance to the contributors and to the peer reviewers of the chapters.

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COTTON PHYSIOLOGY BOOK SERIES

FOREWORD

The Cotton Foundation Reference Book Series started with the publication of *COTTON PHYSIOLOGY* in 1986, edited by J.R. Mauney and J.M. Stewart, followed by a second book, *PHYSIOLOGY OF COTTON*, edited by J.M. Stewart, D.M. Oosterhuis, J.J. Heitholt, and J.R. Mauney published in 2010. In order to facilitate timely publication of pertinent information to the cotton community as a whole, this series has been continued using a smaller book format. Each book is focused on a particular topic within the study of cotton physiology. The books will be published in book form (hard copy) as well as on digitally under the auspices of the National Cotton Council as a continuation of the original Cotton Physiology book published in 1986. Each book has incorporated a special symposium on a topic chosen by members of the National Cotton Council, Agronomy and Physiology Conference and held at the Beltwide Cotton Conferences. Prominent speakers are invited to partake in the symposium, and together with additional invited authorities, will make up the subsequent book. The first of small physiology of cotton book series was on *STRESS PHYSIOLOGY IN COTTON* and was published in 2011. The subsequent book was entitled *FLOWERING AND FRUITING IN COTTON* and was published in 2012. *LINKING PHYSIOLOGY TO MANAGEMENT* was published in 2015. A symposium was held at the Beltwide Cotton Conferences in San Antonio in January 2013 and the subsequent book entitled *COTTON SEED AND SEEDLINGS* will be published in 2020.

PREFACE

As noted in the foreword, a substantial amount of information is available on the physiology of the cotton plant. It has long been recognized that productivity is influenced by genotype, management, and environment, and in the aforementioned books and other reviews on cotton physiology not mentioned herein, it has been argued that profitable cotton production hinges on a thorough understanding of the underlying physiological mechanisms that influence a given cotton genotype's response to management and environment. Therefore, it is the goal of the current book to clearly demonstrate how the physiology of the cotton crop dictates management decisions and how management decisions impact the physiology, growth, development, and yield of the cotton crop. Particular chapter foci include *Remote Sensing for Nitrogen Management*, *Plant-Insect Interactions and Cotton Development*, *Seeds and Planting*, *Irrigation Timing and Application Methods for Improving Water Use Efficiency*, *Plant-Based Irrigation Scheduling in Cotton*, and *Physiology of Host-Pathogen Interaction in Wilt Diseases of Cotton in Relation to Pathogen Management*. The interdisciplinary approach taken with the current book should highlight the importance of physiology in forming management decisions for multiple, seemingly disparate, aspects of the cotton production system.

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Chapter 1

ANATOMY AND MORPHOLOGY OF THE COTTON SEED AND SEEDLING

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INTRODUCTION

The structures of the seed of the cotton plant (*Gossypium* sp.) have been described in great detail by Brown (1927), Baranov and Maltzev (1937), Leahy (1948), Tharp (1965), Mauney (1968), and Oosterhuis and Jerstadt (1999). The definitive structures of the seed and seedling which influence the development of the subsequent crop are: 1) vascular connections, 2) seed coat and chalazal pore connections, 3) cotyledons, 4) hypocotyl and radicle axis, and 5) apex. This review will concentrate upon these five elements of the seed anatomy and morphology.

VASCULAR CONNECTIONS OF THE SEED

During development the seed is connected to the vascular system of the plant through the attachment of the funiculus to the placenta of the boll (Figure 1K). There are vascular strands consisting primarily of phloem in the raphe (Fig 1, J) of the seed extending to the chalazal pore on the distal portion of the developing seed. The vascular strands end at the pore. Thus, all hydration and nutrition for the developing embryo must enter the embryo sac through the spongy (unvascularized) tissue of the chalazal pore. This arrangement enables the plant to bathe the developing embryo in endosperm with very high osmotic potential (Mauney, 1961; Trolinger, et al., 1993) while the canopy operates at familiar, and fluctuating, water potential (Van Iersel et al., 1994, Van Iersel et al., 1995; Van Iersel and Oosterhuis, 1996)

SEED COAT AND CHALAZAL PORE STRUCTURE

Seed dormancy is evolutionarily and culturally necessary for cotton which has a long period of exposure to weather before harvest. The embryos must remain dormant while the bolls are subjected to moisture from dew and rainfall for up to more than 60 days after the first bolls crack. This dormancy may be due to ABA as a biochemical inhibitor (Kloth and Turley, 2010) or due to physical prevention of moisture uptake. It is probable that each of these mechanisms plays a role in preserving germinability in mature seed before harvest.

It has been demonstrated (Simpson, 1935; Christiansen and Moore, 1959) that water for germination of the dormant seed must enter through the chalazal pore. The palisade layer of the mature dry seed is heavily lignified and impermeable to water. (Figure 1G) The seal at the funiculus is also impenetrable until broken by moisture entering through the chalaza. The chalazal seal is a heavily lignified plug of tissue. In "hard" seed cultivars and species this plug may persist for months or years. Placing the seed in hot (80 C) for a short period (1 minute), ethanol,

or ethyl ether disrupts this seal, allowing water to enter and germination to begin (Christiansen and Moore, 1959). In "soft" seed cultivars the normal storage period from harvest to planting is sufficient to break the dormancy tendencies and allow immediate imbibition.

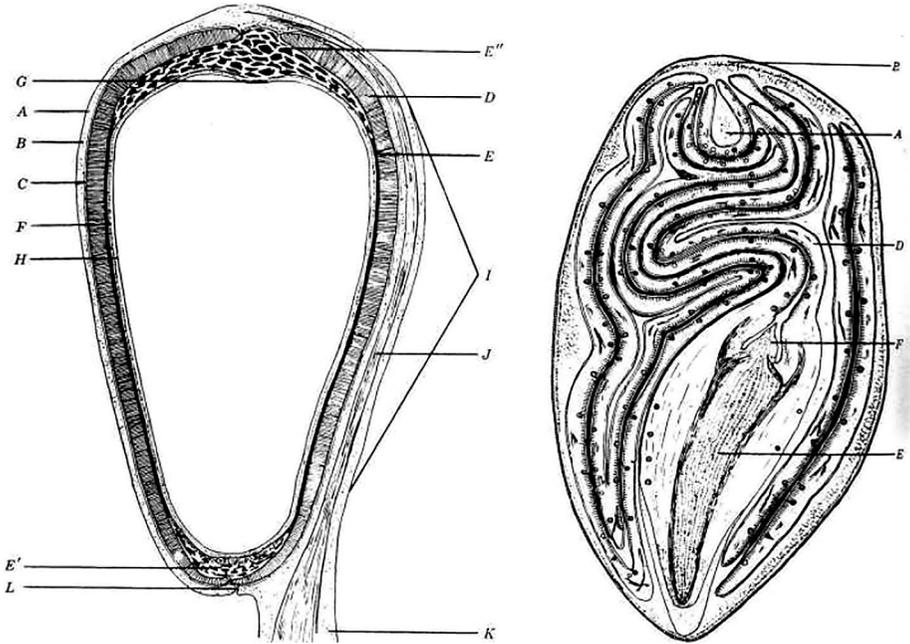


Figure 1. Structure of mature cotton seed. Seed coat (on left): A, outer epidermis; B, outer pigment layer; C, colorless layer; D, palisade layer; E, inner pigment layer; E', inner pericarp layer differentiated at micropyle; E'' inner pigment layer differentiated a chalazal cap; F, fringe cells; G, specialized cells at base of chalazal cap; H, membrane cells; I, raphe; J, vascular bundle; K, funiculus; L, micropyle. Embryo (on right) A, endosperm; B, nucellus; D, cotyledon; E, hypocotyl and radicle; F, epicotyl. From Leahy, 1948, as presented by Tharp, 1960.

COTYLEDONS

Two massive cotyledons occupy the majority of the volume and weight of cotton seed. They begin development as protuberances on the globular embryo at about 9 (Hector, 1936) to 12 (Quintanelha et al., 1962) days after pollination (DAP) to form what is known as the heart stage embryo. From that point the cotyledons expand in a sigmoid growth curve (Figure 2) to fill the embryo sac at about 20 DAP. The morphology of these cotyledons is identical to the primary leaves of the plant. There are resin glands (Fig. 3) which contain gossypol. However, whereas aerial leaves contain starch grains as the principle storage product, cotyledons contain oil and protein bodies in the cytoplasm as storage energy (Reeves and Valle, 1932). Because the cotyledons are the primary source of photosynthate for the seedling, any damage to them through weather and sand abrasion or insect attack (thrips feeding) can produce a severe delay in seedling growth.

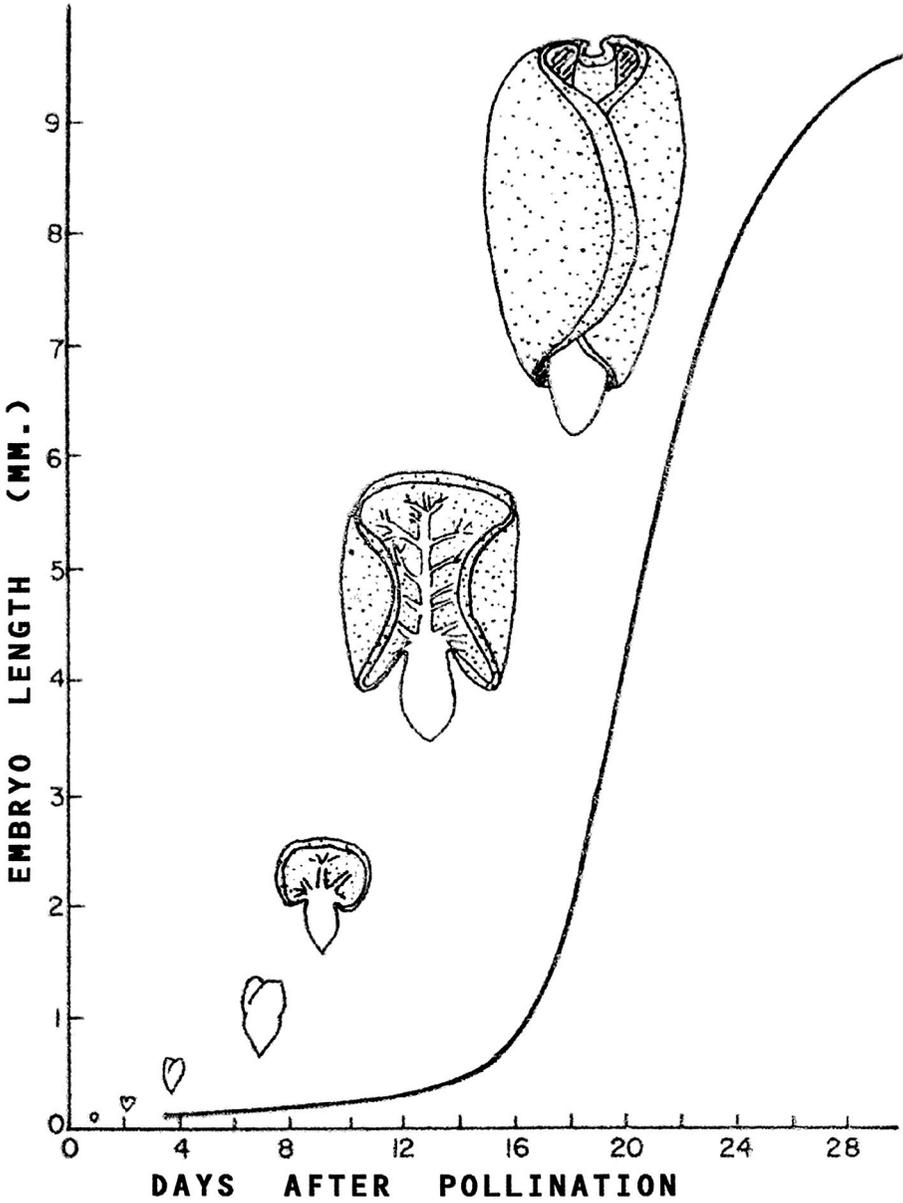


Figure 2. Growth of the cotton embryo. Sigmoid curve is a plot of embryo length with time as measured as days from pollination (DAP). Illustration shows degree of differentiation at various stage of growth in length. From Mauney 2012.

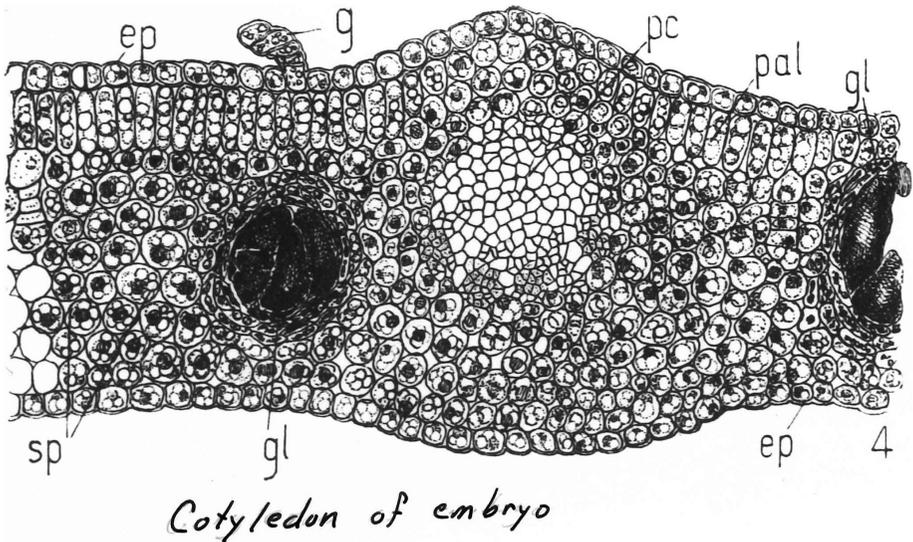


Figure 3. Cross section of the embryonic cotyledon showing resin glands (gl). From Baranov and Maltzev, 1937

HYPOCOTYL AND RADICLE AXIS

The hypocotyl of the cotton seed is a well-developed stem between the cotyledons and the radicle. The transition from hypocotyl and radicle (stem and root) can be seen in Figure 4 at section 5 and 6 where the tetrarch arrangement of the typical root vascular system (McMichael, 1986) changes to the circular stele of the typical aerial stem of the vegetative cotton plant. That these structures are differentiated in the seed means that the elongation of the hypocotyl and radicle into stem and root at germination can be rapid, limited by only imbibition of water.

Upon germination, water entering the chalazal pore travels to the radicle which rapidly elongates and breaks through the micropyle of the seedcoat and becomes the seedling root. Water entering the root hydrates the hypocotyl, which elongates pushing the cotyledons through the soil (Figure 5). Because of the drag of the cotyledons the hypocotyl forms a “hook” as it penetrates the soil. This hook is the first tissue to emerge from the soil. After emergence the hypocotyl straightens (Fig 5) allowing the cotyledons to function as photosynthetic leaves. Under ideal conditions of moisture and temperature the seedling can begin adding dry weight by 4 to 5 days after imbibition (Mauney, unpublished).

The most active tissue of the germinating cotton seed is the radicle. It can penetrate into the soil 15 to 20 cm. by the time the cotyledons unfurl (Tharp, 1960). Secondary roots form from lateral meristems located at each of the tetrach vascular bundles in the root (Figure 6). Thus, secondary roots are observed in rows down each quadrant of the tap root.

The vigorous expansion of the root system during the seedling phase results in the highest root/shoot ratio of any stage in the plant development (Taylor and Klepper, 1974; Mauney, et al., 1994). The high R/S ratio enables rapid expansion of the leaf area when the true leaves begin expansion.

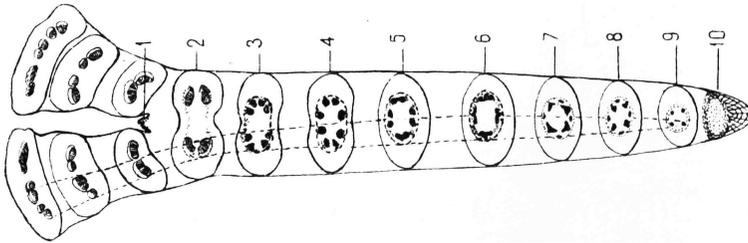


Figure 4. Drawing of the embryonic axis showing the vascular development, 1, epicotyl 2 to 5, hypocotyl; 6 to 10, radicle. From Baranov and Maltzev 1937.

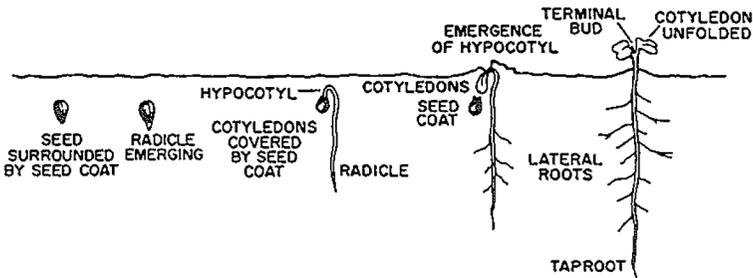


Figure 5. Drawing of stages of seedling establishment from seed to cotyledon unfolding. From Oosterhuis, 1991.

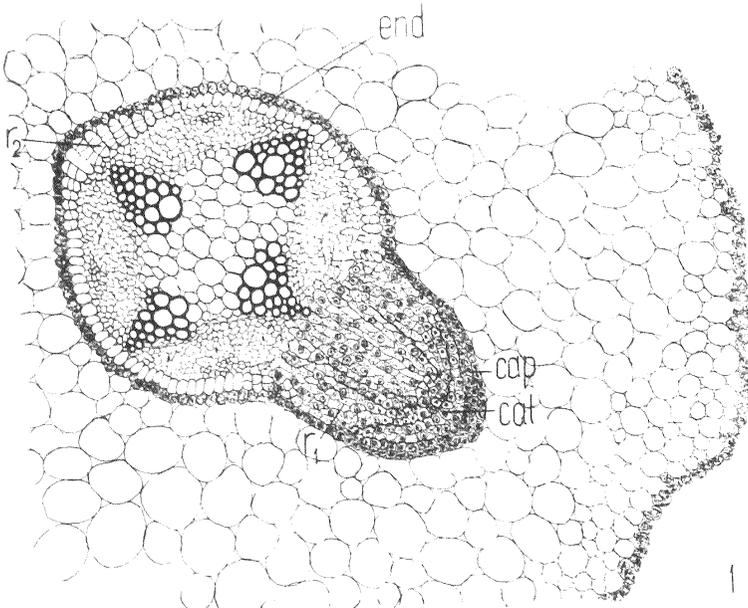


Figure 6. Drawing of the epicotyl of the embryonic axis with a single true leaf primordium and the apical meristematic dome. From Baranov and Maltzev, 1937

APEX

The length of the seedling phase of cotton production derives from the fact that that embryo has no plumule as seen in legumes and grasses. A cotton seed (embryo) contains only a poorly developed epicotyl (apex) consisting of a single leaf primordium and a meristematic dome (Figure 7). Using carbohydrates from the photosynthetic activity of the cotyledons and water from the rapidly expanding root system the epicotyl develops the tissues of the first primary leaf during the first week of seedling establishment.

The first and second leaves are not lobed. Climax leaf lobing occurs at about the sixth true leaf. Axillary branch buds are found at the base of the expanded cotyledons and all true leaves. Axillary buds are not present in the seed. At the time The second true leaf is unfurled about five to seven additional true leaf primordia have been organized into the plumule of the plant. At that time the axillary meristems are sensitive to floral induction which is influenced by plant vigor and environmental conditions (Mauney, 1966).

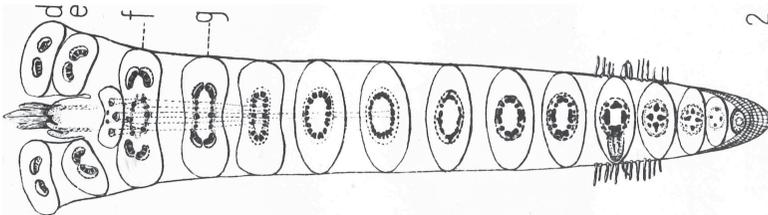


Figure 7. Drawing of the vascular development in the seedling axis. Note secondary root development and root hairs at transition zone to radicle.. From Baranov and Maltzev 1937.

SUMMARY

The structures of the cotton seed determine much of the developmental timing of the juvenile and mature plant. The well-developed seed radicle allows rapid establishment of a tap root to support the seedling. The cotyledons contain energy for seedling expansion and become photosynthetic immediately. The fact that the seed has no plumule delays seedling display of true leaves until the apical meristem can develop leaf primordia. Thus, the seedling stage is a long and vulnerable stage in the development of the crop.

REFERENCES

- Baranov, P.A., and A.M. Maltzev. 1937. The structure and development of the cotton plant: an atlas. Ogis-Isogis, Moscow-Leningrad.
- Brown, H.B. 1927. Cotton. McGraw-Hill Inc. New York, NY.
- Christiansen, M.N. and R.P. Moore. 1959. Seed coat structural differences that influence water uptake and seed quality in hard seed cotton. *Agron. J.* 51:582-584.

- Dennis, R.E., and R.E. Briggs. 1969. Growth and development of the cotton plant in Arizona. Univ. of Ariz. Cooperative Extension Service Bull. A-64. Tucson, AR.
- Hector, J.M. 1936. Introduction to the botany of field crops. Vol II Non-Cereals. Central News Agency, Johannesburg, South Africa. pp. 1127.
- Kloth, R.H., and R.B. Turley. 2010. Physiology of seed and fiber development. In: J.M. Stewart, D.M. Oosterhuis, J.E. Heitholt, and J.R. Mauney, editors, Physiology of cotton. Springer, New York, NY. pp. 111-122.
- Leahy, J. 1948. Structure of the cottonseed. In: A.E. Bailey, editor, Cottonseed and cottonseed products – their chemistry and chemical technology. Interscience Publishers Inc., New York, NY. pp. 105-116.
- Oosterhuis, D.M., 1991. Growth and development of a cotton plant. *MP-University of Arkansas, Cooperative Extension Service (USA)*.
- Oosterhuis, D.M., and J. Jernstedt. 1999. Morphology and anatomy of the cotton plant. In: C.W. Smith, and J.T. Cothren, editors, Cotton: origin, history, technology, and production. John Wiley and Sons, Inc New York, NY. pp. 175-206.
- Mauney, J.R. 1961. The culture *in vitro* of immature cotton embryos. Bot. Gaz. 122:205-209.
- Mauney, J.R. 1966. Floral initiation of upland cotton in response to temperatures. J. Exp. Bot. 17:452-459.
- Mauney, J.R. 2012. Anatomy and morphology of fruiting forms. In: D.M. Oosterhuis, and J.T. Cothren, editors, Flowering and fruiting of cotton. The Cotton Foundation, Memphis. pp. 245.
- Mauney, J.R. 1968. Morphology of the cotton plant. In: F.C. Eliot, M. Hoover, and W. K. Porter, Jr., editors, Advances in production and utilization of quality cotton. Iowa State University Press, Ames, IA.
- Mauney, J.R., B.A. Kimball, P.J. Pinter, Jr., R.L. LaMorte, K.F. Lewin, J. Nagy, and G.R. Hendrey. 1994. Growth and yield of cotton in response to free-air carbon dioxide enrichment (FACE). Agric. For. Met 70:49-67.
- Quintanilha, A.L, S. D'Eca, and A. Cabral. 1962. Desenvolvimento de botao floral do algodoeiro em funcao do tempo. Bol. Sol. Brot. 36:189-215.
- Reeves R.G., and C.C. Valle. 1932. Anatomy and microchemistry of the cottonseed. Bot Gaz. 93:259-277.
- Simpson, D.M. 1935. Relation of moisture content and method of storage to deterioration of stored cotton seed. J. Agr. Res. 50:449-456.
- Taylor, H.M., and B. Keppler. 1974. Water relations in cotton. I. Root growth and water use as related totop growth and soil water content. Agron. J. 66:584-588.
- Tharp, W.H. 1960. The cotton plant. how it grows and why its growth varies. Agric. Handbook No. 178. US Department of Agriculture, Agricultural Research Service, Washington, DC.
- Trolinger, N.L., B.L. McMichael, and D.R. Upchurch. 1993. Water relations of cotton flower petals and fruit. Plant Cell Environ. 16:755-760

- Van Iersel, M.W., D.M. Oosterhuis, and V.M. Harris. 1994. Apoplastic water flow to cotton leaves and fruits during development. *J. Exp. Bot.* 45:163-169.
- Van Iersel, M.W., V.W. Harris, and D.M. Oosterhuis. 1995. Phloem in developing cotton fruits: 6(5) Carbofluorescein as a tracer for functional phloem. *J. Exp. Bot.* 46:321-328.
- Van Iersel, M.W., and D.M. Oosterhuis. 1996. Drought effects on the water relations of cotton fruits, bracts, and leaves during ontogeny. *Environ. Exp. Bot.* 36:51-59.

Chapter 2

SEED CHARACTERISTICS AND SEEDLING VIGOR

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INTRODUCTION

Why does seedling vigor matter? Without question, “high vigor” seedlings are universally desired by cotton producers. Vigorous early season growth and uniform stand establishment provide growers with peace of mind and indicate that the crop is off to a good start. This is particularly important for cotton because it is generally accepted that cotton exhibits poor seedling vigor relative to other major row crops (Pilon et al., 2016). Early work conducted by Wanjura et al. (1969) showed that seedlings which emerged rapidly (five days after planting in this instance) exhibited greater survival and higher relative yields than seedlings emerging eight or 12 days after planting. Furthermore, percent emergence at five days after planting had a pronounced impact on lint yield (Figure 1), indicating that early seedling vigor and stand establishment can be a major factor in realizing cotton’s yield potential. Similarly, heat unit accumulation within a narrow window following planting has been positively associated with lint yield (Kerby et al., 1989). This implies that conditions conducive to vigorous early seedling growth can promote higher yields in some situations. To understand the mechanism by which seedling vigor might impact yield, it is important to view agriculture as a system designed to exploit solar energy through the process of photosynthesis (Gardner et al., 1985). Consequently, yield (Y) can be mathematically defined as the product of total absorbed photosynthetically active radiation (APAR) during a growing season, the efficiency with which the crop converts intercepted radiation into dry matter (RUE), and harvest index (HI) (Earl and Davis, 2003; Monteith, 1977, 1994; Stöckle and Kemanian, 2009), or $Y = \text{APAR} \times \text{RUE} \times \text{HI}$. Furthermore, it is well-established that crops typically do not attain maximum crop growth rate (CGR) until leaf area development is sufficient to intercept 95% of incoming solar radiation, a level of leaf area development known as critical leaf area index (LAI) (Gardner et al., 1985). Thus, poor early season vigor delays canopy development and results in lower APAR and inefficient utilization of available land area for a longer period of time when compared with a more vigorously growing canopy. While differences in seedling vigor do not always impact yield in cotton (Liu et al., 2015; Snider et al., 2016), it should be noted that there are other positive aspects associated with early season vigor. For example, vigorous early season growth lessens the potential damage that can be done by insect herbivory and plant pathogens, improves crop competitiveness with weedy plants, and might promote tolerance to drought at later stages in development (Elliot et al., 2008; Cook and El-Zik, 1992; Liu et al., 2015; Reddy and Boykin, 2010; Snider and Oosterhuis, 2015). Furthermore, the consequences of poor seedling vigor can be costly as growers are

forced to replant to remedy stand establishment issues. Because cotton is not always planted under optimal conditions for seedling growth and development, the most ideal scenario would be for a grower to make planting decisions based on seedling vigor before the seed is ever planted. For this to be a possibility, researchers are required to identify which planting seed characteristics are most indicative of seedling vigor and to develop reliable, quantitative relationships between seed characteristics and seedling performance in the field. The current chapter provides a review of seed and seedling development, studies relating early seedling performance to seed characteristics, factors influencing seed composition and quality, and opportunities and challenges for future research.

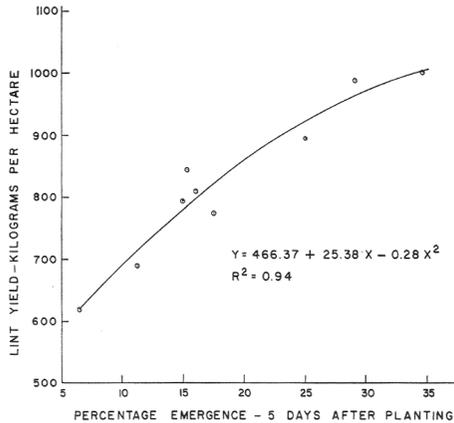


Figure 1. Relation between percentage emergence at 5 days after planting and lint yield for combinations of three seed qualities and three planting depths. [From Wanjura et al., 1969]

SEED AND SEEDLING DEVELOPMENT

Although other authors have provided extensive reviews of seed and seedling development in cotton (Hopper and McDaniel, 1999; Kloth and Turley, 2010; Oosterhuis and Jernstedt, 1999; Turley and Chapman, 2010), the authors feel that the impacts of genotype, management, and environment on seed characteristics and seedling vigor are best understood within the context of ontogeny. For the purposes of this review we will first consider the development of the seed from the initial fertilization event to boll maturity and then from seed germination to the development of photosynthetically self-sufficient, emerged cotyledons.

On the day of anthesis, the cotton flower opens after sunrise following a night of rapid cellular expansion for the floral bud (Beasley, 1975; Stewart, 1986). Subsequently, pollen is transferred to a receptive stigma and germinates; the pollen tube traverses the conducting tissue of the style and carries two sperm nuclei to the ovules; finally, double fertilization occurs (Beasley, 1975; Snider and Oosterhuis, 2011, 2012, 2015; Stewart, 1986). The timing of these events can vary substantially based on environmental factors such as temperature (Snider et al., 2011; Snider and Oosterhuis, 2011; Stewart, 1986), but it is generally accepted that fertilization occurs between 12 and 24 hours after pollination (Stewart, 1986). Beginning roughly on the day of anthe-

sis and ending ~45 to 50 days past anthesis, fiber development occurs in the following phases: fiber cell initiation, elongation, thickening, and maturation (Haigler, 2010; Lee et al., 2007; Oosterhuis and Jernstedt, 1999). After fertilization, seed development occurs in the following phases: morphogenesis, maturation, and desiccation (Turley and Chapman, 2010; Snider and Oosterhuis, 2015). The maturation period is when the embryo is accumulating oil and protein reserves (Turley and Chapman, 2010; Snider and Oosterhuis, 2015) that serve as the primary compounds needed to fuel the earliest stages of seedling growth. While it seems out of place to discuss fiber development within the context of seed development, it is of particular importance that embryo maturation occurs at the same time as fiber thickening, resulting in intra-boll competition between fiber and seed for available photosynthate (Kloth and Turley, 2010).

Just as seed development from zygote formation to embryo desiccation has been extensively characterized, the key developmental events in the germination of the seed are also well-documented and predictable. For example the first stage of seed germination is imbibition, which results in the hydration of embryonic tissues (Cothren, 1999; Christiansen and Rowland, 1986). The time required for the imbibition phase can vary greatly depending upon seed coat characteristics and environmental parameters such as the temperature at which germination occurs, but typically, cotton seeds are fully imbibed within the first 12 h after being placed in a moist environment (Wanjura and Minton, 1974; Cole and Christiansen, 1975; Christiansen and Rowland, 1986). Once embryonic tissues are hydrated, cellular repair and subsequent growth processes resume and are accompanied by an increase in oxygen uptake of the germinating cotton seed (Kuo et al., 1988; Turley and Chapman, 2010). The last stage of seed germination occurs when the radicle visibly protrudes beyond the seed coat. At this point, the radicle grows into deeper layers of the soil profile and the hypocotyl expands to eventually pull the cotyledons above the soil surface, where they will eventually become photosynthetically self-sufficient and begin to fuel additional vegetative growth (Snider and Oosterhuis, 2015). Post-germinative growth that occurs prior to photosynthetic self-sufficiency of the plant is largely driven by mobilized oil and protein reserves that were initially stored in the cotyledons of the quiescent embryo (Bradow and Bauer, 2010). For example, lipid mobilization and gluconeogenesis from lipid precursors provide carbohydrates that can be incorporated into the body of a developing seedling or utilized in respiration.

VARIATION IN SEED VIGOR IN COTTON

While it is not the primary focus of this review to discuss seed vigor, seed vigor can have a pronounced impact on seedling vigor and should at least be considered in this context. As noted elsewhere in this collection of reviews on seed and seedlings, Bourland (2013) defines high vigor seed lots as those that exhibit high germinability and emergence over a range of environmental conditions. Although a number of different tests are available to quantify seed vigor in cotton, seed vigor is commonly assessed by quantifying germination percent after a predefined incubation period at one or two temperatures. Examples include the cool germination test that quantifies percent germination at 18°C following a seven day incubation period and the cool-warm test (sum of germination at 18 and 30°C); these indicators of seed vigor more accurately reflect in-field performance than seed germination experiments conducted at optimal conditions alone (Bourland, in press; Pilon et al., 2016). For example, Bolek (2010) evaluated the germina-

tion response to temperature for 106 cotton cultivars across three different cotton species, and observed significant cultivar differences in seed germination percent under cool temperature conditions (18°C) for both *Gossypium hirsutum* and *G. barbadense*. Importantly, seed vigor assessments at 18°C were correlated with percent emergence in the field, and *G. barbadense* was more cold tolerant than most *G. hirsutum* cultivars. While seed vigor does not necessarily predict seedling vigor under field conditions, the ability of seeds to germinate rapidly under a wide range of conditions could potentially result in earlier emergence under less than optimum temperatures and promote more vigorous early seedling growth. At a minimum, having high emergence rates could potentially contribute to greater early season crop growth rates.

RELATIONSHIPS BETWEEN SEED CHARACTERISTICS & SEEDLING VIGOR

Seedling vigor can be defined in a number of different ways, but most measures of seedling vigor provide an indication of seedling size or growth rates (Bourland, 2013; Liu et al., 2015; Pilon et al., 2016). As noted elsewhere in this review, other measures of seedling vigor such as true leaf differentiation, development of lateral roots, and low incidence of disease may be better indicators of seedling vigor than plant size (Bourland, 2013), but our review will focus on shoot growth parameters since these are the most widely reported indicators of vigor. Chemical and physical characteristics of planting seed can strongly impact seedling vigor and are influenced by genotype, management, and environment (discussed later). Early studies indicated that seed size, density, and degree of seed filling could greatly impact seed and seedling performance, where large, high density seeds exhibited the greatest seed and seedling vigor (Bartee and Krieg, 1974; Ferguson and Turner, 1971; Leffler and Williams 1983; Krieg and Bartee, 1975). Recent work by Snider et al. (2014) characterized seedling vigor (whole-plant fresh weight at the 2-3 leaf stage of development) for 11 different cotton cultivars at 5 locations scattered across much of southern Georgia's cotton production area. When average seedling vigor across all locations for each cultivar was plotted versus seed characteristics such as seed mass, percent oil, and total oil content (mg seed⁻¹), seed mass and seed oil content most strongly and positively impacted seedling vigor (Figure 2). Subsequent work addressed the hypothesis that seed size and total seed oil + protein content (expressed as kcal per seed to account for different energy content in each type of macromolecule) would be strongly predictive of seedling vigor (dry weight per plant) (Snider et al., 2016). In the majority of production environments assessed, oil + protein kcal per seed provided slightly improved relationships over seed mass although the trends were very similar for both seed characteristics (Figures 3 and 4). This is likely because seed mass influences the quantity of reserves available to fuel metabolic processes; a large seed has a larger reserve available to fuel the earliest stages of growth. Another potentially important factor contributing to vigorous seedling growth in larger seeds is that large seeds have the ability to house larger cotyledons (Figure 5), and larger cotyledons should intercept more solar radiation, potentially leading to higher whole-cotyledon photosynthesis. Positive relationships between seed mass and seedling vigor have also been reported in Liu et al. (2015), and in their studies, they observed a strongly positive relationship between seedling dry weight during early growth (17 and 27 DAP) and cotyledon area.

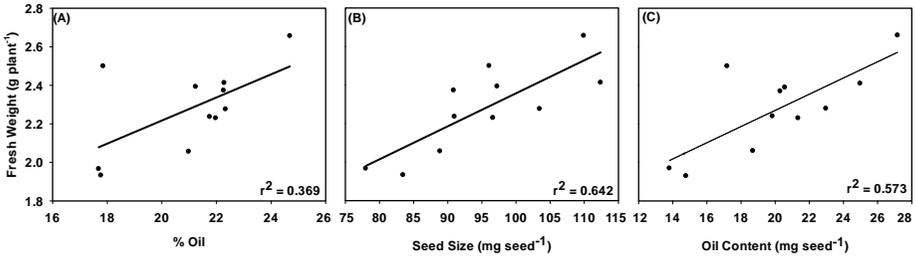


Figure 2. Linear regression of seedling fresh weight at the 2-3 leaf stage versus % oil (A), seed size (B), and total seed oil content (C) for 11 commercially-available cotton cultivars. Fresh weight data were averaged from 5 locations, 4 replicate plots, at each location, and 20 plants per plot (each data point represents the average weight of 400 seedlings). [From Snider et al., 2014]

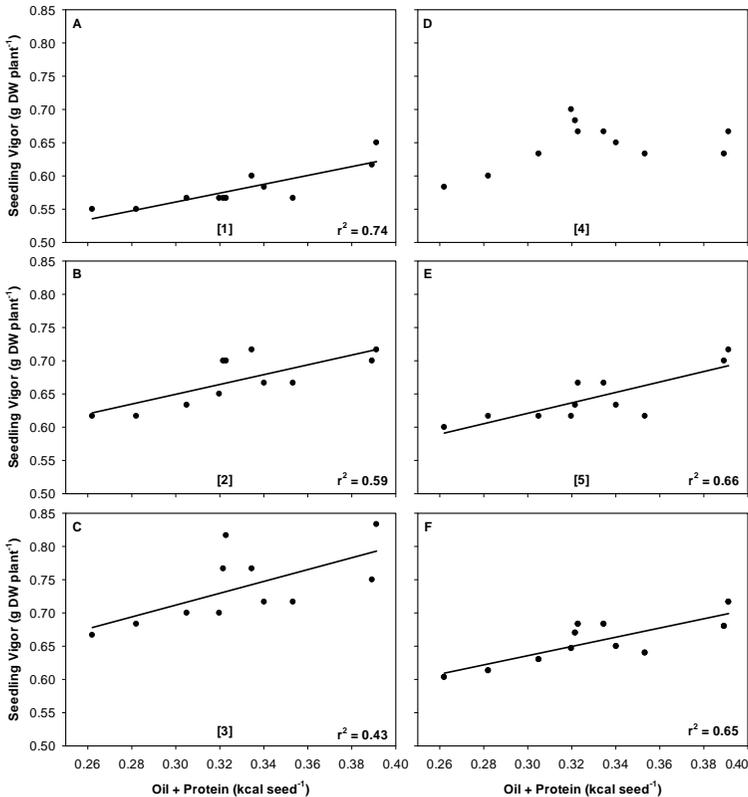


Figure 3. The relationship between planting seed oil + protein content (kcal seed⁻¹) and seedling vigor (dry weight at the 2-3 leaf stage) for 11 different cultivars in each of five different growth environments (A-E; Bracketed numbers represent each growth environment noted in Table 1) or averaged for a given cultivar across all five environments during the 2012 growing season (F). [From Snider et al., 2016]

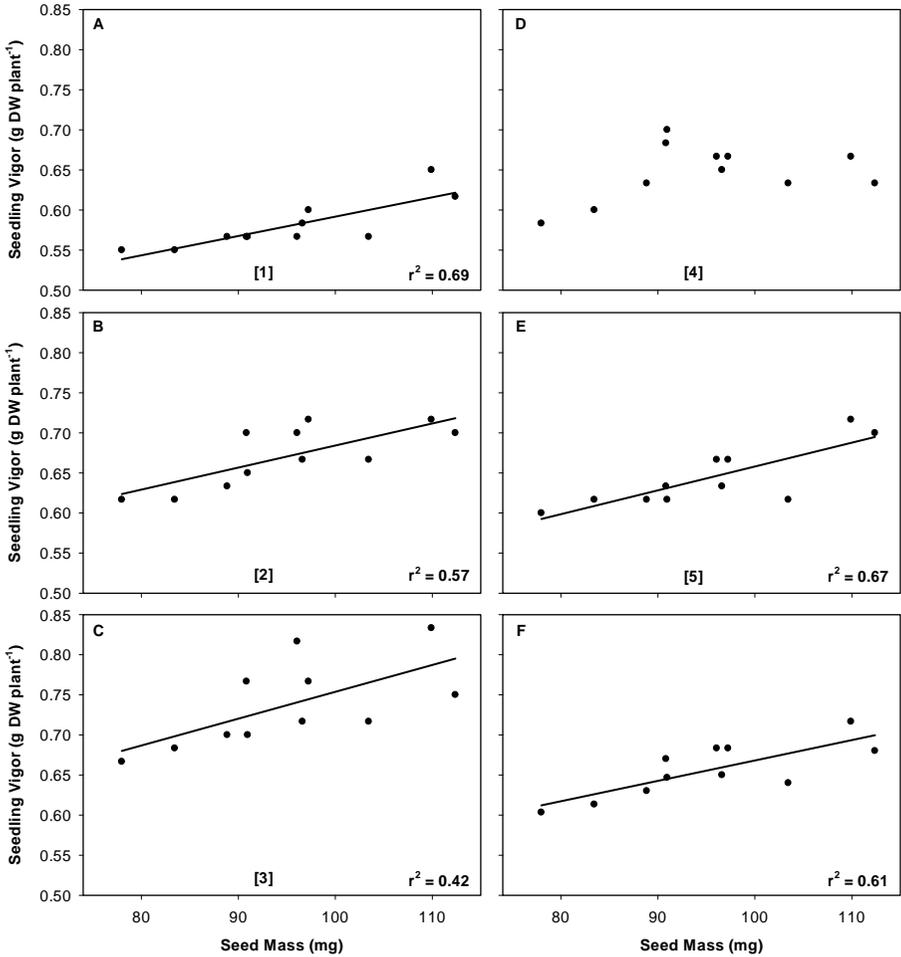


Figure 4. The relationship between planting seed individual seed mass and seedling vigor (seedling dry weight at the 2-3 leaf stage) for 11 different cultivars in each of five different growth environments (A-E; Bracketed numbers represent each growth environment noted in Table 1) or averaged for a given cultivar across all five environments during the 2012 growing season (F). [From Snider et al., 2016]



Figure 5. Visual differences in cotyledon area at approximately two weeks after planting for three cotton cultivars differing in seed mass and planted in Tifton, GA during the 2017 growing season. **1** = lightest seed (Upland, 72 mg seed⁻¹); **2** = intermediate weight seed (Upland, 94 mg seed⁻¹); **3** = heaviest seed (Pima, 138 mg seed⁻¹).

FACTORS AFFECTING SEED CHEMICAL AND PHYSICAL CHARACTERISTICS

As noted above, seedling vigor is influenced by seed mass and composition (oil and protein), so genotypic, cultural, or environmental impacts on these seed properties should have a pronounced impact on seedling vigor. In subsequent sections we provide a general overview of literature addressing the impact of genotype and production environment on seed characteristics.

Cultivar Influence on Seed Characteristics

Cotton breeding programs have dedicated a great deal of effort towards cultivar selection for high yield and fiber quality. Little attention has been given to seed composition and its relevance to vigorous seedling growth and stand establishment. Current commercial cotton cultivars vary in seed composition (USDA, 2015). Some seed characteristics are relatively dependent on genetics, which is the case for crude oil and fatty acid concentrations. For instance, according to research conducted from 1996 through 2013 using data from the Regional High Quality Trial of the National Cotton Variety Testing program, 20 to 57% of oil content variation in cottonseeds were due to genetic diversity, while environment contributed to 44 to 73% of protein content (Zeng et al., 2015). The influence of environment on seed composition is discussed in subsequent sections of this review chapter. A study by Dowd et al., (2010) suggested that approximately two-thirds of the variation in fatty acid composition in seeds is accounted for by genotype. Pettigrew and Dowd (2012) documented genetic

variation in six cultivars for cottonseed composition traits such as for gossypol, oil, protein, carbohydrate, and fatty acid concentrations. A recent assessment of genotypic variation in seed oil and protein from 82 cotton germplasm lines and cultivars indicated that 21% of total variation in oil content is explained by genetics, while only 4% of the variation in protein is due to genetics (Campbell et al., 2016). Although the percentage of total variation in seed oil and protein accounted for by genotype is fairly low, genetic variation is present for these seed traits and can certainly be taken into consideration in cotton breeding programs for improved cottonseed composition. As noted above, seed mass strongly impacts seedling vigor by increasing the total oil (or oil + protein) content available to the growing seedling (Pettigrew and Dowd, 2012; Snider et al., 2014, 2016). Seed mass varies among modern cotton cultivars and breeding efforts over the past several decades have resulted in selection for cultivars with high lint percent and low seed index (g per hundred seed) (Campbell et al., 2011). Thus, seed mass has been strongly influenced by breeding efforts.

Environmental Influence on Seed Characteristics

While seed size and chemical composition can be drastically impacted by genotype, it is important to note that seed production environment and post-harvest storage environment can also influence seed composition. For example, Leffler et al. (1977) demonstrated that cotton seed amino acid profiles varied with sample date, and that total N content in cotton seed increased with later stages of boll development, concomitant with increases in seed protein content (King and Leffler, 1979; Leffler, 1986). Though this finding is intuitive, it illustrates that harvest timing should drastically impact variability in protein composition within a given seed lot. The aforementioned study also illustrated a positive relationship between N fertility and seed N content. A study by Egelkraut et al. (2004) further documented a linear increase in seed N concentration as N fertility levels increased, and defined a critical seed N concentration for attaining maximum relative yield. Because the N content of the cotton seed is largely reflective of seed protein content, other authors have illustrated increases in seed protein as N fertilizer rates increased (Main et al., 2013; Pettigrew and Dowd, 2014). Both the study by Main et al. (2013) and Pettigrew and Dowd (2014) reported a similar result; seed protein content increased in response to increasing N rates, whereas seed oil content declined at the highest N rates. These findings illustrate that N availability can substantially alter the oil and protein balance within the developing cotton seed. Another management factor that has an impact on seed composition is irrigation. For example, Pettigrew and Dowd (2011 and 2014) have found that irrigated cotton exhibits increased seed oil content and decreased seed protein content when compared with dryland cotton. The study conducted by Pettigrew and Dowd (2011) also illustrated a slight decline in the concentration of saturated fatty acids in response to irrigation, relative to dryland cotton. Varying planting dates or irrigation regimes alters cottonseed composition aforementioned study by Pettigrew and Dowd (2011), there was also a significant effect of planting date on seed oil content and a significant interaction between irrigation and planting date on both oil and protein content, depending upon year of the study. While it is likely that planting date

effects were the result of the environment that seeds were exposed to during post-anthesis development, the authors do not identify the key environmental conditions contributing to planting date effects on seed composition. However, early work by Gipson and Joham (1969) illustrated that seed quality could be linked to temperature conditions during seed development. Specifically, they found that seed oil and protein content were lower in seeds that developed under low temperature conditions and that seed germination was positively correlated with growth temperature during seed development. Thus, the environment encountered during seed development can have a pronounced impact on seed composition and viability. Post-harvest seed storage environment can also have a pronounced effect on seed composition and quality. For example, Abdelmagid and Osman (1975) reported that seed oil content declined concomitantly with germination percentage when seeds were stored for a 16 month period. Over shorter storage durations (9 months) declines in seed germination percentage were primarily observed under high-temperature conditions and were associated with decreased seed protein content.

FUTURE DIRECTIONS

- Providing broadly-applicable, quantitative relationships between seed characteristics and seedling vigor has the potential to substantially aid in a producer's cultivar selection decisions. For example, in fields where high seedling vigor is essential, it would be important to know which seed lots have the greatest potential to develop an adequate stand under challenging production conditions. It is also highly likely that planting practices (seeding rate, depth) could be altered to account for seed characteristics. For example, a producer could potentially plant larger seed at deeper depths to access greater soil moisture (compared to small seeded cultivars), which is particularly important in dryland or water-limited production scenarios. However, the balance between protein and oil in the seed will influence the amount of chemical energy available to fuel seedling growth prior emergence. Thus, relatively novel methods that allow for non-destructive determination of seed oil and protein content in whole cotton seed (Horn et al., 2011) could prove useful in this endeavor.
- As noted above, cotton breeding efforts have increased yield by increasing lint percent, which has had the added effect of decreasing individual seed mass. Because smaller seeds typically produce less vigorous seedlings, this could potentially be problematic for producers if the trend toward decreased seed mass continues. It should be noted, however, that modern, commercially available cultivars have been shown to substantially differ in yield component characteristics (e.g. bolls per acre, lint mass per boll, seed number, seed mass), despite having similar per hectare lint yield (Bednarz et al., 2007). Lint yield is the product of seed number per hectare and lint weight per seed. Obviously, there must be an upper limit to the yield improvement that can be attained with increased lint percent while still producing viable seeds that will produce acceptable early season growth. It is the authors' opinion that yield improvement in cotton must eventually be brought about by manipulating other yield components (See Bourland, 2013 for a detailed overview of yield components) if early season risk is to be minimized while simultaneously maximizing economic productivity.

SUMMARY

Rapid and uniform stand establishment along with vigorous seedling growth are desirable characteristics of cotton. "High vigor" seedlings are generally less affected by early season insect herbivory and plant pathogens and are more competitive with weedy plant species, which lessens the potential for early season crop loss. In this review, we emphasize the importance of seed characteristics in determining seedling vigor. Specifically, high planting seed mass and total nutritive reserves (oil and protein) have a positive impact on early seedling vigor. Seed mass and nutrient composition can be influenced by genotype, but it is important to note that production and post-harvest storage environment can have a pronounced impact on these seed characteristics as well. Specifically, practices such as irrigation, fertility, and planting date have all been shown to influence seed oil and protein content as has growth temperature during seed maturation. Long periods of seed storage under high temperature have been closely associated with decreased oil, protein, and seed viability. Because seed quality can be impacted by a number of different factors, we suggest that seed mass and composition could be used as broadly applicable predictors of seedling vigor that integrate a number of variables. This could potentially allow growers to position high vigor seeds in locations where production conditions are challenging during the early season or alter planter settings to account for seed traits based on production needs. Continued breeding for high yielding cultivars has produced cotton genotypes with high lint percent but smaller seeds, which could negatively impact seedling vigor. Future research should be focused on opportunities to increase yield by manipulating yield components other than lint percent.

REFERENCES

- Abdelmagid, A.S., and A.M. Osman. 1975. Influence of storage period and temperature on viability and chemical composition of cotton seeds. *Ann. Bot.* 39:237-248.
- Beasley, C.A. 1975. Developmental morphology of cotton flowers and seed as seen with the scanning electron microscope. *Amer. J. Bot.* 62:584-592.
- Bartee, S.N., and D.R. Krieg. 1974. Cottonseed density: associated physical and chemical properties of 10 cultivars. *Agron. J.* 66:433-435.
- Bednarz, C.W., R.L. Nichols, and S.M. Brown. 2007. Within-boll yield components of high yielding cotton cultivars. *Crop Sci.* 47:2108-2112.
- Bolek, Y. 2010. Genetic variability among cotton genotypes for cold tolerance. *Field Crops Res.* 119:59-67.
- Bourland, F.M. 2013. Novel approaches used in the University of Arkansas cotton breeding program. In *Proc. Beltwide Cotton Conf.*, San Antonio, TX. 7-10 Jan. Natl. Cotton Counc. Am., Memphis, TN. p. 409-418.
- Bradow, J.M., and P.J. Bauer. 2010. Germination and seedling development. In: J.M. Stewart, D.M. Oosterhuis, J.J. Heitholt, and J.R. Mauney, editors, *Physiology of cotton*. Springer, New York, NY p. 48-56.
- Bourland, F.M. 2013. Workable definitions of cotton seed and seedling vigor. In *Proc. Beltwide Cotton Conf.*, San Antonio, TX. 7-10 Jan. Natl. Cotton Counc. Am., Memphis, TN.

- Campbell, B.T., K.D. Chapman, D. Sturtevant, C. Kennedy, P. Horn, P.W. Chee, E. Lubbers, W.R. Meredith Jr. J. Johnson, D. Fraser, and D.C. Jones. 2016. Genetic analysis of cottonseed protein and oil in a diverse cotton germplasm. *Crop Sci.* 56:2457-2464.
- Campbell, B.T., P.W. Chee, E. Lubbers, D.T. Bowman, W.R. Meredith, J. Johnson, and D.E. Fraser. 2011. Genetic improvement of the Pee Dee cotton germplasm collection following seventy years of plant breeding. *Crop Sci.* 51:955-968.
- Christiansen, M.N., and R.A. Rowland. 1986. Germination and stand establishment. In: J.R. Mauney and J.M. Stewart, editors, *Cotton Physiology*. The Cotton Foundation, Memphis, TN. p. 535-542.
- Cook, C.G., and K.M. El-Zik. 1992. Cotton seedling and first-bloom plant characteristics: relationships with drought-influenced boll abscission and lint yield. *Crop Sci.* 32:1464-1467.
- Cothren, J.T. 1999. Physiology of the cotton plant. In: C.W. Smith and J.T. Cothren, editors, *Cotton: origin, history, technology, and production*. John Wiley & Sons, Inc., New York, NY.
- Dowd, M.K., D.L. Boykin, W.R. Meredith Jr., B.T. Campbell, F.M. Bourland, J.R. Gannaway, K.M. Glass, and J. Zhang. 2010. Fatty acid profiles of cottonseed genotypes from the national cotton variety trials. *J. Cotton Sci.* 14:64-73.
- Earl, H.J., and R.F. Davis. 2003. Effect of drought stress on leaf and whole canopy radiation use efficiency and yield of maize. *Agron. J.* 95:688-696.
- Egelkraut, T.M., D.E. Kissel, M.L. Cabrera, G.J. Gascho, and W. Adkins. 2004. Nitrogen concentration in cottonseed as an indicator of N availability. *Nutr. Cycl. Agroecosys.* 68:235-242.
- Elliott, R.H., C. Franke, and G.F.W. Rakow. 2008. Effects of seed size and seed weight on seedling establishment, vigour and tolerance of Argentine canola (*Brassica napus*) to flea beetles, *Phyllotreta* spp. *Can. J. Plant Sci.* 88:207-217.
- Ferguson, D., and J.L. Turner. 1971. Influence of unfilled cotton seed upon emergence and vigor. *Crop Sci.* 11:713-715.
- Gardner, F.P., R.B. Pearce, and R.L. Mitchell. 1985. *Physiology of Crop Plants*. Iowa State University Press, Ames, IA.
- Gipson, J.R., and H.E. Joham. 1969. Influence of night temperature on growth and development of cotton (*Gossypium hirsutum* L.) IV. Seed quality. *Agron J.* 61:365-367.
- Haigler, C.H. 2010. Physiological and anatomical factors determining fiber structure and utility. In: J.M. Stewart, D.M. Oosterhuis, J.J. Heitholt, and J.R. Mauney, editors, *Physiology of cotton*. Springer, New York, NY. p. 33-47.
- Hopper, N., and R. McDaniel. 1999. The cotton seed. In: C.W. Smith and J.S. Cothren, editors, *Cotton: origin, history, technology and production*. John Wiley & Sons, Inc., New York, NY. pp 289-317
- Horn, P.J., P. Neogi, X. Tombokan, S. Ghosh, B.T. Campbell, and K.D. Chapman. 2011. Simultaneous quantification of oil and protein in cottonseed by low-field time-domain nuclear magnetic resonance. *J. Am. Oil Chem. Soc.* 88:1521-1529.

- Kerby, T.A., M. Keeley, and S. Johnson. 1989. Weather and seed quality variables to predict cotton seedling emergence. *Agron. J.* 81:415-419.
- Kloth, R.H. and R.B. Turley. 2010. Physiology of seed and fiber development. In: J.M. Stewart, D.M. Oosterhuis, J.J. Heitholt, and J.R. Mauney, editors, *Physiology of cotton*. Springer, New York, NY. p. 111-122.
- King, E.E., and H.R. Leffler. 1979. Nature and patterns of proteins during cotton seed development. *Plant Physiol.* 63:260-263.
- Krieg, D.R., and S.N. Bartee. 1975. Cottonseed density: associated germination and seedling emergence properties. *Agron. J.* 67:343-347.
- Kuo, T.M., J.F. Van Middlesworth, and W.J. Wolf. 1988. Content of raffinose oligosaccharides and sucrose in various plant seeds. *J. Agric. Food Chem.* 36:32-36.
- Lee, J.J., A.W. Woodward, and Z.J. Chen. 2007. Gene expression changes and early events in cotton fiber development. *Ann. Bot.* 100:1391-1401.
- Leffler, H.R. 1986. Developmental aspects of planting seed quality. p. 465-474. In: J.R. Mauney and J.M. Stewart, editors, *Cotton physiology*. The Cotton Foundation, Memphis, TN.
- Leffler, H.R., C.D. Elmore, and J.D. Hesketh. 1977. Seasonal and fertility-related changes in cottonseed protein quantity and quality. *Crop Sci.* 17:953-956.
- Leffler, H.R., and R.D. Williams. 1983. Seed density classification influences germination and seedling growth of cotton. *Crop Sci.* 23:161-165.
- Liu, S., M. Remley, F.M. Bourland, R.L. Nichols, W.E. Stevens, A. Phillips Jones, and F.B. Fritsch. 2015. Early vigor of advanced breeding lines and modern cotton cultivars. *Crop Sci.* 55:1729-1740.
- Main, C.L., L.T. Barber, R.K. Boman, K. Chapman, D.M. Dodds, et al. 2013. Effects of nitrogen and planting seed size on cotton growth, development, and yield. *Agron. J.* 105:1853-1859.
- Monteith, J.L. 1977. Climate and the efficiency of crop production in Britain. *Phil. Trans. R. Soc. Lond. B.* 281:277-294.
- Monteith, J.L. 1994. Validity of the correlation between intercepted radiation and biomass. *Agr. Forest Meteorol.* 68:213-220.
- Oosterhuis, D.M. and J. Jernstedt. 1999. Morphology and anatomy of the cotton plant. In: W. Smith and J.S. Cothren, editors, *Cotton: origin, history, technology and production*. John Wiley & Sons, Inc, New York, NY. p. 175-206.
- Pettigrew, W.T., and M.K. Dowd. 2011. Varying planting dates or irrigation regimes alters cottonseed composition. *Crop Sci.* 51:2155-2164.
- Pettigrew, W.T., and M.K. Dowd. 2012. Interactions between irrigation regimes and varieties result in altered cottonseed composition. *J. Cotton Sci.* 16:42-52.
- Pettigrew, W.T., and M.K. Dowd. 2014. Nitrogen fertility and irrigation effects on cottonseed composition. *J. Cotton Sci.* 18:410-419.
- Pilon, C., F. Bourland, and D. Bush. 2016. Seeds and Planting. In: J.L. Snider, and D.M. Oosterhuis, editors, *Linking physiology to management*. The Cotton Foundation, Cordova, TN.

- Reddy, K.N., and J.C. Boykin. 2010. Weed control and yield comparisons of twin- and single-row glyphosate-resistant cotton production systems. *Weed Tech.* 24:95-101.
- Snider, J.L., G.D. Collins, J. Whitaker, K.D. Chapman, P. Horn, and T.L. Grey. 2014. Seed size and oil content are key determinants of seedling vigor in *Gossypium hirsutum*. *J. Cotton Sci.* 18:1-9.
- Snider, J.L., G.D. Collins, J. Whitaker, K.D. Chapman, and P. Horn. 2016. The impact of seed size and chemical composition on seedling vigor, yield, and fiber quality of cotton in five production environments. *Field Crops Res.* 193:186-195.
- Snider, J.L., and D.M. Oosterhuis. 2011. How does timing, duration, and severity of heat stress influence pollen-pistil interactions in angiosperms? *Plant Signal. Behav.* 6:930-933.
- Snider, J.L., D.M. Oosterhuis, and E.M. Kawakami. 2011. Diurnal pollen tube growth rate is slowed by high temperature in field-grown *Gossypium hirsutum* pistils. *J. Plant Physiol.* 168: 441-448.
- Snider, J.L., and D.M. Oosterhuis. 2012. Heat stress and pollen –pistil interactions. In: D.M. Oosterhuis and J.T. Cothren, editors, *Flowering and fruiting in cotton*. The Cotton Foundation, Cordova, TN. pp. 59-78.
- Snider, J.L., and D.M. Oosterhuis. 2015. Physiology. In: D. Fang, and R. Percy, editors, *Agronomy Monograph 57, Cotton 2nd Edition*. ASA-CSSA-SSSA, Madison, WI, p. 339-400.
- Stewart, J.M. 1986. Integrated events in flower and fruit. In: J.R. Mauney, and J.M. Stewart, editors, *Cotton physiology*. The Cotton Foundation, Memphis, TN. p. 261-300.
- Stökel, C.O., and A.R. Kemanian. 2009. Crop radiation capture and use efficiency: a framework for crop growth analysis. In: V.O. Sadras and D. F. Calderini, editors, *Crop physiology-applications for genetic improvement and agronomy*. Academic Press, San Diego, CA.
- Turley, R.B. and K.D. Chapman. 2010. Ontogeny of cotton seeds: gametogenesis, embryogenesis, germination, and seedling growth. In: J.M. Stewart, D.M. Oosterhuis, J.J. Heitholt, and J.R. Mauney, editors, *Physiology of cotton*. Springer, New York, NY. p. 332-341.
- USDA. 2015. USDA National Cotton Variety Test. Available at <https://www.ars.usda.gov/ARSUserFiles/60661000/2015NCVTBook.pdf> (Verified 3 Aug. 2017).
- Wanjura, D.F., E.B. Hudspeth Jr., and J.D. Bilbro Jr. 1969. Emergence time, seed quality, and planting depth effects on yield and survival of cotton (*Gossypium hirsutum* L.). *Agron. J.* 61:63-65.
- Wanjura, D.F., and E.B. Minton. 1974. Evolution of cotton seed hydration- chilling treatments for improving seedling emergence. *Agron. J.* 66:217-220.
- Zeng, L., B.T. Campbell, E. Bechere, J.K. Dever, J. Zhang, et al. 2015. Genotypic and environmental effects on cottonseed oil, nitrogen, and gossypol contents in 18 years of regional high quality tests. *Euphytica.* 206:815-824.

Chapter 3

COTTON SEEDLING ROOTS

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INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is classified as a dicotyledonous plant which means that upon germination the organs to first penetrate the soil are the two cotyledons. However, the very first organ to penetrate the seed coat is the radicle or the primary root which is characteristic of all dicot plants. The primary root is generally referred to as the “tap” root and can elongate at the rate of 2 to 3 cm per day depending on the soil conditions of texture and moisture content (Oosterhuis, 1990).

Lateral roots develop from the taproot beginning prior to ten days after germination and continuing on through the life of the plant until harvest (Figure 1). Tertiary roots can also develop from the existing lateral roots to form a mass of roots for each plant that can extend to 50-60 in depth again depending on the soil conditions (Oosterhuis, 1990).

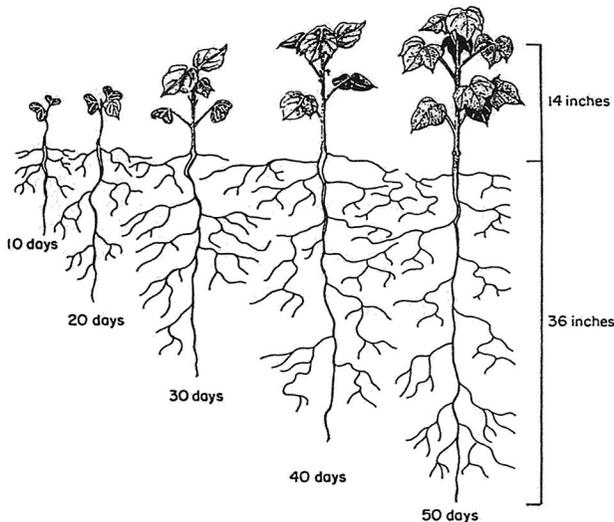


Figure 1. Root Development of the cotton plant. From: Oosterhuis 1990. Arkansas Cooperative Extension Service. Bulletin MP332.

The taproot has been described anatomically as having four to five distinct vascular “bundles” containing both xylem and phloem elements (Figure 2) (McMichael et al., 1985). The xylem elements are the organs in which water and nutrients are taken up by the developing seedling and transported to the entire plant. Also the total number of vascular bundles in the developing

primary or taproot is critical in that the number of lateral roots developed along the taproot must have the connection between the lateral root primordia and the vascular or xylem elements in order for water and nutrients to be transported from the lateral roots to the taproot and finally to other parts of the seedling. The same organization applies to the connection between the lateral roots and the developing tertiary roots in the seedling.

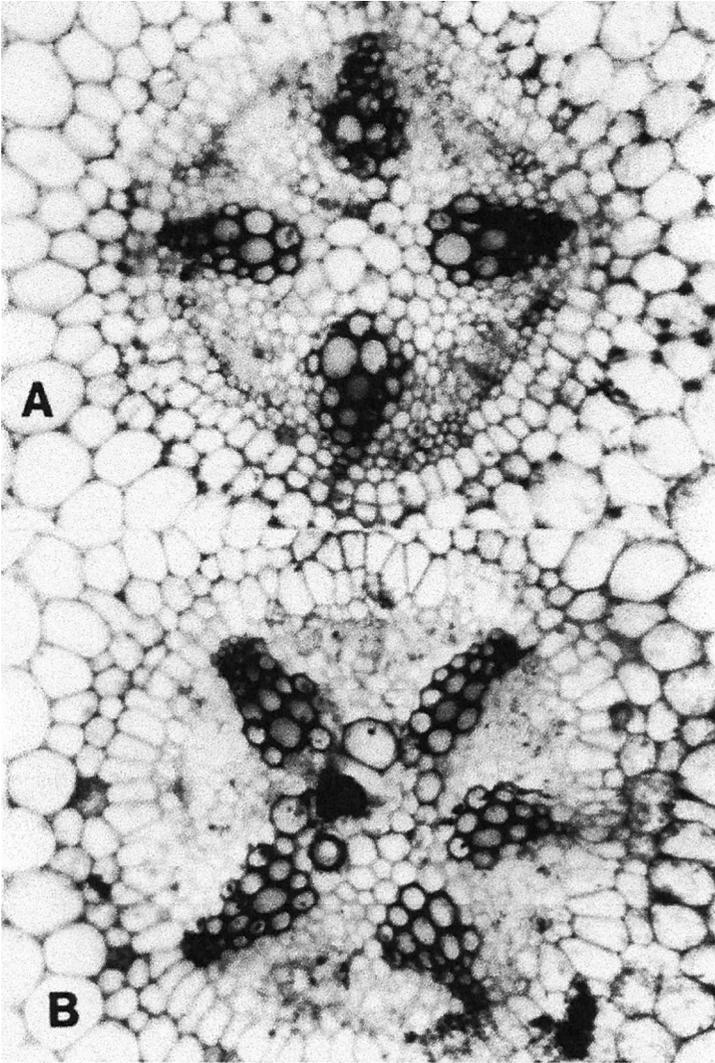


Figure 2. Xylem pattern in primary roots of *G. hirsutum* cv. DPL-16 (A) and *G. hirsutum* cv. T25 (B). The tetraarch arrangement (A) is typical of cultivated cottons. The pentarch arrangement (B) has been found in drought tolerant types (X100). (From McMichael et al., 1983).

The seedling, which for this discussion I would consider to be 10-15 days after germination, develops a root system that is approximately not more than 15-20 g in dry weight. However, if the total length of the seedling root system is considered, these few grams equate to something on the order of several meters in length. Therefore, if the specific root length or length per unit weight is calculated, this number represents the changes in the diameter of the seedling root as development occurs.

PRODUCTION OF PLANT HORMONES

Certain plant hormones are produced in the roots and subsequently transported to the remaining parts of the developing seedling. Specifically, it has been shown that cytokinins are produced in the young root system and used in stems and leaves as the plant develops (MacAdam, 2009). Some gibberellic acid hormones may also be synthesized in the roots (MacAdam, 2009). Other plant hormones may influence root growth but are not specifically produced in the roots.

TEMPERATURE

The impact of temperature on seedling development has been covered extensively by other authors (McMichael et al., 2010; Taylor, 1983). However, the impact of these abiotic factors on seedling root development bears additional discussion. It has been shown that changes in soil temperature can have a significant effect on seedling root growth in cotton. In general, the optimum temperature for seedling root growth and development is 23-25°C (McMichael and Burke, 1994) (Table 1). If the temperature is reduced to 20°C, for example, the growth of the taproot is reduced and more importantly the initiation of lateral roots does occur but over a much longer period of time. Also, if the temperature is increased significantly from the optimum, (up to 40°C for example) the overall seedling root growth is reduced or stopped altogether (Figure 3) (McMichael, 1986).

Table 1. Development of 10-day-old cotton seedlings grown at four temperature regimes. Adapted from McMichael and Burke (1994).

| Treatment | | Root parameters | | | |
|-------------------|------------------|---------------------|-----------------------|-----------------------|-----------------|
| Shoot temperature | Root temperature | Tap root length, cm | Later root length, cm | Total root length, cm | No. of laterals |
| 28 | 20 | 3.08 ± 0.24* | 0.0a | 3.08 ± 0.24 | 0.0a |
| 20 | 28 | 5.06 ± 0.28b | 1.83 ± 0.35b | 6.89 ± 0.54b | 8.00 ± 0.83b |
| 28 | 28 | 7.71 ± 0.59c | 5.82 ± 1.15c | 13.53 ± 1.07c | 8.38 ± 0.90c |
| 20 | 20 | 4.71 ± 0.35ab | 0.0a | 4.71 ± 0.35a | 0.0a |

* Means followed by the same letter are not significantly different at the 0.05 probability level based on Duncan's Multiple Range Test.

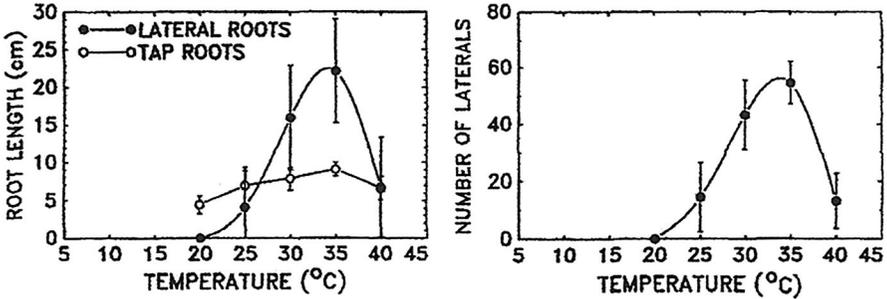


Figure 3. Root development of cotton seedlings as influenced by temperature. Vertical bars about each mean = 95% confidence limits. (From McMichael, 2010).

IMPACT OF STORED RESERVES

McMichael and Burke (1994) have shown that as stored reserves are depleted to supply nutrients to the entire seedling, the sensitivity to temperature changes of the organs including roots is increased therefore reducing the optimum temperature for maximum development.

SOIL COMPACTION

The degree of soil compaction can have a significant influence on the growth and development of seedling roots (Taylor, 1983). It has been shown that as soil compaction, specifically in clay soils, increases the growth of the roots decreases and the roots become thicker as they attempt to increase length (Glinski, 1990).

MYCORRHIZAE

The root systems of cotton plants become mycorrhizal or form a symbiotic association with the roots and the fungi depending on the initial levels of the fungi and other soil conditions (Nehl and McGee, 2010). The most common type of fungi to colonize cotton roots are termed arbuscular mycorrhizae (AM). The hyphae development by the fungi penetrates the cotton roots and moves internally across the cortex of the root. This symbiotic relationship thus provides the host plant (cotton) with additional phosphorus that might not otherwise be available to the plant, while the plant provides carbohydrates to the mycorrhizal fungi (Nehl and McGee, 2010). In general, therefore cotton plants whose root systems are devoid of AM fungi have been shown to have significantly reduced growth and development compared to plants that have formed mycorrhizae (Nehl and McGee, 2010).

SUMMARY

In summary, the growth of the root system of a cotton seedling is very dynamic with conditions that ultimately impact the outcome either act alone or in combination with other factors to produce a result. This brief account covers only a few of the abiotic as well as biotic factors

that impact cotton seedling root growth in general. A more comprehensive account of the growth of cotton roots at this stage may be found in journal articles aimed specifically at the subject as well as books also written specifically describing cotton development (Pearson et al., 1970; Taylor, 1983).

REFERENCES

- Glinski, J. 1990. Soil physical conditions and plant roots. CRC press, Inc. Boca Raton, FL.
- MacAdam, J.W. 2009. In: Structure and Function of Plants. Wiley-Blackwell Press, Ames, IA. pp. 215-234.
- McMichael, B.L., J.J. Burke, J.D. Berlin, J.L. Hatfield, and J.E. Quisenberry. 1985. Root vascular bundle arrangement among cotton strains and cultivars. *Environ. Exp. Bot.* 25:23-30.
- McMichael, B.L., and J.J. Burke. 1994. Metabolic activity of cotton roots in response to temperature. *Environ. Exp. Bot.* 34:201-206.
- McMichael, B.L. 1986. Growth of Roots. In: J.R. Mauney and J.M. Stewart, editors, Cotton physiology. The Cotton Foundation, Memphis, TN. pp 29-38.
- McMichael, B.L., D.M. Oosterhuis, J.C. Zak, and C.A. Beyrouty. 2010. Growth and development of root systems. In: J.M. Stewart, D.M. Oosterhuis, J.J. Heitholt, and J.R. Mauney, editors, Physiology of cotton. Springer, New York, NY. pp. 57-71.
- Nehl, D.B., and D.A. McGee. 2010. Ecophysiology of arbuscular mycorrhizas in cotton. In: J.M. Stewart, D.M. Oosterhuis, J.J. Heitholt, and J.R. Mauney, editors, Physiology of cotton. Springer, New York, NY. pp. 206-212.
- Oosterhuis, D.M. 1990. Growth and development of a cotton plant. Arkansas Cooperative Extension Service. Bulletin MP332-4m-9-92R.
- Pearson, R.W., L.F. Ratliff, and H.M. Taylor. 1970. Effect of soil temperature, strength and pH on cotton seedling root elongation. *Agron. J.* 62:243-246.
- Taylor, H.M. 1983. Managing root systems for efficient water use: an overview. In: W.R. Jordan and T.R. Sinclair, editors, Limitations to efficient water use in crop production. Am. Society of Agronomy, Madison, WI. pp. 87-113.

Chapter 4

QUANTITATIVE AND SPATIAL ANALYSIS OF LIPID METABOLITES IN SEEDS OF DIVERSE *GOSSYPIUM* GENOTYPES

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Abbreviations- MALDI: matrix assisted laser desorption/ionization, MS: mass spectrometry, ESI: electron sprayionization, TD-NMR: time domain ¹H nuclear magnetic resonance, PC: phosphatidylcholine, TAG: triacylglycerol, TLE: total lipid extract, P: palmitic (16:0), O: oleic (18:1), L: linoleic (18:2) numerical designation of lipids indicates number of carbons in acyl chains: number of double bonds.

INTRODUCTION

Although cotton is farmed for its production of spinnable fibers, the residual seed after ginning is a plentiful source of vegetable oil. In fact, the yield of seed on a per acre basis is about 1.6 times that of the harvested fiber, and in 2012 this amounted to 5.37 million tons of cottonseed produced in the United States (USDA-Oil Crops Outlook). Currently, less than half of the seed produced in the United States is crushed and processed into refined vegetable oil, and with world demand for vegetable oils on the rise, this may represent a place for farmers to recognize additional value from their overall crop. Oilseeds and their refined vegetable oils vary in price based on their compositional formulations and end-use markets. Due to its oxidative stability and flavor enhancing properties, cottonseed oil enjoys a reputation as an excellent frying oil, but with changes to its fatty acid composition, cottonseed oil might enter other markets (Lui *et al.*, 2009; 2012). As with all natural products, the compositions of extracted products may vary from season to season, with environmental and genetic factors contributing to both desirable and undesirable components. A more complete understanding of the many components in refined cottonseed oils and the factors which influence their formation within the embryo may help to develop new varieties with consistent and highly desirable vegetable oil compositions. Detailed chemical analysis of seed oils (including minor components) within the context of different genotypes or environmental conditions could help to provide breeders with rich resources to enhance the overall value of the cotton crop. Moreover, the detailed analysis of lipid metabolites within embryos may offer insights into pathways and postharvest processes that influence seed viability and seedling vigor.

ANALYSIS OF COTTONSEED CONSTITUENTS

Oil and protein reserves in cotton seeds have been traditionally quantified using destructive and time-intensive chemical extractions (AOCS, 2009a; 2009b; 2009c). Advances in non-invasive methodologies, including time domain ^1H - nuclear magnetic resonance (TD- ^1H NMR), have made it possible to quantify oil and protein content in seed samples without destroying viability (Horn *et al.*, 2011a). Cottonseed from current commercial cultivars typically averages about 20% oil and 25% protein by weight (Jones and King, 1996). A recent survey of oil and protein content within the genetically diverse U.S. National Cotton Germplasm Collection (Horn, Hinze, Percy and Chapman, unpublished observations) suggested that there was considerable variation in seed reserve composition within the *Gossypium* background. Here, eight accessions were identified with extreme levels of oil and/or protein reserves (Table 1) and were selected for further detailed lipid analysis. For example, oil content ranged from 8.2% by weight in *G. stocksii* to 25.5% by weight in *G. barbadense* (cv. Pima-S6), whereas protein content ranged from 11.4% in *G. thurberi* to over 30% by weight in a couple of *G. hirsutum* accessions (Table 1). Seed sizes ranged dramatically as well in these different accessions (Figure 1), and generally the larger seeds had a larger percentage of oil, but this positive trend was not observed for protein (i.e., there were large seeds with low protein like Pima-S6 and small seeds with higher protein like *G. stocksii* accession E01-3). Cultivated varieties examined included *G. barbadense*, cv Pima- S6, *G. hirsutum*, accession SA-1254 and *G. hirsutum* cv Coker 312 while others mostly were wild accessions (Table 1).

Table 1. Comparison of seed size, % oil (by weight), and % protein (by weight) for selected *Gossypium* accessions representing a broad range of oil and protein content across diverse genomes in the U.S. National Cotton Germplasm Collection. Oil and protein content was determined by TD-NMR in triplicate batches of seeds at approximately 1g each. Values are means and standard deviations.

| Sample ID | Species | Genome | Status/Origin | Seed Size (mg/seed) | Oil (%) | Protein (%) |
|-----------|----------------------|--------|-----------------------------------|---------------------|------------|-------------|
| B01-1 | <i>G. anomalum</i> | B1 | Wild/Africa | 24.4 | 12.8 ± 0.3 | 20.2 ± 0.3 |
| D01-10 | <i>G. thurberi</i> | D1 | Wild/North America | 21.0 | 16.6 ± 0.3 | 11.4 ± 0.3 |
| E01-3 | <i>G. stocksii</i> | E1 | Wild/Arabia | 24.1 | 8.2 ± 0.3 | 23.8 ± 0.3 |
| PIMA-S6 | <i>G. barbadense</i> | (AD)2 | Cultivated/South America | 119.2 | 25.5 ± 0.3 | 16.3 ± 0.4 |
| SA-1254 | <i>G. hirsutum</i> | (AD)1 | Cultivated/Central, North America | 98.7 | 20.0 ± 0.4 | 37.4 ± 2.1 |
| TX-2236 | <i>G. hirsutum</i> | (AD)1 | Wild/Central, North America | 59.5 | 14.4 ± 0.1 | 31.0 ± 0.4 |
| TX-2500 | <i>G. hirsutum</i> | (AD)1 | Wild/Central, North America | 90.2 | 24.8 ± 0.1 | 23.0 ± 0.5 |
| Coker 312 | <i>G. hirsutum</i> | (AD)1 | Cultivated/Central, North America | 83.9 | 20.6 ± 0.3 | 24.0 ± 1.5 |

While TD-NMR provides accurate quantitative information on total seed lipid content, it does not provide detailed lipid composition information. Instead, direct-infusion, “shotgun” lipidomics analysis of total lipid extracts (TLE) of seeds by electrospray ionization mass spectrometry (ESI-MS) has made it possible to quantify individual lipid molecular species in cottonseeds in a relatively high throughput manner (Horn *et al.*, 2011b; 2012). Generally this “shotgun” lipidomics methodology complements well other analytical approaches historically used for the analysis of cottonseed lipids, such as gas chromatography (GC) (Metcalf *et al.*, 1966; Bland *et al.*, 1991; Yunusova *et al.*, 1991; Dowd *et al.*, 2010) and high performance liquid chromatography (HPLC) (Bland *et al.*, 1991; Lisa and Holcapek, 2008) which often can be coupled to detection by mass spectrometry (e.g., GC-MS and LC-MS). A major advantage of the direct-infusion approach compared to the chromatographic approaches is a reduced time of sample handling/analysis without compromising the detailed, comprehensive and sensitive quantitative information for lipid composition (Horn and Chapman, 2012).

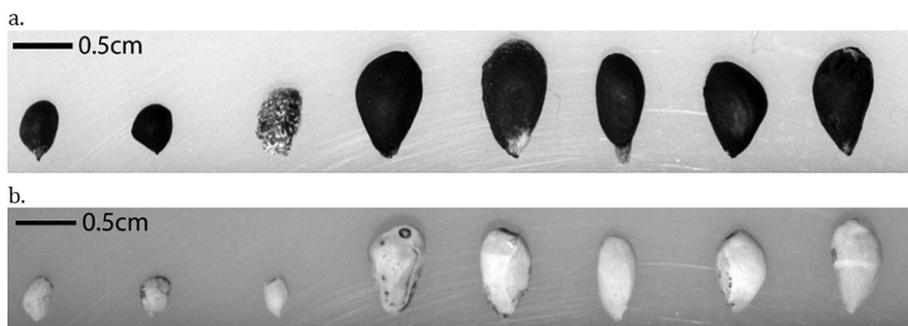


Figure 1. Representative mature seeds from genetically diverse *Gossypium* species with (a) and without seed coat (b). Sample IDs from left to right: B01-1, D01-10, E01-3, PIMA-S6, SA-1254, TX-2236, TX-2500 and Coker-312. See Table 1 for species, origins, and characteristics.

Cottonseed oil is mostly comprised of triacylglycerols (TAGs, ~98 % of the total lipid), and the molecular species profiles (differing in acyl chain composition) together make up the overall fatty acid composition of the oil. The major fatty acid species represented in cottonseed TAGs are palmitic (16:0), oleic (18:1), and linoleic (18:2) acids. TAGs from seeds of these selected accessions were quantified individually by ESI-MS, and there were clear differences in the fatty acid composition in TAGs among the seven genotypes examined (Figure 2A). One obvious difference was in the mol% of TAG 52:4, which contains acyl chains of 16:0/18:2/18:2. This molecular species made up a larger percentage of the total TAGs in several diploid exotic species and less in several (but not all) of the tetraploid genotypes. However, since few accessions were profiled here, it is also possible that similar variation would be seen across all genotypes. In any case, such marked variation in TAG composition suggests considerable genetic diversity in *Gossypium* to realize breeder-directed changes in seed oil composition.

Like in other oilseeds, TAGs are synthesized in cotton embryos from the metabolic precursor, phosphatidylcholines (PCs), by a relatively complex, and incompletely understood set of reactions (Chapman and Ohlrogge, 2012). The acyl chains that are assembled into TAGs are first incorporated and modified on PCs before being transferred to the glycerol backbone of TAGs by either acyl CoA-dependent or acyl CoA-independent pathways. Regardless of the route of entry, analysis of the acyl chains on PCs reflects a major precursor pool of metabolites for TAG synthesis, especially when analyzed in the same samples as for TAGs. As might be expected, the PC molecular species profiles were different among these diverse genotypes in a manner consistent with their corresponding TAGs (Figure 2B). For example, the accession E01-3, *G. stocksii*, was notably high in TAG 54:6, which is a TAG species with 18:2 at each position. Similarly, E01-3 was the genotype with the highest proportion of PC 36:4, which is a PC with 18:2 at each position, consistent with a precursor-product relationship between these PC and TAG molecular species.

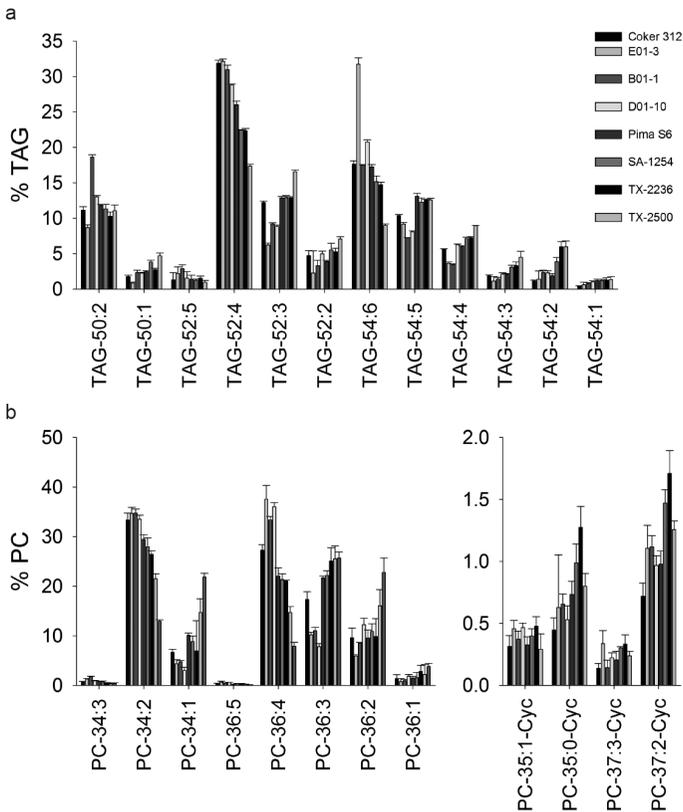


Figure 2. Direct infusion, “shotgun” lipidomics analysis of total lipid extracts (TLE) from seeds of diverse *Gossypium* species (see Table 1). Relative quantification of major TAG (a) and PC (b) molecular species are presented on a mol% basis within the class and were measured in triplicate with 5 seeds each in each replicate (bars represent standard deviation). Tandem MS scanning mode confirmed acyl chain composition (not shown here).

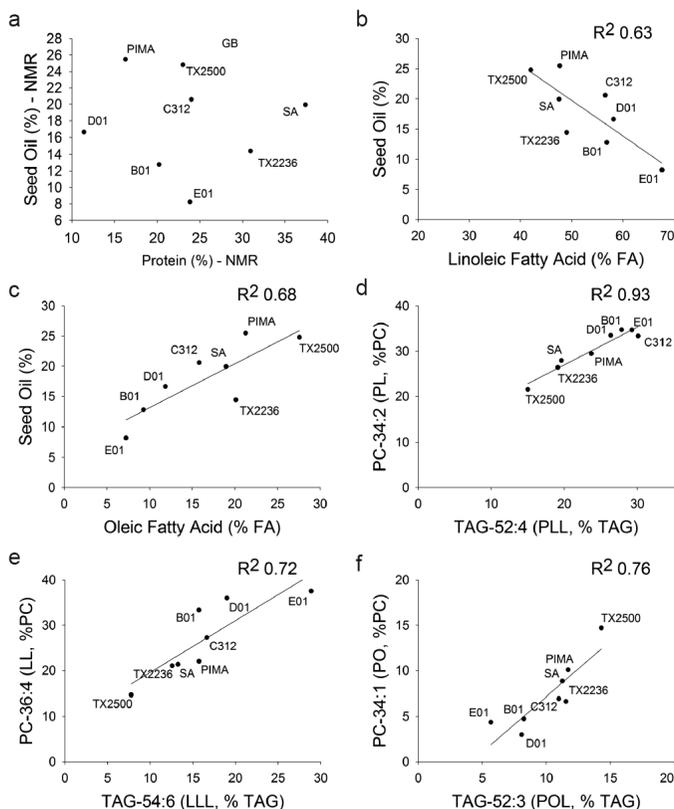


Figure 3. Lipids in seeds of diverse *Gossypium* species (see Table 1 for list). (a) Comparison of the seed oil and protein % by TD-NMR demonstrating variability in extremes chosen for further analysis. (b) Relationship of seed oil content and linoleic acid %. (c) Relationship of seed oil content and oleic acid %. (d) Relationship of mol% PC 34:2 (PL) and mol% TAG-52:4 (PLL). (e) Relationship of mol% PC-36:4 (LL) and mol% TAG-54:6 (LLL). (f) Relationship of mol% PC-34:1 (PO) and mol% TAG-52:3 (POL). (b,c) suggest association of seed oil content and fatty acid composition, while (d,e,f) support metabolic precursor-product relationships between PC species and TAG species.

The several genotypes analyzed here actually could be divided into “high linoleic (18:2)” (like E01-3) or “high oleic (18:1)” (like TX-2500) phenotypes (Figure 3), and these differences in overall fatty acid composition were reflected in the molecular species profiles of both PC and TAG (Figure 3). For example there was a strong relationship between PC 34:2 content and TAG 54:4 content; similarly, there was a strong relationship between PC34:1 and TAG 52:3—and these were associated with high linoleic and high oleic phenotypes respectively, even in the few selected species and genotypes examined here. These differences in lipid compositions suggest that there may be much wider variation than realized across diverse *Gossypium* genotypes in monounsaturated fatty acid content, and that *FAD2* gene expression/activity may represent a

good target for molecular marker-assisted breeding. Since high-oleic seed oils have meant price premiums or increased market share for other oilseed crops, like sunflower, safflower, soybean, and corn (Dyer *et al.*, 2008), this variation in *Gossypium* may represent a potentially valuable association for cotton breeders to examine more closely. Additional incentive may come from the positive relationship that was noted between oleic acid percentage and overall seed oil content. In other words, among these diverse genotypes with wide-ranging oil contents, there was a tendency for seeds that had the highest oil content to have the highest oleic acid content. By contrast, highest linoleic acid percentage was noted for those with lowest oil content, suggesting that those with lowest FAD2 activity in embryos tended to accumulate more seed oil. Certainly, this is not a simple relationship, and there are far too few accessions compared here to draw definitive conclusions, but it is an interesting relationship that may warrant further attention. It is worthwhile to note that the desaturation reaction by the FAD2 enzyme requires reducing equivalents and molecular oxygen, both of which if not required might prove for a more efficient overall accumulation of seed lipids.

IMAGING LIPID METABOLITES IN SITU IN COTTONSEEDS

Shotgun lipidomics is indeed a valuable tool for identifying and quantifying individual molecular species of lipid classes in cottonseeds; however, the location of these lipids within the embryos is lost during solvent-based extraction of seed tissues. Application of matrix-assisted laser desorption/ionization (MALDI)-MS imaging has recently been developed as a new technique to combine the high-resolution chemical information of mass spectrometry with the localization of these metabolites in tissue sections ((Horn *et al.*, 2012); see also Figure 4). Such studies have revealed surprising heterogeneity within cottonseed tissues (Horn *et al.*, 2012; 2013)), and have suggested that this heterogeneity results from differences in the metabolic pathways that are involved in the assembly of PC and the production of TAGs at these different locations. Understanding the basis for this heterogeneity will provide important insights into the pathways and enzymes that are responsible for seed oil content and composition.

Based on the compositional diversity in seeds from these diverse genotypes, we suspected that there might be differences in the tissue localization of TAG and PC metabolites among some of these embryos. Indeed, variation in compartmentalization of these lipid metabolites was evident in the four genotypes examined (Figures 5, 6, 7, 8). These results suggest marked differences in the organization of seed lipid metabolism in these species.

The spatial distribution of gossypol and six different molecular species of PC are shown for embryos of four different *Gossypium* species in Figure 5. Images are reconstructed on a gray scale (black is highest concentration; maximum units are listed on the scale) on an absolute ion basis (for gossypol; Figure 5b) or on a mol fraction basis of the total lipid class (for PC species; Figures 5c-g). Two-dimensional images are constructed using software, Metabolite Imager, written for the conversion of MALDI-MS data to spatial images (Horn and Chapman, 2013). These chemical maps are oriented with bright-field images of tissue sections (Figure 5a), to provide the tissue-based context of metabolite distributions. For validation and by way of example of the

methodology, the spatial distribution of gossypol metabolites is associated with pigmented glands throughout the embryos of these *Gossypium* species, although *G. stocksii* with few glands had little gossypol (Figures 5a,b). Heterogeneity in phospholipid composition was obvious immediately. Some PC species were distributed throughout the embryo uniformly, whereas others varied on a mol% basis between cotyledonary and embryonic axis tissues. Further, these patterns were variable across genotypes. For example, PC-34:1 (palmitic and oleic acids at the sn-1 and -2 positions) was more prevalent in outer cotyledonary tissues of *G. hirsutum* accession SA-1254, similar in pattern (but at a higher amount) compared to *G. anomalum* accession B01-1. This was different from the *G. barbadense* cv Pima-S6, or the *G. stocksii*, which showed a more uniform distribution of this PC species throughout the cotyledons and embryonic axis. PC 34:2 was more prevalent in the embryonic axis tissues relative to cotyledons, but the Pima-S6 embryos showed the reverse distribution (Figure 5d). Further, PC 36:3 showed opposing distributions (cotyledons vs axis) in *G. anomalum* and *G. stocksii* embryos (Figure 5f). Together these differences in PC distribution suggest differences in pathway enzymes leading to the assembly of these phospholipids, and because of the precursor-product relationship between PC and TAG, these patterns are predicted to be reflected in tissue-based heterogeneity of seed oil compositions.

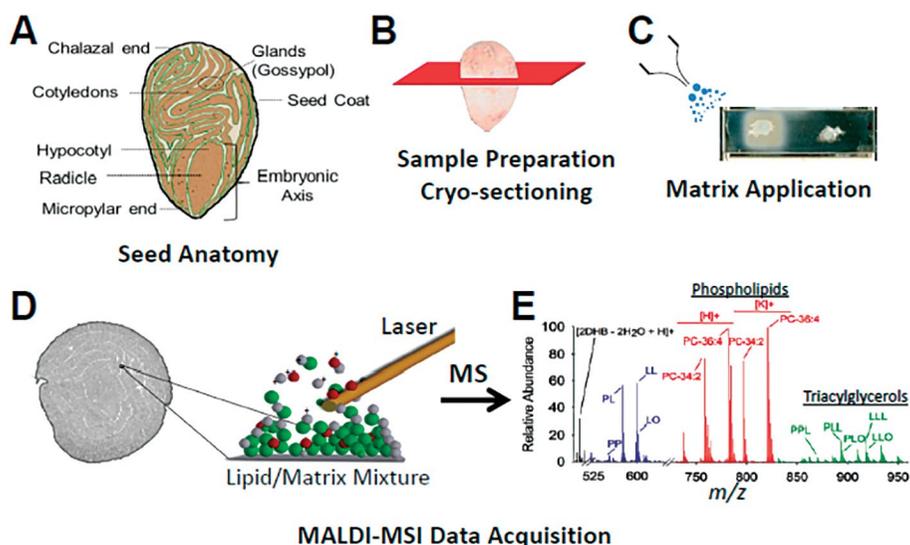


Figure 4. Schematic overview of MALDI-MS imaging process. (A) Longitudinal diagram of mature cotton embryo primarily composed of folded cotyledon tissues surrounding the embryonic axis (hypocotyl and radicle). (B) Embryos are sliced into thin cross- or longitudinal-sections with a cryostat. Sections of approximately 30 microns are coated with matrix (C) to promote the formation of ions. (D) A laser is rastered across the section at ~50 microns intervals. Lipid ions as $[M + H]^+$, $[M + Na]^+$, or $[M + K]^+$ adducts are directed into a mass spectrometer (Thermo LTQ Orbitrap-XL). (E) Each detection cycle generates a raw data spectrum for lipid species at x, y position of the seed section. Images are reconstructed using Metabolite Imager (www.metaboliteimager.com). Legend and image modified from Horn et al. *Plant Cell* 2012 24: 622-636 with permission.

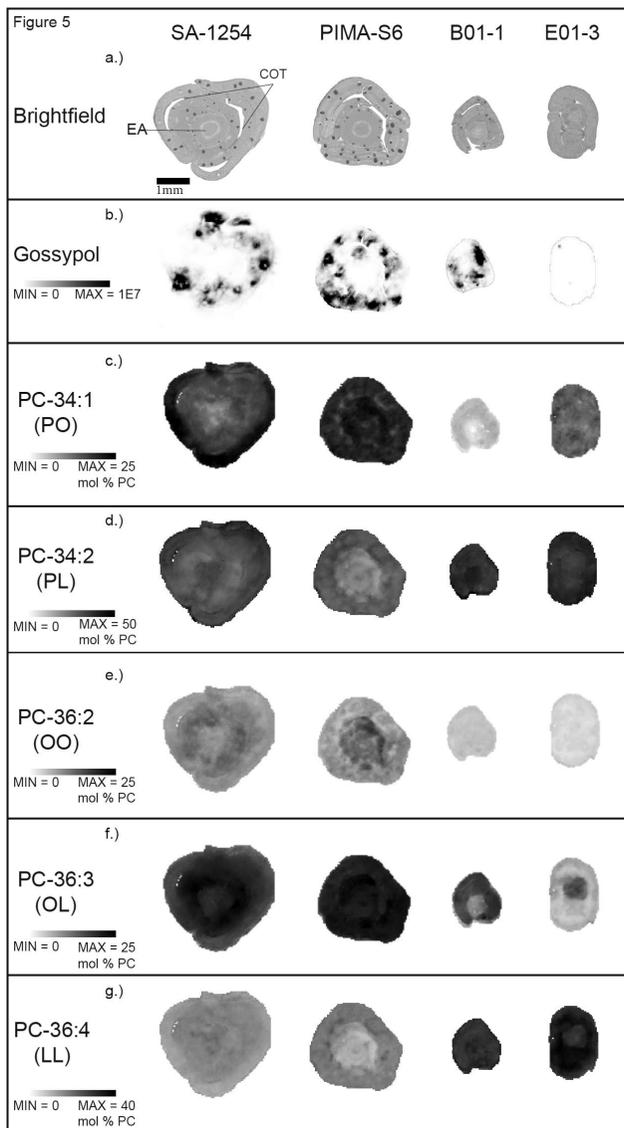


Figure 5. Images of lipid metabolites in cross-sections of embryos (middle) from *G. hirsutum* (SA1254), *G. barbadense* (Pima S6), *G. anomalum* (B01-1), *G. stocksii* (E01-1). Ion maps are generated from MALDI-MS for gossypol (b) and phosphatidylcholine (PC) molecular species (c-g), and compared to bright-field micrographs (a) for orientation. PC molecular species are denoted as total number of acyl carbons and number of total double bonds. (P is palmitic (16:0), O is oleic (18:1) and L is linoleic (18:2)). Gray scale images are converted from ion counts (b) or mol% of class (c-g) with black as highest.

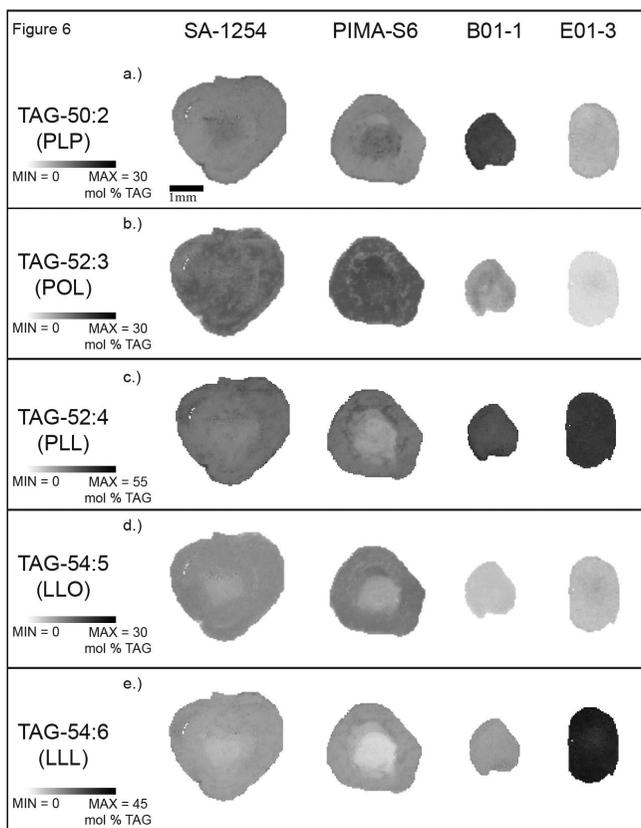


Figure 6. Images of lipid metabolites in cross-sections of embryos (middle) from *G. hirsutum* (SA-1254), *G. barbadense* (Pima-S6), *G. anomalum* (B01-1), *G. stocksii* (E01-1). Ion maps are generated from MALDI-MS for triacylglycerol (TAG) molecular species. Compare to bright-field micrographs (Figure 5a) for orientation. TAG molecular species are denoted as total number of acyl carbons and number of total double bonds. (P is palmitic (16:0), O is oleic (18:1) and L is linoleic (18:2)). Gray scale images are converted from mol% of class with black as highest relative amount.

The distribution of the major triacylglycerol species in embryos is shown in Figure 6 for four *Gossypium* species. Most obvious were the differences in the content of each of these TAGs in the different *Gossypium* backgrounds (e.g., the mol fraction of TAG-50:2, or PLP, was much greater relative to total TAG in B01-1 embryos than in E01-3 embryos (Figure 6a). Although there were some heterogeneous distributions of TAGs evident within embryo tissues (like PLP, PLL, LLO in Pima S6; Figure 6), the large variation in amounts of each of the TAGs in the different *Gossypium* embryos masked somewhat the tissue-based heterogeneity within the species (Figure 6).

Differences in spatial distributions of TAG metabolites were considerable when comparing within a single *G. hirsutum* genotype (Horn *et al.*, 2012; 2013), and this was also the case for these embryos when they are analyzed individually (Figure 7). Plots of TAG distributions in transects from left to right over embryo cross-sections showed that some TAGs were more concentrated in cotyledonary tissues than in the embryonic axis tissues (and some vice versa), and this was especially evident in *G. hirsutum* (SA-1254) and *G. barbadense* (Pima-S6). For example, TAG-52:4 (PLL) and TAG54:6 (LLL), were present at higher mol fractions of the total TAGs in cotyledons compared to the axis (Figure 7). These differences in distribution were not obvious for the smaller seed species, *G. anomalum* (B01-1) or *G. stocksii* (E01-3), and may reflect differences in TAG metabolic pathways among these diverse species.

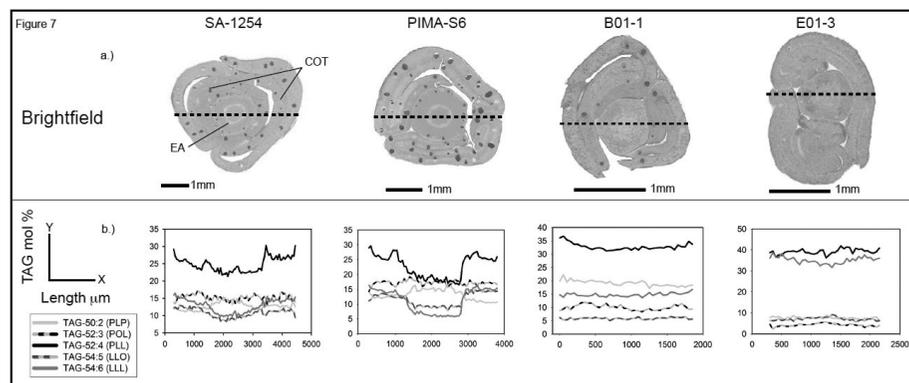
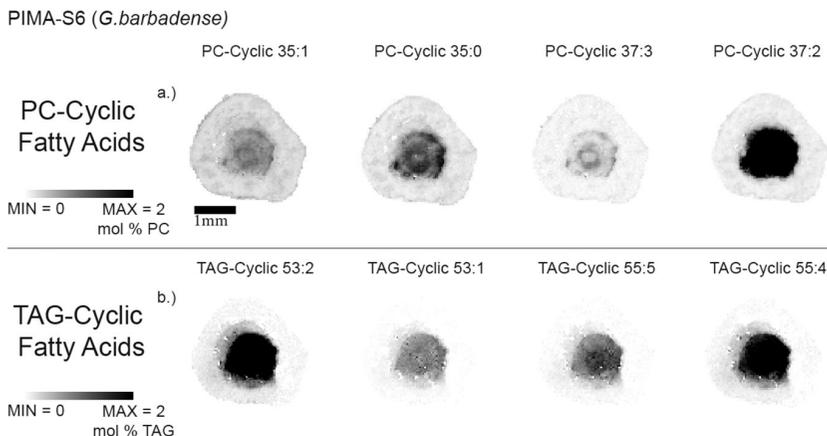


Figure 7. Triacylglycerol (TAG) distributions in transects across embryos of four *Gossypium* species. (a) Bright-field images of cross sections through embryos with the embryonic axis (EA) and cotyledon (COT) tissues labeled. (b) Plots of mol% values for five major TAGs in tissue sections crossing from left to right through cotyledon, axis and cotyledon tissues. Substantial spatial differences in distributions of TAGs were evident in *G. hirsutum* (SA-1254) and *G. barbadense* (Pima-S6), but were not so apparent in *G. anomalum* (B01-1) or *G. stocksii* (E01-3).

To illustrate the marked heterogeneity and metabolic relationship of PC and TAG metabolites within a species, the distribution of several PC and TAG species with a cyclic fatty acid are shown for embryos of *G. barbadense* cv Pima-S6 (Figure 8). Consistent with previous results for *G. hirsutum* embryos (Horn *et al.*, 2012; 2013), the PC and TAG molecular species with a cyclopropane/ene fatty acid are restricted to the embryonic axis regions of the *G. barbadense* embryo. The metabolic relationships are consistent with this distribution since the cyclic group is introduced into the acyl chain when it is esterified to PC and these acyl groups on PC are utilized for the synthesis of TAGs. PC-cyclic 35:1 and PC-cyclic 35:0 are precursors for TAG-cyclic 53:2 and TAG-cyclic 53:1, respectively (in each case adding an 18:1 acyl group); the PC-cyclic 37:3 and PC-cyclic 37:2 are precursors for TAG-cyclic 55:5 and TAG-cyclic 55: 4 (in each case adding an 18:2 acyl group). So the heterogeneous distribution of these metabo-

lites suggests that the cyclopropane fatty acid synthase is mostly active in the embryonic axis and that the production of TAGs in these tissues may vary from that in cotyledonary tissues, at least in this respect. Since this pattern of cyclic fatty acid distribution holds across two diverse *Gossypium* tetraploid species, it suggests some fundamental importance in the *Gossypium* evolutionary history, perhaps as defense compounds during radicle emergence and early seedling growth (Liu *et al.*, 2009; 2012).



SUMMARY

The domestication and breeding of cotton for elite, high-fiber cultivars has led to reduced genetic variation of seed constituents within currently cultivated accessions. A screen of the genetically diverse U.S. National Cotton Germplasm Collection identified accessions/species with dramatic differences in seed oil and protein content. Several genotypes were analyzed for quantitative and spatial differences in seed lipid compositions by mass spectrometric approaches. Results indicated marked variation in pathway metabolites for triacylglycerol biosynthesis in embryos across *Gossypium* species, and suggest that this variation might be exploited by breeders for seed composition traits. Given the large amounts of cottonseed produced in the U.S. that is currently not processed into higher value products, these efforts might be one avenue to raise the overall value of the cotton crop for producers. More comprehensive profiling of seed lipid metabolites across the *Gossypium* genus would appear to be warranted.

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REFERENCES

- AOCS. 2009a. Generic combustion method for determination of crude protein (Ba 4e-93). Official and recommended practices of the AOCS, 6th edn. AOCS Press, Champaign, IL.
- AOCS. 2009b. Nitrogen–ammonia-protein modified kjeldahl method (Ba 4d-90). Official and recommended practices of the AOCS, 6th edn. AOCS Press, Champaign, IL.
- AOCS. 2009c. Oil (Aa 4-38). An Official and recommended practices of the AOCS, 6th edn. AOCS Press, Champaign, IL.
- Bland, J.M., E.J. Conkerton, and G. Abraham. 1991. Triacylglyceride composition of cottonseed oil by HPLC and GC. *J. Am. Oil Chem. Soc.* 68:840-843.
- Chapman, K.D., and J.B. Ohlrogge. 2012. Compartmentation of triacylglycerol accumulation in plants. *J. Biol. Chem.* 287:2288-2294.
- Dyer, J. M., S. Stymne, A.G. Green, and A.S. Carlsson. 2008. High-value oils from plants. *Plant J.* 54:640-655.
- Dowd, M.K., D.L. Boykin, W.R. Meredith Jr., B.T. Campbell, F.M. Bourland, J.R. Gannaway, K.M. Glass, and J. Zhang. 2010. Fatty acid profiles of cottonseed genotypes from the National Cotton Variety Trials. *J. Cotton Sci.* 14:64-73.
- Horn, P.J., and K.D. Chapman. 2012. Lipidomics in tissues, cells and subcellular compartments. *Plant J.* 70:69-80.
- Horn, P.J., and K.D. Chapman. 2013. Metabolite imager: customized spatial analysis of metabolite distributions in mass spectrometry imaging. *Metab. J. In Press.*
- Horn, P.J., C.N. James, S. Gidda, A. Kilaru, J.M. Dyer, R.T. Mullen, J.B. Ohlrogge, and K. Chapman. 2013. Identification of a new class of lipid droplet-associated proteins in plants. *Plant Physiol. In Press.*
- Horn, P.J., A.R. Korte, P.B. Neogi, , E. Love, J. Fuchs, K. Strupat, L. Borisjuk, V. Shulaev, Y.J. Lee, and K.D. Chapman. 2012. Spatial mapping of lipids at cellular resolution in embryos of cotton. *Plant Cell* 24:622-636.
- Horn, P.J., P. Neogi, X. Tombokan, S. Ghosh, B.T. Campbell, and K.D. Chapman. 2011a. Simultaneous quantification of oil and protein in cottonseed by low-field time-domain nuclear magnetic resonance. *J. Am. Oil Chem. Soc.* 88:1521-1529.

- Horn, P.J., N.R. Ledbetter, C.N. James, W.D. Hoffman, C.R. Case, G.F. Verbeck, and K.D. Chapman. 2011b. Visualization of lipid droplet composition by direct organelle mass spectrometry. *J. Bio. Chem.* 286:3298-3306.
- Horn, P.J., D. Sturtevant, and K.D. Chapman. 2013. Modified oleic cottonseeds show altered content, composition and tissue-specific distribution of triacylglycerol molecular species. *Biochimie*. In Press.
- Jones, L.A., and C.C. King. 1996. Cottonseed oil. In: Y. Hui, editor, *Bailey's industrial oil and fat products*, John Wiley & Sons, New York, NY. p. 159-240.
- Lisa, M., and M. Holcapek. 2008. Triacylglycerols profiling in plant oils important in food industry, dietetics and cosmetics using high-performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. *J. Chrom. A.* 1198:115-130.
- Liu Q., D.J. Llewellyn, S.P. Singh, and A.G. Green. 2012. Cotton seed development: Opportunities to Add value to a byproduct of fiber production. In: D.M. Oosterhuis, and T. Cothren, editors, *Flowering and fruiting in cotton*. The Cotton Foundation Reference Book Series, number 8, Cotton Foundation, Cordova, TN. p. 133-162.
- Liu, Q., S. Singh, K. Chapman, and A. Green. 2009. Bridging traditional and molecular genetics in modifying cottonseed oil. In: A.H. Patterson, editor, *Genetics and genomics of cotton*. Springer, New York, NY. p. 353-382.
- Metcalf, L.D., A.A. Schmitz, and J.R. Pelka. 1966. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. *Analytical Chem.* 38:514-515.
- Yunusova, S.G., S.D. Gusakova, A.I. Glushenkova, U.K. Nadzhimov, S. Turabekov, and S.A. Musaev, S.A. 1991. A comparative investigation of the fatty acid compositions of the seeds of a number of lines of a genetic collection of *Gossypium hirsutum*. *Chem. Nat. Comp.* 27:147-150.
- Yunusova, S.G., S.D. Gusakova, and A.U. Umarov. 1982. Stereospecific analysis of the triacylglycerols of a cottonseed oil hydrogenate and its fractions. *Chem. Nat. Comp.* (Translation of *Khimiya Prirodnykh Soedinenii*). 18:396-399.

CHAPTER 5

SOIL CLIMATIC AND EDAPHIC EFFECTS ON COTTON GERMINATION AND THE FINAL STAND

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INTRODUCTION

Germination, emergence, and seedling survival are critical aspects of obtaining a profitable cotton (*Gossypium hirsutum* L.) crop, and this period of growth represents an extremely stressful time for the grower. Historically, a grower principally only had to fear the embarrassment of a skippy or failed stand and having to replant, and possibly ending up with a crop later than desired as well as a crop with mixed maturity. Currently, the cost of replanting genetically modified cultivars containing multiple stacked technologies on a large scale is becoming cost prohibitive. Nonetheless, growers in an attempt to plant extensive acres in a timely manner as well as try to avoid stresses due to water and insects, continue to take the risk of planting when the probability of unfavorable weather conditions is high. Various rules exist for obtaining a successful stand including, do not plant until the soil temperature is at least 20°C at a 5-cm depth for three days in a row followed by a five-day favorable weather forecast or 50 Degree Day 60's (MSU Cares, 2014). Implied in such rules and recommendations are the known detrimental effects that cool soil temperatures have on a tropical perennial oilseed. Less than optimal soil temperatures during the rapid phase of water imbibition by a cotton seed planted in moist soil prevents efficient re-organization of cellular membranes and initiation of biochemical processes and results in the leakage of essential electrolytes such as sugars and proteins. The loss of electrolytes not only can hamper germination, but it can also lead to the growth of pathogenic organisms.

Other soil factors to consider when trying to optimize germination, emergence and seedling survival of cotton includes soil texture, tilth, aggregation, soil potential for crusting, moisture, aeration, compaction, organic matter, surface residues, surface smoothness, and overall planting conditions. Silt to silt loam textured soils are somewhat optimal for cotton growth and provide ideal conditions for emergence as long as rainfall following planting does not result in soil crusting. Sandy textured soils are prone to rapid drying after the disturbance caused by planting and may form a crust following rainfall, while clayey soils present obstacles such as excessive moisture, less than optimal O₂, poor seed-to-soil contact due to large aggregate size, and excessive soil strength. The placement of cotton seed at a proper depth of 2.0-cm to no more than 3.2-cm is critical for rapid germination and emergence. Seed-to-soil contact is important to allow for adequate moisture delivery to the seed and the emerging radical. Soil friability and tilth are extremely important for unimpeded emergence of the epicotyl and cotyledons so that the seedling can get off to a rapid start.

CLIMATIC AND EDAPHIC EFFECTS

Soil temperature is a primary factor influencing cotton seed germination and emergence. Generally, a minimum temperature of 15°C has been recommended for germination under controlled conditions as cooler temperatures can reduce germination (Christiansen and Rowland, 1986). The maximum temperature at which cottonseed germinate has been defined as 42°C (Nabi, 1998). Thus, soil temperatures in the zone of seed placement should be greater than 15°C and less than 42°C for a long enough period to allow for germination. The optimal temperature for cotton seed germination itself has not been well-defined and studies have focused mainly on the effects of chilling temperatures on radicle growth and metabolic activity (Krieg and Carroll, 1978). Christiansen (1967) noted that as little as 4 h of chilling temperatures during imbibition can cause aborted root tips. Pre-hydrating to 12-13% of the weight of the seed prevented chilling injury. Chilling temperatures following 24 h of imbibition at 31°C can have season-long effects on growth. Plant height at maturity was reduced up to 36 cm with 6 d of chilling at 10°C, 14 cm with 4 d, and 5 cm with 2 d (Christiansen and Thomas, 1969). Ultimately lint yield was not affected, but micronaire readings were reduced with the length of chilling from 3.89 to 3.38, presumably due to a delay in flowering and maturity. Although conditions may be favorable for a cotton seed to germinate and grow a radicle 2- to 3-cm in length, chilling temperatures following germination can have a negative impact on vascular tissue sloughing, electrolyte leakage, slowing of growth and development, and increase susceptibility to fungal diseases (Christiansen, 1963, 1967; Christiansen et al., 1970).

The rapid growth of both the radicle and hypocotyl is important for seedling emergence. The role of the radical is to anchor the plant and seek out water and nutrients essential for growth, while the hypocotyl, located between the radicle and cotyledons, facilitates the pulling of cotyledons through to the soil surface and then straightens to expose the cotyledons to sunlight. Increasing soil temperature from 15.6°C to 32.2°C has been shown to increase the rate of growth as well as total growth of cotton radicles and hypocotyls, while a temperature of 37°C caused a decline in growth of radicles and hypocotyls (Wanjura and Buxton, 1972). Krieg and Carroll (1978) demonstrated at a temperature of 15°C that radicle growth rate was initially related to lipid metabolism, but by 6 d it became dependent on the metabolism of the non-lipid fraction with protein being the most important. Cultivars were also studied and the differences between them were mostly found in the utilization of N compounds. More recent research by Mills et al. (2012) examining seedling root growth response of several cotton cultivars to cool temperatures noted no significant trends on root dry matter production after germination, but substantial variation from differing varieties. The overall effects of colder temperatures were to reduce metabolism in the radicle. Under field conditions, the time to reach 45% of the final stand decreased with an increase in temperature from 10 to 21°C for the cotton seed planted 5-cm deep (Wanjura et al., 1969b). The effects of temperature on hypocotyl elongation rate of cotton were modeled and studied by Wanjura et al. (1970). A near linear response in hypocotyl elongation was noted for a temperature range of 14.44°C up to 32.5°C and a decrease with temperatures above 35°C.

Optimal soil moisture supply is critical for rapid cotton seed germination and emergence. Cotton seeds generally reach full hydration in 4 to 6 h (Dewez, 1964; Wanjura and Minton,

1974). Maximum hydration of cotton seeds occurred at 80% of the initial seed weight as compared to corn which hydrated up to 155% of initial seed weight (Stiles, 1948). Phillips (1968) noted species differences in the imbibition rate of water with soybean (*Glycine max* L.) seed hydrating faster than corn (*Zea mays* L.), and corn faster than cotton. Germination times varied accordingly with soybean germinating in 20 h, corn in 54 h, and cotton in 62 h. Murungu et al. (2003) observed 94 % emergence at a soil moisture content of -10 kPa, 35 % at - 50 kPa, and 2 % at -100 kPa. No emergence was found at a soil moisture content of -200 to -1500 kPa. A decline in the ratio of hypocotyl elongation to radicle elongation with an increase in soil moisture stress suggests that the plant preferentially develops a deeper taproot seeking out soil water (Wanjura and Buxton, 1972). Some evidence exists that suggests water vapor transport may play an important role in germination and emergence, which implies that soil porosity is important (Wuest et al., 1999; Wuest, 2002). Soil porosity is dependent on aggregation of individual sand, silt, and clay particles. Murungu et al., (2003) in a study on whether seed priming with water for 24 h is beneficial for cotton seed germination, suggested that priming helped as soil aggregate size increased and seed to soil contact decreased. Seed priming was not effective with soil having small aggregates. These results suggest that water uptake was optimal in finer aggregated soil, but as aggregates became larger that water diffusivity to the cotton seed became restricted.

In areas where a high probability of rainfall can occur between the time of planting and seedling emergence, soil crusting can occur which can slow or impede emergence. Efforts to overcome a crusted soil include using a rotary hoe to disturb the surface crust, switching to a hill drop planting configuration, or reducing tillage to increase crop residue protection of the soil surface. Susceptibility to surface crusting is dependent on soil aggregate stability and decreases with increasing clay and organic matter contents. Soil crusting increases bulk density, decreases porosity, and proportionally decreases macropore space (aeration and drainage) relative to micropore space (capillarity or water holding capacity and conductivity). Bulk density and air porosity are inversely related to each other, although the ratio of aeration pores to capillary pores is dependent on soil texture and degree of aggregation or structure. Nabi et al. (2001) studied the effects of planting depth and irrigation on cotton germination to simulate the effects of hot conditions and soil crusting following planting and irrigation. They determined that germination itself was relatively unaffected and was completed by day three after planting. Slight differences were found in cotton seedling emergence with planting depths of 23 and 46 mm, while no seedlings emerged when planted at a depth of 92 mm. Simulated rainfall prevented seedling emergence due to the formation of a hard crust which produced a strong mechanical impedance. Since cracks in the soil crust formed, the prevention of emergence was not believed to be related to poor soil aeration, but rather to soil strength. Increasing soil bulk density and increasing aggregate size has been shown to delay and reduce total seedling emergence in wheat (*Triticum aestivum* L.) but for opposing reasons (Nasr and Selles, 1995). Increased aggregate size caused an increase in tortuosity for the emerging coleoptile while increasing bulk density increased interfacial stress. Montemayor (1995) demonstrated a reduction in cotton emergence from 66.5% to 32.1 % with an increase in soil sheer strength from 5.5 kPa to 8.2 kPa from mechanically induced soil compaction from press wheels.

On an Amarillo fine sandy loam, Wanjura and Minton (1981) tested the effects of delaying the time of emergence by 50 and 100% of the control to simulate the physiological effects of a crusted soil with high penetration resistance. Hypocotyl diameter was measured and the greatest increase occurred with the first 50% delay in emergence. Taylor and Gardner (1963) investigated the effects of soil moisture, bulk density, and soil strength on cotton root growth under controlled conditions. Root penetration decreased with increasing bulk density across soil moisture contents of $-1/3$ to $-2/3$ bars but was preferentially decreased with increasing soil moisture tension. Soil strength increased with increasing bulk density across all soil moisture tensions and increased with increasing soil moisture tension from $-1/3$ to $-2/3$ bars. Soil strength appeared to be the critical impedance factor rather than bulk density for this sandy soil as the strongest linear correlation found was declining soil root penetration with increasing soil strength. Taylor and Ratliff (1969) confirmed the primary effect of decreasing cotton root elongation with increasing penetration resistance and minimal effects of soil moisture tension at a given penetrometer resistance. Wanjura and Buxton (1972) noted that cotton hypocotyl elongation was reduced preferentially more than radicles with increasing soil impedance. Emergence can also be delayed due to excessive depth of planting. Wanjura et al. (1969a) noted a decrease in seedling survival with increasing planting depth and time to emergence negatively correlated with lint yield. Also, seed of known poorer quality had lower survival rates with increasing planting depth and when seedling days to emergence increased.

PRODUCTION MANAGEMENT EFFECTS

With an increase in adoption of conservation tillage systems for cotton, questions have arisen with regards to whether soil conditions or the inclusion of cover crops would be detrimental or beneficial to cotton germination and emergence. An early survey of no-till cotton studies found that obtaining a stand could be problematic (McWhorter and Jordan, 1985). Bauer and Bradow (1993) noted from the literature increases in seedling disease due to legume cover crops, NH_3 toxicity due to soil incorporation of legumes, emittance of volatile compounds during cover crop decomposition, and potential for soil moisture depletion by cover crops prior their termination and cotton planting. Megie et al. (1967) using conventional tillage showed that soil incorporation of crop residues can be detrimental to cotton germination when high protein residues such as alfalfa (*Medicago sativa* L.) or peanut (*Arachis hypogaea* L.) were used. It was believed that elevated NH_3 levels were damaging to germinating cottonseed. Hicks et al. (1989) found reduced emergence of cotton planted into wheat stubble and identified intolerant and tolerant cotton cultivars to wheat extracts during germination. Allelopathic type compounds can be leached from crop residues or produced during the initial decomposition of certain crop residues. Straw incorporation reduced germination 26% and dry matter 14% as compared to when wheat straw was applied to the soil surface in a greenhouse study. Similar results were found in a field study where standing wheat stubble improved emergence relative to wheat stubbled removed or tilled into the soil. Triplett et al. (1996) found a reduced stand of cotton with no-till and a wheat cover crop versus conventional till the first year of a 4-year study, a greater stand the 3rd year and no difference either the 2nd or 4th year with essentially no difference when averaged across the 4 years of the study. No-till planting

into cover crops by Touchton et al. (1984) did show a 20 to 30% seedling mortality with a crimson clover (*Trifolium incarnatum* L.) cover crop. Stevens et al. (1992) noted a 3-y average reduction in plants/ha for cotton planted no-till into a hairy vetch (*Vicia villosa* Roth) cover crop, but not wheat or no cover crop. Average maximum soil temperature was 5.0°C cooler when planted into wheat and 1.7°C to 2.8°C cooler when planted into hairy vetch for the first 5-d following planting. Varco et al. (1999) did not monitor cotton stand in a no-till cover crop experiment, but found the greatest profitability across a 3-yr period with a hairy vetch cover crop, followed by the use of a rye (*Secale cereale* L.) cover as compared to winter fallow. Stand loss due to cutworm spp. has been shown to increase with the adoption of no-till and favors where legume cover crops are used relative to grass cover crops (Gaylor, 1989; Leonard et al., 1993). Terminating the cover crops at least four weeks before planting minimizes the detrimental effects of cutworms (Leonard et al., 1993). Chambers (1995) noted an increased incidence of seedling disease severity with no-till versus conventional till and the severity increased with earlier planting dates and an increase in stress caused by poor weather conditions.

Nyakatawa and Reddy (2000) studied specifically the effects of tillage, cover cropping, and poultry litter addition on cotton germination and seedling growth. Seedling emergence was more rapid with no-till than with conventional till, but no differences in stands were noted on day four after planting the first year and a 14% advantage the second year when a rye cover crop was included. The more rapid emergence with no-till was attributed to the greater soil volumetric water content. No-till resulted in greater volumetric water content from day 1 through seedling emergence (day 4), while the greatest soil water content was found with no-till and a rye cover crop. Both were compared to conventional tillage. Poultry litter addition which supplied an N equivalent of 200 kg ha⁻¹ also increased soil water content and enhanced emergence up to 4 days from planting, but only increased the final stand the second year. Soil temperature in the surface 7 cm of soil was 2°C cooler with no-till than conventional till up through day 4 both years of the study. When a rye cover crop was included, soil temperature one year was 4°C cooler on day 4 only but averaged 3.5°C cooler up through day 4 the second year than without a rye cover crop. Poultry litter also had a tendency to reduce soil temperature 2.4°C. No-till alone tended to be the average 2°C cooler in the top 7 cm of soil than for conventional till. Overall, seedling emergence was enhanced with no-till, rye cover crop, and poultry litter addition due to great soil water availability, and although cooler temperatures were observed, they were not cold enough to be detrimental. These results support an altered root: shoot ratio in favor of the growth of the hypocotyl compared to the radicle with increasing soil water availability (Wanjura and Buxton, 1972; Xiao-tang et al., 2009).

Potential problems with increased soil strength when tillage is reduced or eliminated has been explored. Bauer and Busscher (1996) did not find an increase in soil strength for a conservation tillage system when compared to a conventional till system, especially in areas where normal row traffic occurred. When a tillage pan does exist, subsoiling has been shown to be beneficial in increasing cotton rooting depth (Salih et al., 1998). Grant and Lafond (1993) noted increased soil penetration resistance in the top 10 cm depth only when tillage was eliminated on a clay soil. Burmester et al. (1995) noted an increase in soil penetration resistance in conservation tillage systems down to a depth of 30 cm with the greatest difference occurring within the top 10 cm. A wheat cover crop in a conservation tillage system resulted in lower penetration resistance

than with cotton stalks only. More recently, Raper et al. (2000) examined the response of soil compaction and cotton lint yields in a silt loam to a combination of conventional, conservation, and no-till practices both with and without cover crops. Results suggested that soil compaction beneath the row could be reduced by shallow, in-row tillage or cover crops and that these treatments resulted in comparable seedcotton yields to the conventional tillage treatment. Further research by Raper et al. (2007) examined the response of seed-cotton yields in a fine sandy loam to a combination of no-tillage, variable depth sub-soiling (15-45 cm, based on depth to hardpan), and deep sub-soiling (45 cm) both with and without cover crops. No overall effect of cover crop on seed cotton yield was noted, but both subsoiling treatments yielded greater than no-tillage systems. These studies highlight the need to address soil compaction in the root zone, with either cover crops or a hardpan-disrupting subsoil tillage event, in order to reach full cotton yield potentials.

Consideration should be given to the effects of fertilizer application near cotton seed on the soil osmotic potential as well as the production of NH_3 /rise in pH. Hood and Ensminger (1964) concluded that the detrimental effect of diammonium phosphate (DAP), when placed with the seed, was not an osmotic or NH_3 effect alone. They suggested that magnesium utilization may be adversely affected. In contrast, Bremner and Krogmeier (1989) studied the effects of urea placement with four different seed species and concluded that production of NH_3 with resulting toxicity was the cause of reduced germination. Bennett and Adams (1970) demonstrated the toxic effects of aqueous NH_3 on cotton root growth of seedlings in solution culture and soil. In nutrient solution culture, increasing pH increased the deleterious effects of NH_3 on root growth. The critical concentration for both solution culture and soil for incipient toxicity was between 0.17 mM and 0.22 mM. Excessive salinity from either salt buildup in the soil or from too close of contact with fertilizer is detrimental to cotton seed germination as it increases the soil osmotic potential which restricts imbibition of soil water by seed. Little to no effect on cotton seed germination was found at a soil salinity level of 1.9 dS m^{-1} , 16 to 43% reduction at 10.0 dS m^{-1} , and 46 to 83 % reduction at 20 dS m^{-1} (Qadir and Shams, 1997). There was also clear genotype tolerances/susceptibility to salinity.

SUMMARY

Given the sensitivity of the cotton seed to adverse weather and soil conditions, seed costs, and availability of seed of desired cultivars, planting the seed of the highest quality and vigor under the most optimal conditions should be a priority. This includes waiting until the soil temperature is 20°C or above at a depth of 5-cm, soil moisture is slightly less than field capacity, and the weather forecast is favorable. Soil should be conditioned or prepared to provide the greatest seed to soil contact in both conventional and no-till planting systems. For soils prone to crusting, consideration should be given to switching to a minimum till or no-till system as well as including a cover crop to provide residues for soil protection. Organic matter additions such as poultry litter or gin trash compost can also provide aggregate stabilizing benefits and improve moisture relations. Plowing under cover crop residues, especially legumes, should be done at least two weeks prior to planting to allow enough time for decomposition processes to reach their peak

and then taper off to minimize NH_3 levels as well as other decomposition products which may have allelopathic effects. In no-till conditions, cover crops should be terminated at least 30 days prior to intended planting to minimize cutworm populations and potential stand damage. Cover crops with known strong allelopathic effects on cotton should be avoided. Planting under more ideal weather conditions will also reduce the likelihood of disease pressure and the need for fungicides. In-furrow fertilizer application at the time of planting should be kept to low rates and sources with low salt effect as well as low NH_3 production potential. In order to maximize profitability, planting under the most ideal conditions will increase stand vigor and minimize the risk of stand loss or failure, increase the growth rate and yield potential, and minimize the stress level of all those involved in the production of the crop.

REFERENCES

- Bauer P.J., and J.M. Bradow. 1993. Cover crops. In: M.R. McClelland, T.D. Valco, and R.E. Frans, editors, Conservation-tillage systems for cotton. Special rep. 160. Ark. Agric. Exp. Stn. Fayetteville, AR. p. 18-22.
- Bauer, P.J., and W.J. Busscher. 1996. Winter cover and tillage influences on coastal plain cotton production. *J. Prod. Agric.* 9:50-54.
- Bennett, A.C., and F. Adams. 1970. Concentration of NH_3 (aq) required for incipient NH_3 toxicity to seedlings. *Soil Sci. Soc. Amer. Proc.* 34:259-263.
- Bremner, J.M., and M.J. Krogmeier. 1989. Evidence that the adverse effect of urea fertilizer on seed germination in soil is due to ammonia formed through hydrolysis of urea by soil urease. *Proc. Natl. Acad. Sci.* 86:8185-8188.
- Burmaster, C.H., M.G. Patterson, and D.W. Reeves. 1995. Challenges of no-till cotton production on silty clay soils in Alabama. In: M.R. McClelland, T.D. Valco, and R.E. Frans, editors, Conservation-tillage systems for cotton. Special rep. 169. Ark. Agric. Exp. Stn. Fayetteville, AR. p. 5-7.
- Chambers, A.Y. 1995. Comparative effects of no-till and conventional tillage on severity of three major cotton diseases in Tennessee. In: M.R. McClelland, T.D. Valco, and R.E. Frans, editors, Conservation-tillage systems for cotton. Special rep. 169. Ark. Agric. Exp. Stn. Fayetteville, AR. p. 96-99.
- Christiansen, M.N. 1963. Influence of chilling upon seedling development of cotton. *Plant Physiol.* 38:520-522.
- Christiansen, M.N. 1967. Periods of sensitivity to chilling in germinating cotton. *Plant Physiol.* 42:431-433.
- Christiansen, M.N., H.R. Carns, and J.D. Slyter. 1970. Stimulation of soluble loss from radicles of *Gossypium hirsutum* L. by chilling anaerobiosis, and low pH. *Plant Physiol.* 46:53-56
- Christiansen, M.N., and R.A. Rowland. 1986. Germination and stand establishment. In: J.R. Mauney and J. M. Stewart, editors, Cotton physiology. Ref. Book Series number one, The Cotton Foundation, Memphis TN. p. 535-541.

- Christiansen, M.N., and R.O. Thomas. 1969. Season-long effects of chilling treatments applied to germinating cottonseed. *Crop Sci.* 9:672-673.
- Dewez, J. 1964. Water uptake and heat evolution by germinating cotton seed. *Plant Physiol.* 39:240-244.
- Gaylor, M.J. 1989. Direct and indirect effects of conservation tillage on the management of insect pests of cotton.. Proc. 1989 Southern Cons. Till. Conf. Special Publ. 89-1. IFAS, Gainesville, FL. p. 10-12.
- Grant, C.A., and G.P. Lafond. 1993. The effects of tillage systems and crop sequences on soil bulk density and penetration resistance on a clay soil in southern Saskatchewan. *Can J. Soil Sci.* 73:223-232.
- Hicks, S.K., C.W. Wendt, J.R. Gannaway, and R.B. Baker. 1989. Allelopathic effects of wheat straw on cotton germination, emergence, and yield. *Crop Sci.* 29:1057-1061.
- Hood, J.T., and L.E. Ensminger. 1964. The effect of ammonium phosphate and other chemicals on the germination of cotton and wheat seeds. *Soil Sci. Soc. Amer. Proc.* 28:251-253.
- Krieg, D.R., and J.D. Carroll. 1978. Cotton seedling metabolism as influenced by germination temperature, cultivar, and seed physical properties. *Agron. J.* 70:21-25.
- Leonard, B.R., R.L. Hutchinson, J.B. Graves, and E. Burris. 1993. In: M.R. McClelland, T.D. Valco, and R.E. Frans, editors, Conservation-tillage systems for cotton. Special rep. 160. Ark. Agric. Exp. Stn. Fayetteville, AR. p. 80-85.
- McWhorter, C.G., and T.N. Jordan. 1985. Limited tillage in cotton production. In: A.F. Wiese, editor, Weed control in limited tillage systems. *Weed Sci. Monogr.* 2 *Weed Sci. Soc. Am., Champaign, IL.* p. 61-76.
- Megie, C.A., R.W. Pearson, and A.E. Hiltbold. 1967. Toxicity of decomposing crop residues to cotton germination and seedling growth. *Agron. J.* 59:197-199.
- Mills, N.B., D.M. Oosterhuis, and B.L. McMichael. 2012. Seedling root growth response to cool environmental conditions for diverse cotton cultivars. *Am. J. Exp. Ag.* 2:269-287.
- Montemayor, M.B. 1995. The effect of soil compaction during planting on cotton emergence. *J. Agric. Eng. Res.* 61:129-136.
- MSU Cares. 2014. Cotton production in Mississippi. Mississippi State University, MS. http://muscares.com/crops/cotton/soil_temp/html.
- Murunga, F.S., P. Nyamugafata, C. Chiduza, L.J. Clark, and W.R. Whalley. 2003. Effects of seed priming, aggregate size and soil matric potential on emergence of cotton (*Gossypium hirsutum* L.) and maize (*Zea mays* L.). *Soil Till. Res.* 74:161-168.
- Nabi, G. 1998. The effect of soil physical factors on the germination and emergence of cotton. Ph.D. diss. University of Aberdeen, Aberdeen, UK.
- Nabi, G., C.E. Mullins, M.B. Montemeayor, and M.S. Akhtar. 2001. Germination and emergence of irrigated cotton in Pakistan in relation to sowing depth and physical properties of the seedbed. *Soil Till. Res.* 59:33-44.

- Nasr, H.M., and F. Selles. 1995. Seedling emergence as influenced by aggregate size, bulk density, and penetration resistance of the seedbed. *Soil Till. Res.* 34:61-76.
- Nyakatawa, E.Z., and C. Reddy. 2000. Tillage, cover cropping, and poultry litter effects on cotton: I. Germination and seedling growth. *Agron. J.* 92:992-999.
- Phillips, R.E. 1968. Water diffusivity of germinating soybean, corn, and cotton seed. *Agron. J.* 60:568-571.
- Qadir, M., and M. Shams. 1997. Some agronomic and physiological aspects of salt tolerance in cotton (*Gossypium hirsutum* L.). *J. Agron. Crop Sci.* 179:101-106.
- Raper, R.L., D.W. Reeves, E.B. Schwab, and C.H. Burmester. 2000. Reducing soil compaction of Tennessee valley soils in conservation tillage systems. *J. Cotton Sci.* 4:84-90.
- Raper, R.L., D.W. Reeves, J.N. Shaw, E. van Santen, P.L. Mask. 2007. Benefits of site-specific subsoiling for cotton production in Coastal Plain soils. *Soil Till. Res.* 96:174-181.
- Salih, A.A., H.M. Babikir, and S.A.M. Ali. 1998. Preliminary observations on effects of tillage systems on soil physical properties, cotton root growth and yield in Gezira Scheme, Sudan. *Soil Till. Res.* 46:187-191.
- Stevens, W.E., J.R. Johnson, J.J. Varco, and J. Parkman. 1992. Tillage and winter cover management effects on fruiting and yield of cotton. *J. Prod. Agric.* 5:570-575.
- Stiles, I.E. 1948. Relation of water to the germination of corn and cotton seeds. *Plant Physiol.* 23:201-22.
- Taylor, H.M., and H.R. Gardner. 1963. Penetration of cotton seedling taproots as influenced by bulk density, moisture content and strength of soil. *Soil Sci.* 96:153-156.
- Taylor, H.M., and L.F. Ratliff. 1969. Root elongation rates of cotton and peanuts as a function of soil strength and soil water content. *Soil Sci.* 108:113-119.
- Touchton, J.T., D.H. Rickerl, R.H. Walker, and C.E. Snipes. 1984. Winter legumes as a nitrogen source for no-tillage cotton. *Soil Till. Res.* 4:391-401.
- Triplett, G.B. Jr., S. Dabney, and J.H. Siefker. 1996. Tillage systems for cotton on silty upland soils. *Agron. J.* 88:507-512.
- Varco J.J., S.R. Spurlock, and O. Sanabria-Garro. 1999. Profitability and nitrogen rate optimization associated with winter cover management in no-tillage cotton. *J. Prod. Agric.* 12:91-95.
- Wanjura, D.F., and D.R. Buxton. 1972. Hypocotyl and radicle elongation of cotton as affected by soil environment. *Agron. J.* 64:431-434.
- Wanjura, D.F., D.R. Buxton, and H.N. Stapleton. 1970. A temperature model for predicting initial cotton emergence. *Agron. J.* 62:741-743.
- Wanjura, D.F., E.B. Hudspeth, Jr., and J.D. Bilbro, Jr. 1969a. Emergence time, seed quality, and planting depth effects on yield and survival of cotton (*Gossypium hirsutum* L.). *Agron. J.* 61:63-65.
- Wanjura, D.F., E.B. Hudspeth, Jr., and J.D. Bilbro, Jr. 1969b. Temperature effect on emergence rate of cotton (*Gossypium hirsutum* L.) under field situations. *Agron. J.* 61:387-389.

- Wanjura, D.F., and E.B. Minton. 1974. Evaluation of cottonseed hydration-chilling treatments for improving seedling emergence. *Agron. J.* 66:217-220.
- Wanjura, D.F., and E.B. Minton. 1981. Delayed emergence and temperature influences on cotton seedling vigor. *Agron. J.* 73:594-597.
- Wuest, S.B. 2002. Water transport from soil to seed: the role of vapor transport. *Soil Sci. Soc. Am. J.* 66:1760-1763.
- Wuest, S.B., S.L. Albrecht, and K.W. Skirvin. 1999. Vapor transport vs. seed-soil contact in wheat germination. *Agron. J.* 91:783-787.
- Xiao-tang, H., C. Hu, W. Jing, M. Xiao-bin, and C. fu-Hong. 2009. Effects of soil water content on cotton root growth and distribution under mulch drip irrigation. *Agric. Sci. China.* 8:709-716.

Chapter 6

LOW TEMPERATURE AND MOISTURE STRESS EFFECTS ON COTTON SEED GERMINATION

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INTRODUCTION

Seed germination is an orderly process that begins with imbibition of the seed and ends with the emergence of the radicle from the seed coat (Rajjou et al., 2012). Seed germination is considered one of the most vulnerable stages to biotic and abiotic stress (Rajjou et al., 2012). Upland cotton (*Gossypium hirsutum* L.) grown in the U.S. cotton belt is commonly exposed to varying temperatures ranging from 20° to 35°C (Reddy et al., 1991). Little information is, however, available for cold and drought stress during the early growth stages of cotton (Pearson et al., 1970; Reddy et al., 1992a). The reason may be that past studies have identified the reproductive stage as the most sensitive growth stage to these abiotic stresses (Reddy et al., 1992b; Reddy et al., 1992c; Reddy et al., 1995; Reddy et al., 1999). Despite the extensive research, studies which investigated the cold and drought effect on seed germination and seedling growth characteristics in upland cotton were more than two decades old (Arndt, 1945; Ashraf et al., 1994; Pearson et al., 1970). Seed germination in cotton is considered to be very sensitive to sub-optimal temperatures (< 30°C) at planting. Cotton production guides for the southern US recommend for cotton producers to plant early season cotton (Late-April) at about 2.54 cm deep when the five-day forecast of soil temperature is above 18.3°C, provided adequate soil moisture conditions are present (Bradow and Bauer, 2010). Early planting of cotton is always favored in the U.S. cotton belt which lengthens the growing season, minimizes late-season insect pressure, and improves lint yields (Bradow, 1991; Pettigrew, 2002; Wrather et al., 2008). However, early season planting may very often expose early growth to low temperatures (< 30°C) and variable soil moisture conditions that result in poor seed germination and establishment (Barpete et al., 2015). These negative effects of low temperature are aggravated by varying soil moisture conditions, especially during seed imbibition. While soil temperature cannot be altered to facilitate optimal seed germination under field conditions, supplemental irrigation can alter soil moisture at the time of planting. Therefore, information about cotton seed germination in response to sub-

optimal temperature and soil moisture is a matter of utmost importance. This chapter provides the review of effects of low temperature and moisture stress on cotton seed germination.

SEED IMBIBITION

Seed imbibition is the first stage of seed germination that involves water uptake, and increases in respiration soon follow (Rajjou et al., 2012). The rate of seed imbibition is considered to be highly controlled by the properties of the seed and water, in particular seed coat permeability, seed moisture content, temperature of water, and rate at which water is taken up by seed (Vertucci et al., 1989). LOW temperatures ($< 20^{\circ}\text{C}$) can substantially change the viscosity of water as well as membrane properties of the seed, influencing water uptake (Murphy and Noland, 1982). During imbibition, low temperatures inhibit the reorganization of the seed membrane upon rehydration into one with semipermeable properties but make it completely permeable to heavy metals, like lead (Wierzbicka and Obidzińska, 1998). Additionally, excessive permeability can cause leakage of cellular components such as lipids, enzymes and phenolic compounds from the embryo to the outside medium, which makes the embryo more susceptible to microbial attack (Woodstock, 1988). Membrane leakage due to chilling (5°C) during imbibition can substantially be prevented with the addition of calcium to the system (Christiansen et al., 1970). Cotton seeds also showed more rapid uptake of water at high temperatures ($> 30^{\circ}\text{C}$) than low temperatures ($< 30^{\circ}\text{C}$) (Wanjura and Buxton, 1972a). Christiansen (1964) observed the symptoms of radicle injury after seeds were imbibed at 10°C , and these symptoms were more apparent and severe when imbibed at 5°C . Soil water potential also impacts the rate of water uptake but has a lesser influence than low temperatures during imbibition (Wanjura and Buxton, 1972a). However, low moisture in combination with low temperature can reduce water uptake to a greater extent when compared to low temperature alone (Bradow and Bauer, 2010). Genotypic differences in the rate of water uptake do exist among different species or cultivars under low temperatures. Phillips (1968) observed that water diffusivity of soybean seeds was 18 times greater than cotton seeds and four times greater than corn seeds when germinated at $28 \pm 1^{\circ}\text{C}$. The time to seed imbibition has also influenced the sensitivity of cotton seeds to chilling environments. Chilling during imbibition is the most damaging. So, if the seeds are kept warm during water uptake, they can withstand chilling temperatures later. Both upland and Pima cotton showed reduced germination under chilling temperature (5°C) effects, but the sensitivity to chilling temperatures declined in both species after cotton seeds were preconditioned to warm temperatures for a few hours (Buxton et al., 1976; Christiansen, 1968). BÓLEK et al. (2013) observed decreasing germination percentage with increasing period of seed priming at 5°C among all cotton cultivars. However, the studies have reported significant genotype x chilling time interactions for percent seed germination. Two species, *G. barbadense*, and *G. hirsutum*, had significantly different responses to the duration of chilling time (Cole and Christiansen, 1975). Cole and Christiansen (1975) observed reduced percent germination of Pima S-4' (*G. barbadense* L.) than genetic selection M-8 (*Gossypium hirsutum* L.) over same temperature range (5°C in water) after seeds were subjected to different durations of chilling time (0, 1, 2, 4, 8, 16, and 32 days). The author further reported higher germination and emergence rate in pre-conditioned seeds than chilled seeds, where the

rate of water uptake by Pima S-4 was greater than M-8. Therefore, the selection of genotypes for cold tolerance varies depending upon the duration of chilling and imbibition. Buxton et al. (1976) observed similar responses of commercial Pima type cultivars on exposure to chilling at 5°C, but the cultivars differed significantly at 7°C and 10°C. Thus, the authors concluded that higher temperatures (>5°C) are more desirable for selection of cold tolerant genotypes in cotton. Likewise, Christiansen (1968) observed that genotypes of *G. hirsutum* L., M-8, and Deltapine 14, showed a similar response to exposure to chilling temperatures (5°C) during the initial hours of imbibition. The author further reported that after the cotton seeds hydrated for 4 hours at 31°C, subsequent seedling growth occurred normally under chilling temperatures (5°C) for 48 hours. In nutshell the above evidences reveals imbibition as the most chilling-sensitive stage of seed germination.

SEED RESERVE MOBILIZATION AND CONVERSION EFFICIENCY

Seed imbibition is followed by activation and formation of enzymes to metabolize storage compounds and synthesize new proteins (Rajjou et al., 2012). The activity of certain enzymes such as α -amylase and protease is regulated through phytohormones including gibberellins, cytokine, ethylene, and abscisic acids and is controlled by genotype and environmental factors like temperature (Srivastava, 2002), salinity (Ashraf, 2002) and drought (Ashraf et al., 1995). The metabolic activity and respiration rate increase as germination progresses and eventually drop at radicle emergence (Srivastava, 2002). High temperatures (>30°C) can accelerate seed aging which can have a marked influence on the heterotrophic growth of the seedling. The ability of a seedling to sustain itself during the heterotrophic phase of growth can be determined from seed reserve mobilization (SRM) also sometimes called seed reserve utilization and conversion efficiency of seed reserve utilized (SUE) (Nik et al., 2011; Sikder et al., 2009). Seed reserve mobilization depends upon two components: a) initial seed weight and b) seed depletion ratio (SDR), which describes a fraction of seed reserve that is mobilized. By comparison, conversion efficiency of seed reserve utilized (SUE) describes the efficiency with which mobilized or utilized seed reserve is converted to seedling tissue. Therefore, heterotrophic growth based on seed reserve can be determined as follow:

$$\text{SRM (mg seed}^{-1}\text{)} = \text{initial seed dry weight} - \text{unutilized seed dry weight}$$

$$\text{Seed reserve depletion ratio (SDR)} = \text{SRM} / \text{initial seed dry weight}$$

$$\text{SDR (\%)} = (\text{SRM} / \text{initial seed dry weight}) * 100$$

$$\text{SUE (\%)} = (\text{Seedling dry weight} / \text{SRM}) * 100$$

The decline in either of the components of SRM as a result of biochemical deterioration in the seed can reduce seedling growth (Nik et al., 2011). Being an oil-seed crop, cotton contains a substantial amount of seed storage reserve, especially lipids and proteins (Bradow and Bauer, 2010). The embryo serves as a sink for the energy derived from photosynthesis and other metabolic activities during seed development and accumulates storage reserves primarily in the cotyledons. These reserves are mobilized post-germination to fuel early seedling growth until

the seedling becomes photosynthetically active. (Ichie et al., 2001). Thus, SRM declines with the change from heterotrophic growth to autotrophic growth. Seed size determines the amount of storage reserve available to the developing seedling and contributes to improved seedling vigor (Soltani et al. 2002; Snider et al., 2014). However, seed size may not positively relate to improved seedling vigor under stress conditions (Soltani et al. 2002) or necessarily to improved lint yields in cotton (Liu et al., 2015; Snider et al., 2016).

Past studies have recognized that SRM is more sensitive to various abiotic stresses than SUE (Soltani et al., 2002; Soltani et al., 2006; Nik et al., 2011). The growing conditions, particularly high temperatures during the reproductive stage of the parent plant, can adversely affect SRM at post-harvest seed germination (Rao and Sinha, 1993) which is very specific to the genotypes or species depending upon their tolerance levels (Hasan et al., 2013). Genetic variation for SRM under heat stress has been observed in corn (*Zea mays*) (Sikder et al., 2009), sorghum (*Sorghum bicolor*) (Gophane et al., 2005; Rao and Sinha, 1993), wheat (*Triticum aestivum*) (Khatun et al., 2015), and moringa (*Moringa oleifera*) (Tsfay et al., 2016). In addition, various other factors, including drought (Ansari et al., 2012; Soltani et al., 2002; Soltani et al., 2006), salinity (Soltani et al., 2002), nutrients (Seyyedi et al., 2015), seed aging (Mohammadi et al., 2012; Nik et al., 2011; Sung, 1996), and seed coat (Gorim and Asch, 2012) can significantly affect SRM. Higher SRM and SUE are desirable to prevent uneven and delayed emergence, especially under drought stress in the field (Sikder et al., 2009). Soltani et al. (2006) observed decline in seed reserve mobilization with increasing drought stress, while no effect was observed on SUE in Chickpea (*Cicer arietinum*) Further, osmo-or-hydro priming the seeds can significantly lower the reduction in SRM under drought stress compared to unprimed seeds (Maasoumeh and Mohammad, 2014; Ansari et al., 2012). Quantitative trait loci (QTL) approaches have also been exploited that successfully investigate the genetic characteristics of seed reserve utilization during seed germination stages (Cheng et al., 2013). N-acetylphosphatidylethanolamine, a phospholipid, has been found to be responsible for maintaining membrane integrity of cotton seed and promote SRM (Bewley, 1997). There is limited information available on cultivar-specific SRM across a wide range of temperatures and drought conditions for cotton in the peer-reviewed literature. This indicates the need for further research on SRM along with other seed germination parameters to a better understanding of seed and seedling vigor responses to multiple stress conditions.

RADICLE EMERGENCE AND GROWTH

Seed emergence is characterized by protrusion of the radicle and marks the completion of the seed germination process (Weitbrecht et al., 2011; Rajjou et al., 2012). The rapid increase in water and nutrient uptake determines radicle emergence which involves cell division and elongation. As a result, the hypocotyl elongates and pulls the cotyledons and epicotyl above the soil surface. This is followed by a subsequent drop in respiration level as seedling cotyledons begin to photosynthesize. Studies have generally considered seed as germinated after the radicle had protruded beyond the seed coat and attained a certain length (Christiansen and Rowland, 1986; Oosterhuis and Jernstedt, 1999; Bradow and Bauer, 2010; Turley and Chapman, 2010; Snider et al., 2014; Singh et al., 2017a). Extensive research has been conducted in the past to

determine the environment x genotype interaction on radicle growth and development (BÖLEK et al., 2013; Barpete et al., 2015). Several abiotic factors, including temperature (McQuigg and Calvert, 1966; Wanjura and Buxton, 1972a; Ashraf et al., 1994;), seeding depth (Wanjura et al., 1969), salinity (Fuxin and Fuquan, 2000; Ashraf, 2002; Wang et al., 2007), and drought (Naim and Ahmed, 2015; Singh et al., 2017a, b; Singh et al. 2018) were found to influence germination and early seedling growth. Cotton seeds exposed to chilling temperatures (<10°C) during initial water uptake exhibit radicle abortion followed by profuse lateral roots and a swollen hypocotyl (Bradov and Bauer, 2010). The radicle abortion is primarily determined by the degree of damage to cortex cells under low temperatures (Christiansen, 1968). Christiansen et al. (1970) found increased solute (amino acids and sugars) loss from the radicle with increasing length of chilling (5°C) time. Longer periods of chilling treatments can significantly induce chilling injury. Christiansen (1968) observed reduced germination and induced root abnormalities after cotton seeds were exposed to chilling temperatures for 30 minutes. Further, the author noted that longer periods of chilling for 12 hours at the time of imbibition caused permanent damage to the seeds and hence, failure of the radicle to emerge. The rate of radicle emergence and subsequent growth in responses to low temperatures have also been studied to identify cold avoidance or tolerance mechanisms in cotton (BÖLEK et al., 2013; Barpete et al., 2015). Barpete et al. (2015) studied changing germination and seedling growth behavior at different temperatures to identify cold tolerance among cotton cultivars. They found cultivar SG-125 showed maximum germination and radicle length at 30°C among the five cotton cultivars including SG-125, ST-468, Ozbek-100, Ozaltin-404, and Ayhan-107. The values for maximum germination and radicle length declined significantly at 20°C and no germination was observed at 4°C for all cultivars. Also, radicle emergence was more sensitive to low temperatures than drought stress in cotton (Wanjura et al., 1971; Singh et al., 2018). Singh et al. (2018) observed a significant increase in time to reach 50 % emergence (TTE) under low temperatures (22°C) for seedlings of nine elite cotton lines representing nine different breeding programs when compared to optimum temperatures (30°C). Further, the study observed significant variability among the lines for emergence rate, where Ark 0504-4 took longest, and PD05064 took the shortest time to emerge, and no effect of drought stress (50 % of the optimum moisture) on seedling emergence of the cotton lines was observed.

Several methods have been developed to induce germination and emergence rate at low temperature (BÖLEK et al., 2013). BÖLEK et al. (2013) observed hydropriming at 25°C could induce germination and seedling emergence in cotton. However, primed cotton seeds at 5°C showed significantly lower germination percentage and emergence rate than 25°C, especially under longer priming duration (> 2 hours) (BÖLEK et al., 2013). The authors further identified cultivar Stoneville-468 had the highest germination and emergence rate among the three cotton cultivars, including Stoneville-468, Maraş-92, and Sayar-314, when primed in distilled water at 5°C and 25°C for 2, 4, 6, 8, or 10 hours. Secondly, they observed increased germination and emergence rate at low temperature after the cotton seeds were subjected to high temperature for 10 seconds which was applied using hot water (96 ± 1°C) bath. Cole and Wheeler (1974) observed that preconditioning Pima S-4' cottonseed with hot water at 70°C for 5 min significantly reduced sensitivity of seeds to gibberellic acid, thereby reducing percent emergence relative to

seeds preconditioned with water at 30°C. The author further reported that preconditioning cotton seeds with water or exogenous gibberellic acid or adenosine-3':5'-cyclic monophosphate can substantially reduce the chilling effect on germination and emergence at the time of planting. Soil physical properties such as soil aggregates (Murungu et al., 2003), soil crusting (Bilbro and Wanjura, 1982; Awadhwal and Thierstein, 1985), and cultural practices that include cover crops and conservation tillage can also alter cotton germination and seedling growth (Nyakatawa and Reddy, 2000). Sowing-time during a day can also alter the rate of seedling emergence and subsequent development under cold soil conditions (Steiner and Jacobsen, 1992). Steiner and Jacobsen (1992) observed cotton seeds planted at 0800 hours in early spring under cool temperatures showed reduced seedling emergence (SE) compared to those planted at 1600 h, while the rate of seedling development (SD) remained unaffected by planting time. However, genotypic differences were observed at 0800 h and 1600 h planting times for both SE and SD in response to cool soil conditions. Seedling emergence and SD were significantly reduced in cultivar GC-510 relative to SJ-2 for the first 100 h and 30 h from planting, respectively, for both sowing times. Hence, the interaction between year, time of planting and genotype could determine the whole-plant rate of development and lint yield in cotton (Bauer and Bradow, 1996).

Variation in seed moisture has been considered an important physiological trait during radicle emergence (Wanjura and Buxton, 1972a). Both temperature and drought stress can cause considerable variability in seed moisture leading to variability in the rate of germination, and variable subsequent growth and development (Wanjura and Buxton, 1972a). Although, the responses to temperature and drought stress can be variable during this stage of physiological development, the combination of two stresses causes a synergistic effect (Wanjura et al., 1971; Wanjura and Buxton, 1972a, b; Singh et al., 2018). A significant increase in the radicle emergence time and rate of hypocotyl elongation with decreasing temperatures (< 30°C) and increasing soil moisture tension (> 0 bar) was observed (Wanjura et al., 1971). However, no germination occurred under the very low temperature (15°C) and very high moisture tension (10 bars) treatment combination (Wanjura et al., 1971). Further, the ratio of hypocotyl to radicle length significantly declines under drought stress due to an increased rate of radicle elongation (Wanjura and Buxton, 1972b; Wanjura, 1986). While, studies have also reported that under a certain range of temperature (38 to 15°C) and soil moisture stress (-0.3 to -10 bars), decreasing temperature can cause a similar change in radicle length as soil moisture stress increased (Wanjura and Buxton, 1972a).

SEED GERMINATION MODELS

Seed germination studies have developed various seed germination models using polyethylene glycol (Singh et al., 2017a), thermotolerance assays (Gajanayake et al., 2011), and hydropriming assays (BÖLEK et al., 2013) to quantify the response of seed germination to abiotic stresses. Such models estimate seed germination parameters and correlate them with relative seedling vigor, growth, and development. Seed germination models aim to observe seed germination time course data that provide maximum seed germination percentage (MSG) and seed germination rate (SGR) (Gajanayake et al., 2011; Singh et al., 2017a). Some seed testing laboratories use cool germination test to determine cottonseed quality and seedling vigor

(Drummond and Savoy, 1996). Under the cool germination test, seeds are incubated at $18 \pm 0.5^\circ\text{C}$ (AOSA 1983) for 7 days, and percent germination is recorded after that. This criterion has been used to predict the performance (vigor) of cotton cultivars in the field for early season planting in the United States (Smith and Varvil, 1984). Using cool germination test protocols, BÖLEK et al. (2013) considered cotton cultivars showing MSG more than 60% at $18 \pm 0.5^\circ\text{C}$ temperature over a 7-day period as tolerant. However, cultivars with similar MSG may vary for SGR (Maguire, 1962) and therefore, SGR is considered a valuable tool in selecting and evaluating the cultivar tolerance to various stress factors (Maguire, 1962; Gajanayake et al., 2011; Singh et al., 2017a). The thermotolerance models are unique because they estimate MSG and SGR under a wide temperature range among different cultivars (Gajanayake et al., 2011). This determines cultivar-specific optimum temperatures for seed germination and emergence. The response function developed from MSG and SGR can also be useful to estimate cultivar-specific cardinal temperatures which may be useful to classify cultivars for cold and heat tolerance. The information can also be applied to determine cultivar-specific optimum planting times for a specific region.

Seed parameters such as seed weight, seed size, seed storage reserves, seed mobilization efficiency, seed coating, radicle length and dry weights, also contribute to the difference in seedling vigor among cotton cultivars (Snider et al., 2014; Liu et al., 2015; Snider et al., 2016). However, certain seed quality parameters that are not necessarily linked to genotype may lower the validity of cultivar-specific seed germination models. The best way to nullify such effects is to collect the seeds from parent populations grown at the same time under a uniform seed production and seed storage environment for use in future experiments. Post-harvesting treatments and seed storage conditions can also influence seed germination, irrespective of cultivars or species. The time of seed storage can affect the seed quality and subsequent germination processes. Storing cotton seeds for a longer period than recommended could cause deterioration, membrane leakage, and decreased germinability. Goel and Sheoran (2003) identified differences in germinability of two cotton cultivars, HS6, and H1098 based on electrolyte leakage and correlated them with seed aging. They concluded that seed aging could be determined from membrane lipid peroxidation due to lower activity of antioxidant enzymes. Further, the ambient conditions, especially high temperature and humidity, during seed aging can accelerate the seed deterioration processes. Goel et al. (2003) observed increased lipid peroxidation and reduced activity of peroxide scavenging enzymes in cotton seeds that were stored at $40 \pm 1^\circ\text{C}$ and 100% relative humidity for four days. The author also found that the aging treatment of 96 hours had a greater decrease in enzyme activity for cv. HS6 than H1098. The differences between cultivars in response to aging treatment in the studies above necessitate further work aimed at identifying cultivar-specific optimum conditions for seed storage.

Seed dormancy prevents the germination of seeds under otherwise favorable conditions and therefore, is an important consideration in conducting seed germination tests. It can be described as the inability of viable seeds to germinate under favorable conditions (Bewley, 1997). Embryo dormancy is a type of primary dormancy caused by internal factors. For instance, a higher concentration of abscisic acid is likely to induce transient dormancy (Halooin, 1986). In contrast, secondary seed dormancy is caused by unfavorable external/environmental factors (Vincent et

al., 2010). Temperature, water, light, and oxygen are the major environmental factors that control this type of dormancy. Many plants such as oak (*Quercus spp.*) and apple (*Malus spp.*) require a certain number of chilling degree days or cold temperature treatment of about 5°C to 7°C to break primary dormancy and induce germination (Srivastava, 2002). While species that are sensitive to chilling temperatures like cotton on exposure to very high or low temperatures would induce secondary dormancy and fail to germinate even if favorable conditions are provided (Vincent et al., 2010). While cottonseed hydrated to 12-13% can significantly lower the chilling injury effect, seeds with moisture less than 10% develop a hard seed coat (Bradow and Bauer, 2010), making the seed membrane impermeable for water uptake. One way to eliminate the dormancy on freshly harvested cotton seeds is to dry and store them for a short period (Simpson, 1935).

SUMMARY

Cotton planted in early spring in the US does not necessarily exhibit uniform stand establishment across the field. The major reason is that environmental conditions, especially temperature and soil moisture, that vary across the field and during different phases of seed germination. Initially, exposure of cotton seeds to sub-optimal temperatures causes decreased membrane integrity during seed imbibition, making the embryo more susceptible to seedling disease. Further, variable soil moistures developed across field under minimum tillage practices can reduced rate of water uptake. The lowered seed moisture, as a result, reduces the rate of respiration causing a reduction in enzymatic metabolism of the seed reserves. As a consequence, heterotrophic phase of growth based on seed reserve slows down leading to reduced rate of hypocotyl elongation. Both hypocotyl and radicle elongation decreases with decreasing temperature and soil moisture from optimum, but show differential sensitivity to given stress. Thus, the ratio of hypocotyl/radicle is another important to study extensively under drought and cold stress during this stage of development. However, significant genotypic variability has been identified among cotton germplasm under low temperature and moisture environment for germination and physical characteristics of seed. Appropriate seed germination models are being developed studying these characteristics under controlled conditions to correlate with the post-harvesting treatments and early planting practices under field conditions. Future studies are however needed to focus on studying the mode of resistance identified in the cotton gene pool to improve region-specific cotton cultivars. The development of resistant cultivars could provide long-term benefits to cotton producers along with simultaneous advantage from early season cotton planting.

REFERENCES

- Ansari, O., H. Chogazardi, F. Sharifzadeh, and H. Nazarli. 2012. Seed reserve utilization and seedling growth of treated seeds of mountain rye (*Secale montanum*) as affected by drought stress. *Cercetari Agronomice in Moldova* 45:43-48.
- AOSA. 2013. Rules for testing seeds. Association of Official Seed Analysts, Ithaca, NY.
- Arndt, C. 1945. Temperature-growth relations of the roots and hypocotyls of cotton seedlings. *Plant Physiol.* 20:200-220.

- Ashraf, M. 2002. Salt tolerance of cotton: some new advances. *Critical Rev. Plant Sci.* 21:1-30.
- Ashraf, M., A. Azmi, A. Khan, and S. Naqvi. 1995. Alpha-amylase and protease activities of wheat seedling grown under water stress conditions. *Pak. J. Scienti. Indust. Res* 38:430-434.
- Ashraf, M., M. Saeed, and M. Qureshi. 1994. Tolerance to high temperature in cotton (*Gossypium hirsutum* L.) at initial growth stages. *Environ. Exper. Bot.* 34:275-283.
- Awadhwal, N., and G. Thierstein. 1985. Soil crust and its impact on crop establishment: a review. *Soil Tillage Res.* 5:289-302.
- Barpete, S., M.C. Oğuz, S. Özcan, E. Anayol, H.A. Ahmed, K.M. Khawar, and S. Özcan. 2015. Effect of temperature on germination, seed vigor index and seedling growth of five Turkish cotton (*Gossypium hirsutum* L.) cultivars. *Fresenius Environ. Bull.* 24: 1-7.
- Bauer, P.J., and J.M. Bradow. 1996. Cotton genotype response to early-season cold temperatures. *Crop Sci.* 36:1602-1606.
- Bewley, J.D. 1997. Seed germination and dormancy. *The Plant Cell* 9:1055-1066.
- Bilbro, J., and D. Wanjura. 1982. Soil crusts and cotton emergence relationships. *Trans. ASAE* 25:1484-1487.
- BÖLEK, Y., M.N. Nas, and H. Cokkizgin. 2013. Hydropriming and hot water-induced heat shock increase cotton seed germination and seedling emergence at low temperature. *Turk. J. Agric. For.* 37: 300-306.
- Bradow, J.M. 1991. Cotton cultivar responses to suboptimal postemergent temperatures. *Crop Sci.* 31:1595-1599.
- Bradow, J.M., and P.J. Bauer. 2010. Germination and seedling development. In: J.M. Stewart, D.M. Oosterhuis, J.J. Heitholt, and J.R. Mauney, editors, *Physiology of cotton*. Springer, Netherlands. p. 48-56.
- Buxton, D., P. Sprenger, and E. Pegelow. 1976. Periods of chilling sensitivity in germinating Pima cottonseed. *Crop Sci.* 16:471-474.
- Cheng, X., J. Cheng, X. Huang, Y. Lai, L. Wang, W. Du, Z. Wang, and H. Zhang. 2013. Dynamic quantitative trait loci analysis of seed reserve utilization during three germination stages in rice. *PLoS One* 8:e80002.
- Christiansen, M. 1964. Influence of chilling upon subsequent growth and morphology of cotton seedlings. *Crop Sci.* 4:584-586.
- Christiansen, M. 1968. Induction and prevention of chilling injury to radicle tips of imbibing cottonseed. *Plant Physiol.* 43:743-746.
- Christiansen, M.N., and R.A. Rowland. 1986. Germination and stand establishment. In: J.R. Mauney and J.M. Stewart, editors, *Cotton physiology*. The Cotton Foundation, Memphis, TN. p. 535-541.
- Christiansen, M., H. Carns, and D.J. Slyter. 1970. Stimulation of solute loss from radicles of *Gossypium hirsutum* L. by chilling, anaerobiosis, and low pH. *Plant Physiol.* 46:53-56.
- Cole, D., and J. Wheeler. 1974. Effect of pregermination treatments on germination and growth of cottonseed at suboptimal temperatures. *Crop Sci.* 14:451-454.

- Cole, D., and M. Christiansen. 1975. Effect of chilling duration on germination of cottonseed. *Crop Sci.* 15:410-412.
- Drummond, E.A., and B.R. Savoy. 1996. Influence of cotton planting-seed quality on seedling health. Proceedings of Beltwide Cotton Conferences, Nashville, TN, USA, January 9-12, 1996. National Cotton Council, Memphis, TN.
- Gajanayake, B., B.W. Trader, K.R. Reddy, and R.L. Harkess. 2011. Thermotolerance classification of ornamental pepper cultivars using seed germination traits. *Seed Technol.* 33:134-146.
- Goel, A., A.K. Goel, and I.S. Sheoran. 2003. Changes in oxidative stress enzymes during artificial ageing in cotton (*Gossypium hirsutum* L.) seeds. *J. Plant Physiol.* 160:1093-1100.
- Goel, A., and I. Sheoran. 2003. Lipid peroxidation and peroxide-scavenging enzymes in cotton seeds under natural ageing. *Biologia Plantarum* 46:429-434.
- Gophane, S., S. Mate, and Y. Suryawanshi. 2005. Studies on the mobilization efficiency of seed reserves in sorghum (*Sorghum bicolor* (L.) Moench). *Seed Res.* 33:215-217.
- Gorim, L., and F. Asch. 2012. Effects of composition and share of seed coatings on the mobilization efficiency of cereal seeds during germination. *J. Agron. Crop Sci.* 198:81-91.
- Halloin, J.M. 1986. Weathering: changes in planting seed quality between ripening and harvest. In: J.R. Mauney and J. M. Stewart, editors, *Cotton physiology*. The Cotton Foundation, Memphis, TN. p. 475-481.
- Hasan, M.A., J.U. Ahmed, T. Hossain, M.A.K. Mian, and M.M. Haque. 2013. Evaluation of the physiological quality of wheat seed as influenced by high parent plant growth temperature. *J. Crop Sci. Biotechnol.* 16:69-74.
- Ichie, T., I. Ninomiya, and K. Ogino. 2001. Utilization of seed reserves during germination and early seedling growth by *Dryobalanops lanceolata* (Dipterocarpaceae). *J. Tropical Ecology* 17:371-378.
- Khatun, S., J.U. Ahmed, and M. Mohi-Ud-Din. 2015. Variation of wheat cultivars in their relationship between seed reserve utilization and leaf temperature under elevated temperature. *J. Crop Sci. Biotechnol.* 18:97-101.
- Liu, S., M. Remley, F. Bourland, R. Nichols, W. Stevens, A.P. Jones, and F. Fritschi. 2015. Early vigor of advanced breeding lines and modern cotton cultivars. *Crop Sci.* 55:1729-1740.
- Maasoumeh, A.A., and S. Mohammad. 2014. The effect of osmo and hormone priming on germination and seed reserve utilization of millet seeds under drought stress. *J. Stress Physiol. Biochem.* 10:214-221.
- Maguire, J.D. 1962. Speed of germination-aid in selection and evaluation for seedling emergence and vigor. *Crop Sci.* 2:176-177.
- McQuigg, J.D., and O.H. Calvert. 1966. Influence of soil temperatures on the emergence and initial growth of upland cotton. *Agric. Meteorol.* 3:179-185.
- Mohammadi, H., A. Soltani, H. Sadeghipour, and E. Zeinali. 2012. Effects of seed aging on subsequent seed reserve utilization and seedling growth in soybean. *Int. J. Plant Prod.* 5:65-70.

- Murphy, J.B., and T.L. Noland. 1982. Temperature effects on seed imbibition and leakage mediated by viscosity and membranes. *Plant Physiol.* 69:428-431.
- Murungu, F., P. Nyamugafata, C. Chiduzza, L. Clark, and W. Whalley. 2003. Effects of seed priming, aggregate size and soil matric potential on emergence of cotton (*Gossypium hirsutum* L.) and maize (*Zea mays* L.). *Soil Till. Res.* 74:161-168.
- Naim, A.H., and F.E.G. Ahmed. 2016. Interactive effect of temperature and water stress induced by PEG on germination and early seedling growth of two chickpea (*Cicer arietinum* L.) cultivars. *Open Access Library Journal* 10:1-7.
- Nik, S.M.M., H.G. Tilebeni, E. Zeinali, and A. Tavassoli. 2011. Effects of seed ageing on heterotrophic seedling growth in cotton. *American-Eurasian J. Agr. Environ. Sci.* 10:653-657.
- Nyakatawa, E.Z., and K.C. Reddy. 2000. Tillage, cover cropping, and poultry litter effects on cotton: I. germination and seedling growth. *Agron. J.* 92:992-999.
- Oosterhuis, D.M., and J. Jernstedt. 1999. Morphology and anatomy of the cotton plant. In: C.W. Smith and J.T. Cothren, editors, *Cotton origin, history, technology, and production*. John Wiley & Sons, New York. p. 175–206.
- Pearson, R., L. Ratliff, and H. Taylor. 1970. Effect of soil temperature, strength, and pH on cotton seedling root elongation. *Agron. J.* 62:243-246.
- Pettigrew, W.T. 2002. Improved yield potential with an early planting cotton production system. *Agron. J.* 94:997-1003.
- Phillips, R.E. 1968. Water diffusivity of germinating soybean, corn, and cotton seed. *Agron. J.* 60:568-571.
- Rajjou, L., M. Duval, K. Gallardo, J. Catusse, J. Bally, C. Job, and D. Job. 2012. Seed germination and vigor. *Ann. Rev. Plant Biol.* 63:507-533.
- Rao, D., and S. Sinha. 1993. Efficiency of mobilization of seed reserves in sorghum hybrids and their parents as influenced by temperature regimes. *Seed Res.* 2:97-100.
- Reddy, K., H. Hodges, and V. Reddy. 1992c. Temperature effects on cotton fruit retention. *Agron. J.* 84:26-30.
- Reddy, K., H. Hodges, J. McKinion, and G. Wall. 1992b. Temperature effects on Pima cotton growth and development. *Agron. J.* 84:237-243.
- Reddy, K., V. Reddy, and H. Hodges. 1992a. Temperature effects on early season cotton growth and development. *Agron. J.* 84:229-237.
- Reddy, K.R., G.H. Davidonis, A.S. Johnson, and B.T. Vinyard. 1999. Temperature regime and carbon dioxide enrichment alter cotton boll development and fiber properties. *Agron. J.* 91:851-858.
- Reddy, K.R., H.F. Hodges, and J.M. McKinion. 1995. Carbon dioxide and temperature effects on pima cotton development. *Agron. J.* 87:820-826.
- Reddy, V., D. Baker, and H. Hodges. 1991. Temperature effects on cotton canopy growth, photosynthesis, and respiration. *Agron. J.* 83:699-704.

- Seyyedi, S.M., M. Khajeh-Hosseini, P.R. Moghaddam, and H. Shahandeh. 2015. Effects of phosphorus and seed priming on seed vigor, fatty acids composition and heterotrophic seedling growth of black seed (*Nigella sativa* L.) grown in a calcareous soil. *Ind. Crops Prod.* 74:939-949.
- Sikder, S., M. Hasan, and M. Hossain. 2009. Germination characteristics and mobilization of seed reserves in maize varieties as influenced by temperature regimes. *J. Agric. Rural Dev.* 7:51-58.
- Simpson, D. 1935. Dormancy and maturity of cottonseed. *J. Agr. Res.* 50:429-434.
- Singh, B., E. Norvell, C. Wijewardana, T. Wallace, D. Chastain, and K.R. Reddy. 2018. Assessing morphological characteristics of elite cotton lines from different breeding programmes for low temperature and drought tolerance. *J. Agron. Crop. Sci.* 4:467-476.
- Singh, B., K.R. Reddy, E.D. Redoña, and T. Walker. 2017a. Developing a screening tool for osmotic stress tolerance classification of rice cultivars based on in vitro seed germination. *Crop Sci.* 57:387-394.
- Singh, B., K.R. Reddy, E.D. Redoña, and T. Walker. 2017b. Screening of rice cultivars for morpho-physiological responses to early-season soil moisture stress. *Rice Sci.* 24: 322-335.
- Smith, C.W., and J. Varvil. 1984. Standard and cool germination tests compared with field emergence in upland cotton. *Agron. J.* 76: 587-589.
- Snider, J., G. Collins, J. Whitaker, K. Chapman, P. Horn, and T. Grey. 2014. Seed size and oil content are key determinants of seedling vigor in *Gossypium hirsutum*. *J. Cotton Sci* 18:1-9.
- Snider, J.L., G.D. Collins, J. Whitaker, K.D. Chapman, and P. Horn. 2016. The impact of seed size and chemical composition on seedling vigor, yield, and fiber quality of cotton in five production environments. *Field Crops Res.* 193:186-195.
- Soltani, A., M. Gholipoor, and E. Zeinali. 2006. Seed reserve utilization and seedling growth of wheat as affected by drought and salinity. *Environ. Exp. Bot.* 55:195-200.
- Soltani, A., S. Galeshi, E. Zeinali, and N. Latifi. 2002. Germination, seed reserve utilization and seedling growth of chickpea as affected by salinity and seed size. *Seed Sci. Technol.* 30: 51-60.
- Srivastava, L. 2002. Seed germination, mobilization of food reserves, and seed dormancy. In: L.M. Srivastava, editor, *Plant growth and development: hormones and environment*, Academic Press, London, UK. p. 447-471.
- Steiner, J., and T. Jacobsen. 1992. Time of planting and diurnal soil temperature effects on cotton seedling field emergence and rate of development. *Crop Sci.* 32:238-244.
- Sung, J. 1996. Lipid peroxidation and peroxide-scavenging in soybean seeds during aging. *Physiologia Plantarum* 97:85-89.
- Tesfay, S., A. Modi, and F. Mohammed. 2016. The effect of temperature in moringa seed phytochemical compounds and carbohydrate mobilization. *South Afr. J. Bot.* 102:190-196.

- Turley, R.B., and K.D. Chapman. 2010. Ontogeny of cotton seeds: gametogenesis, embryogenesis, germination, and seedling growth. In: J.M. Stewart, D.M. Oosterhuis, J.J. Heiholt and J.R. Mauney, editors, *Physiology of cotton*. Springer, New York. p. 332-341.
- Vertucci, C.W. 1989. The kinetics of seed imbibition: controlling factors and relevance to seedling vigor. In: P.C. Stanwood and M.B. MacDonald, editors, *Seed moisture*. CSSA Special Publication No. 14. CSSA, Madison, WI. p. 93-115.
- Vincent, S., A. Selvakumari, and S. Kalaivani, S. 2010. Dormancy breaking treatments in rice, wheat, oats, cotton, groundnut, sunflower, pulses, bhendi and gourds. In: S. Kalaivani, G. Sasthri, A. Selvakumari, P. Srimathi, and K. Vanangamudi. editors, *Seed quality enhancement: principles and practices*. Scientific Publishers, Jodhpur, India. p. 44-65.
- Wang, J.J., W.W. Ye, D.Y. Zhou, Y.J. Lü, B.X. Fan, and L.Y. Song. 2007. Studies on germination characteristics of different salinity-resistant cotton under salt stress. *Cotton Sci.* 19:315-317.
- Wanjura, D., and D. Buxton. 1972a. Water uptake and radicle emergence of cottonseed as affected by soil moisture and temperature. *Agron. J.* 64:427-431.
- Wanjura, D., E. Hudspeth and J. Bilbro. 1969. Emergence time, seed quality, and planting depth effects on yield and survival of cotton (*Gossypium hirsutum* L.). *Agron. J.* 61:63-65.
- Wanjura, D.F. 1986. Field environment and stand establishment. In: J.R. Mauney and J.McD. Stewart, editors, *Cotton physiology*. The Cotton Foundation. Memphis, TN. p. 551-554.
- Wanjura, D.F., and D.R. Buxton. 1972b. Hypocotyl and radicle elongation of cotton as affected by soil environment. *Agron. J.* 64:431-434.
- Wanjura, D.F., D.R. Buxton, and H.N. Stapleton. 1971. The effect of temperature, soil moisture, and physical impedance. *Cotton: A College of Agriculture Report*. College of Agriculture, Univ. of Arizona, Tucson.
- Weitbrecht, K., K. Müller, and G. Leubner-Metzger. 2011. First off the mark: early seed germination. *J. Exp. Bot.* 62:3289-3309.
- Wierzbicka, M., and J. Obidzińska. 1998. The effect of lead on seed imbibition and germination in different plant species. *Plant Sci.* 137:155-171.
- Woodstock, L. 1988. Seed imbibition: a critical period for successful germination. *J. Seed Technol.* 12:1-15.
- Wrather, J. A., B.J. Phipps, W.E. Stevens, A.S. Phillips, and E.D. Vories. 2008. Cotton planting date and plant population effects on yield and fiber quality in the Mississippi Delta. *J. Cotton Sci.* 12:1-7.
- Xie, D., H. Wang, F. Wang, and F. Feng. 2000. Effects of cotton seeds germination and seedling growth under salt stress. *Chin. Cotton* 27:12-13.

Chapter 7

TEMPERATURE, WATER STRESS AND PLANTING DEPTH EFFECTS ON COTTON SEED GERMINATION PROPERTIES

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INTRODUCTION

Seed germination and seedling growth are exceptionally sensitive to environmental variability. Subsequently, these stages in plant development are critical to successful establishment, survival and crop performance. The increased frequencies of environmental stresses due to climate change have made significant impacts on the world's agricultural production. Anthropogenic and natural factors influence global surface air temperature, which increased by 0.2°C per decade between 1950 and 1993 and is projected to increase by 2 to 4.5°C by 2100 (Meehl et al., 2007). The changes in temperature associated with climate change are associated with changes in the precipitation patterns (Giorgi and Lionello, 2008) as drought affected areas are expanding at an accelerating rate (Delmer, 2005). Changes in the environment modify growth, physiological and biochemical processes in cotton at different stages of development (Gibson and Mullen, 1996; Oosterhuis, 1999; Singh et al., 2008).

Seed germination is a complex biological process commencing with the uptake of water by the quiescent dry seed and concluding with elongation of the embryonic axis (Bewley, 1997). The mechanism of seed germination involves timing of water absorption, membrane reorganization, metabolic restructuring and cell expansion (Hake et al., 1990; Toole et al., 1956). In the first phase of germination, uptake of water and enzyme activation in the endosperm leads to cell elongation and subsequent radical lengthening (Bewley, 1997; Obroucheva, 1999). The splitting of the seed coat allows oxygen to penetrate the seed during the second phase. At this stage, stored endospermic reserves are metabolized and synthesized enzymes stimulate the loosening of cell walls, which leads to further cell elongation and increase in volume. During the third phase, the seedling emerges by rupturing the seed coat. This process is followed by rapid cell elongation and cell division. After radicle emergence, the embryo can access water and nutrients from the environment which leads to a continuous increase in seedling dry weight (Bewley and Black, 1985).

Several growth and developmental processes are affected by ambient growing temperature. In the seed germination process, temperature plays an important role in the removal of dormancy and determination of the germination capacity (Christiansen, 1967; Roundy and Biedenbender, 1996). Temperature response varies across and within species (Farooq et al., 2004; Larsen and

Andreasen, 2004; Ungar, 1978). During seed germination, the increase in thermal activity of chemically reacting molecules is mainly due to an increase in thermal response between minimum and optimum temperatures (Probert, 1992; Roberts, 1988). At high temperature above optimal conditions, there is molecular dysfunction caused by alteration in protein and physiological effects of temperature on membrane components (Hill and Luck, 1991; Hsu et al., 1985; Moot et al., 2000). Exceptionally high or low soil temperature conditions relative to optimum during planting period therefore influences the germinative capacity of the seed.

Water availability is the most critical factor, since water drives germination and emergence (Hegarty, 1978; Vertucci et al., 1989). Several studies have successfully simulated the effects of osmotic stress on seed germination properties using polyethylene glycol (PEG) as an osmoticum (Murillo-Amador et al., 2002; Okcu et al., 2005). Germination rates generally decrease with decreasing water potential (Emmerich and Hardegee, 1990). Seeds generally experience periods of alternating dry and wet conditions (Baskin and Baskin, 1982; Berrie and Drennan, 1971) due to periodic nature of precipitation (Groisman et al., 2005).

Modeling is a powerful approach to understanding complexity of biological systems (Meng et al., 2004). Crop models are developed for various purposes of predicting plant growth, development, yield testing and decision support through synthesis of plant genetics, physiology, and environmental interactions (Boote et al., 1998). Biological systems modeling is based on quantifying the environmental effect on various plant physiological and phenological processes from sowing to maturity (Reddy et al., 1997). The extent, uniformity, and speed of germination are important attributes of all seeds, and these parameters can be modified by existing environmental conditions. The germination rate and germination percentage are two important parameters seeds and affected by temperature and moisture. Studies conducted by Gracia-Huidobro et al in (1982) and Schimpf et al. (1977) reported positive correlation of germination rate and percentage with temperature up to an optimum. After this point, the rate of germination decreases at maximum temperature to zero (Ellis et al., 1986). The effect of a specific environmental factor on germination is commonly characterized by a sigmoidal curve, relating the germination percentage to time and quantified by standard normal distribution (Janssen, 1973). The purpose of these studies were to test alternative modelling schemes for estimating cotton seed germination capacity and rate response to temperature, water stress and planting depth.

TEMPERATURE EFFECTS ON COTTON SEED GERMINATION

Seed germination is a complex process involving many individual reactions and phases, each of which is affected by temperature. The temperature effect on germination can be expressed in terms of 3 cardinal temperatures: minimum, optimum, and maximum temperatures at which germination can occur. The minimum temperature is sometimes difficult to define since germination may actually be proceeding but at such a slow rate that determination of germination is often made before actual germination is completed. The optimum temperature may be defined as the temperature resulting in the greatest percentage of germination in the shortest period of time. The optimum temperature for most seeds is between 15 and 30°C. The maximum temperature is governed by the temperature at which essential proteins for germination are denatured. The maximum temperature for most species is between 30 and 40°C. This drastic over-simpli-

fication of; therefore, the temperature response may change throughout the germination period because of the complexity of the germination process.

The response to temperature depends on a number of factors, including the species, variety, growing region, quality of the seed, and duration of time from harvest. As a general rule, temperate-region seeds require lower temperatures than do tropical-region seeds, and wild species have lower temperature requirements than do domesticated plants. High-quality seeds are able to germinate under wider temperature ranges than low-quality seeds. The severity of the effects that cool temperatures have on the cotton plant varies according to the age of the plant and the duration of exposure to low temperatures.

The successful emergence and initial growth of cotton seedlings is important for the establishment of healthy and improved productivity. Wanjura and Buxton, (1972 a, b) showed that when the minimum soil temperature at planting depth dropped from approximately 20°C to 12°C, the hours required for initial seedling emergence increased from 100 to approximately 425 hours. In many cotton-growing areas the soil temperature can be significantly lower than the optimum when seeds are planted thus impacting the final yield.

Protocol and Curve Fitting Techniques to Quantify Seed Germination Related Parameters

Availability of heat tolerant cotton cultivars, in particular to seed germination, can overcome high temperature related germination problems. Similarly, the identification of cold tolerant cultivars becomes important for cold weather conditions. The rate of both germination and emergence significantly decreases at low temperatures. Thermal time (degree-day or hour), the heat unit for plant development is a well established developmental principle for plants. The thermal time model has been successfully used to predict seed germination under non-water limiting conditions. According to the model, based on many crops, the existing soil temperature determines both the maximum seed germination (MSG) and the rate of germination (SGR). Using this model, cardinal temperatures (T_{opt} , T_{max} , and T_{min}) for both MSG and SGR, and maximum SGR and MSG can be identified. Such thermal responses from each cultivar are used to identify temperature tolerance.

Time-series seed germination data at various temperature treatments was carried out as a simple experiment to determine above parameters related to seed germination of given cotton cultivars. Seeds in replicates of 4 containing 100 seeds each were placed in a 9-cm sterilized plastic tray layered with two sheets of moistened, vaslin clothes and then placed in incubator (Fisher Scientific, Suwanee, GA) in the dark under a range of temperatures from 5 to 55°C at 5 °C intervals. The trays were covered to minimize moisture loss and the vaslin clothes containing seeds were moistened with distilled water daily, as needed. Germinated seeds were counted, recorded, and discarded every six hours. A seed is considered germinated when the radical length is equal or longer than the diameter of the seed. Temperature and cumulative seed germination time-course data were fitted with a 3-parameter sigmoidal function given below (Equation 01; Figure 1) using Sigma Plot 1.

$$Y = SG_{max} / \{1 + \exp[-(x - x_{50})/Grate]\} \quad [1]$$

This function estimates a , the maximum cumulative germination percentage (germination capacity); b , the shape and steepness of the curve; and x_0 , time to reach germination half maximal (time to 50% of maximum germination).

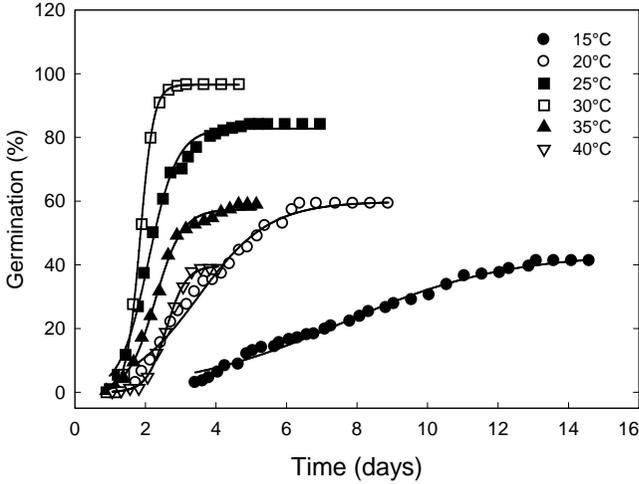


Figure 1. Observed (symbols) and predicted (lines) germination time course of cotton germinated at a range of temperatures (15 to 40°C). The symbols indicate the observed cumulative germination and the lines indicate the germination time courses fitted using a three-parameter sigmoidal function. Data are means and \pm SE of four replications.

The rate of development was derived by the reciprocal of time to 50% of maximum seed germination, T_{50} . The maximum percentage germination and the reciprocal over time to 50% germination (GR) were used for further analysis to determine cardinal temperatures explained below.

Both linear and nonlinear models can be used to analyze maximum seed germination (MSG) and germination rate (GR) responses to temperature. The best models are determined based on the overall highest coefficient of determination (R^2) and the least root mean square error (RMSE) values using non-linear regression procedure. Based on these criteria, the model which best described the MSG responses and seed GR responses to temperature can be estimated by linear or non-linear regression procedures using a statistical software (PROC NLIN in SAS) by a modified Newton Gauss iterative method (Garcia-Huidobro et al., 1982). The modified bilinear model was best fit for MSG. Optimum temperature (T_{opt}) in modified bilinear model was generated using [Eq. 2], by PROC GLM in SAS, while T_{min} and T_{max} were estimated by the following equations [3, 4 and Figure 2].

$$SGR = a + b_1 (T - T_{opt}) + b_2 \times ABS (T_{opt} - T) \quad [2]$$

$$T_{min} = [a + (b_2 - b_1) \times T_{opt}] / b_1 - b_2 \quad [3]$$

$$T_{max} = [a - (b_2 + b_1) \times T_{opt}] / b_1 + b_2 \quad [4]$$

Where T is the treatment temperature and a , b_1 , and b_2 are cultivar specific constants generated (PROC NLIN in SAS). A mean curve is fitted to MSG to determine the parameter estimates. The above proposed protocol based on sigmoidal curves and subsequent model fitting provide a valuable approach to determine temperature related cotton seed germination parameters.

The quadratic model was the best fit model for SGR. For the quadratic function model [Eq.

5], the three cardinal temperatures (T_{min} , T_{opt} , and T_{max}) can be estimated using the following equations [6, 7, and 8 and Figure 2].

$$MSG = a + bT - cT^2 \tag{5}$$

$$T_{opt} = -b / (2c) \tag{6}$$

$$T_{min} = -b + (\sqrt{b^2 - 4ac}) / 2c \tag{7}$$

$$T_{max} = -b - (\sqrt{b^2 - 4ac}) / 2c \tag{8}$$

Where T is the treatment temperature at which MSG were determined for each cultivar and a , b , and c are cultivar specific constants generated by statistical software (PROC GLM by SAS).

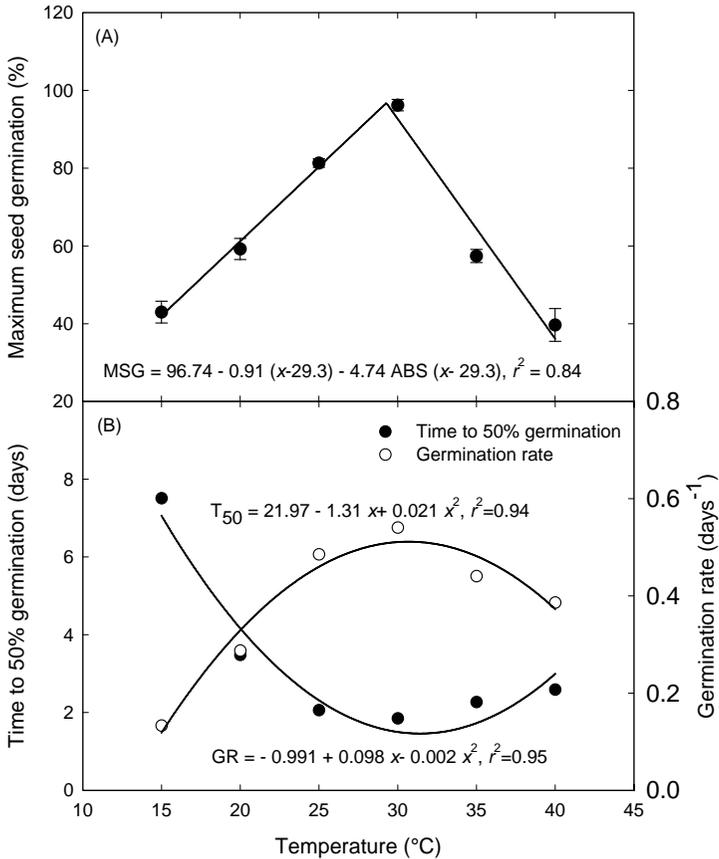


Figure 2. Temperature effects on (A) maximum seed germination for cotton and (B) time to 50% of maximum seed germination and germination rate for cotton along with the fitted linear and quadratic equations. In (A), the symbols are recorded maximum seed germination and the lines are fitted using modified bilinear equations. In (B), the symbols are derived time to 50% of maximum seed germination and germination rates and the lines are fitted using quadratic equations. Data are means and \pm SE of six replications.

Germination of cotton seed is favored by high soil oxygen concentration, adequate soil moisture, and soil temperature above 17.5°C. Temperature affects the seed germination process (percentage and rate) through at least three separate physiological processes namely (1) seed continuously deteriorate and rate of deterioration depends on both moisture content and temperature, (2) seeds are initially dormant and relatively dry seeds continuously lose dormancy at a rate which is temperature dependent. The rate of losing viability however remains constant over a wide range of temperatures, and (3) once seeds have lost dormancy; their seed germination rate shows a positive linear relation between the base temperature and the optimum temperature and negative linear relationship between the optimum temperature and the ceiling temperature.

Temperature effects on cotton seed germination was extensively studied under laboratory conditions. According to Toole and Drummond (1924), cotton seed showed fast germination at 25°C as well as 15–36°C alternating temperatures. The optimum temperature for both germination rate and percentage lies at alternating temperatures of 15 and 36°C. According to the modified bilinear function which describes the response of MSG to temperature, T_{opt} for MSG lies around 29.3°C and T_{opt} for SGR lies around 31.1°C. A recent study by Krzyzanowski and Delouche (2011) reported that the optimum temperature for cotton seed germination lies between 28 to 30°C.

Even though cotton can withstand many stresses, planting at optimum temperature conditions producers can get the maximum emergence and survival. During the critical germination period, soil temperatures below 10°C can cause chilling injury to germinating cotton seed resulting in malformed seedlings, loss of the tap roots resulting reduced vigor and stand establishment, and the increased seedling diseases. The factors affecting cotton seed germination including temperature is well documented, any interaction between temperature and other factors and their influence on seed germination require more investigations.

Temperature and Root Growth

The temperature of the soil can have a significant influence on the growth of cotton root systems. Most research has shown that in general, the growth of cotton roots increases with increasing soil temperature until an optimal temperature is reached beyond which growth declines. Early work suggested that the optimal soil temperature for the growth of cotton roots was approximately 35°C (Bloodworth, 1960; Lety et al., 1961; Pearson et al., 1970; Taylor et al., 1972). Kaspar and Bland (1992) indicted that changes in soil temperature can affect growth of a number of root system components. For example, low temperatures generally reduced cotton root branching (Brower and Hoagland, 1964), while higher temperatures approaching the optimum tend to increase branching (Nielsen, 1974). When the root temperature was low (20°C), root growth was reduced regardless of the temperature of the air (shoot) (McMichael and Burke, 1994). The cotton taproot may be more adapted to adverse soil temperatures than subsequent branch roots at least until the taproot had developed to approximately 10 cm in length (Arndt, 1945; McMichael and Burke, unpublished data). Genetic diversity has been shown to exist in cotton for root development between genotypes (Bourland et al., 1985; Quisenberry and McMichael, 1996).

Effect of Low Temperature on The Root System

Cotton is often planted into cool, wet soils in the US eastern and midsouth regions, leading to problems in germination, emergence, root growth and subsequent seedling development. Christiansen (1967) found that the cotton seedling is hypersensitive to chilling at certain growth stages. One of these stages is when the seed is imbibing water, and the other is 18 to 30 hours after germination when the radicle and the hypocotyl elongate. In these chilled conditions root membranes lose permeability, and this lack of control in the membranes reduces water uptake by plants (Kramer, 1942; Yoshida and Eguchi, 1990). Chilling effects are expressed as reduced vigor, abnormal growth, and death of the seedling (Kaspar et al., 1981). Chilling injury might result in plant stunting later in the life of the plant because, during early germination and hydration, activation of RNA synthesis and active protein synthesis occur (Christiansen, 1967). Lateral root growth is reduced in cool temperatures as cotyledon storage is depleted (McMichael and Burke, 1998), which could retard the growth of the seedling. Also, low temperatures result in a reduced leaf expansion of the plant by slowing metabolism and reducing the size of the plant at harvest (Christiansen and Thomas, 1969). Leaf area of the cotyledons and true leaves of cotton are negatively affected by root exposure to cool temperatures, while the stem is least affected, since it recovers turgidity more rapidly than other components (Nelson, 1967). Another effect of cool temperature on roots is chilling injury, where the taproot is swollen and aborted and described as “nub rooted” (Cole and Christiansen, 1975). The swollen root structure is caused by a buildup of starch and sugars at low temperatures due to slow metabolism (Schrier et al., 2000; Hesketh and Wiginton, 1980). In nub root, when the taproot is aborted, the lateral roots emerge from the swollen hypocotyl to bring nutrients and water to the plant. Aborted root meristem damage is not as serious as injury to the cortex, because the lateral root growth supplements the taproot functions (Christiansen, 1963). Low temperatures can cause a reduction in water uptake by decreased permeability in seedling root systems (Kramer, 1940) and a reduction in cell expansion in cotton (Quisenberry et al., 1994; Nelson, 1967). Guinn and Hunter (1968) also reported that low root temperatures caused rapid increases in sugar contents of all plant parts (leaves, epicotyls, hypocotyls and roots). Hypocotyls contained the most sucrose whereas epicotyls contained the most glucose, fructose and total sugars. Roots contained the lowest concentrations of glucose and fructose. In addition, low root temperatures increased the starch contents of leaves, epicotyls and hypocotyls, except that a root temperature of 10C caused wilting and a very low starch content of the leaves. The authors attributed the increase in carbohydrate concentration to altered respiration rates due to lower than optimum temperatures. Once the roots have been established and the seedling emerged, 35°C is the optimal temperature for 10 days after planting (McMichael and Burke, 1994). The metabolism of the roots and ability to take up water and nutrients are hindered if the seedling is grown in less than optimal temperatures (Nelson, 1967, 1974). Roots are also highly vulnerable to pathogens during this time of slow metabolism and stress (McMichael and Burke, 1998). Genetic diversity has been shown to exist in cotton for root development between genotypes (Bourland et al., 1985; Quisenberry and McMichael, 1996). Mills et al. (2012) also reported significant variation in response to low temperatures of a number of root parameters such as lateral root number, length and branching after studying four genetically and geographically diverse cotton cultivars. Therefore, improvement of cold tolerant cotton should be possible. If the root system is better adapted to a cool environment, the

uptake of nutrients and water will improve, and therefore, the metabolism and maturity level of the plant would increase (McMichael et al., 2010). An earlier planting time and an increased metabolism should mean a greater profit for cotton producers as well as increased economic revenues for the U.S. economy.

WATER STRESS EFFECTS ON COTTON SEED GERMINATION

Drought tolerance is a complex quantitative multigenic trait with considerable intraspecific variation existing for cotton morphology and physiological processes (Cushman and Bohnert, 2000). Seed germination is a triphasic process initiating with imbibition (phase I) resulting from the water potential gradient between the seed and imbibition solution, followed by a plateau phase with minimal changes in water content (phase II) and culminating in an increase in water content coinciding with radicle emergence (Bradford, 1990; Wanjura and Buxton, 1972a). Water stress influences the rate of each phase thereby reducing the rate of water uptake, germination rate, and germination percentage (Heikal and Shaddad, 1982). In addition, water availability influences cotton emergence, seedling vigor, stand establishment and ultimately crop performance. Water stress induced by the long chain polymer, inert, non-ionic and highly water soluble polyethylene glycol (PEG) has been successfully used to study physiological processes in cotton (Nepomuceno et al., 1998).

Using seed germination parameters such as maximum seed germination and germination vigor, Meneses et al. (2011) classified genotypes based on their sensitivity to water stress. Water stress was simulated with PEG 8000 using Michel's (1983) equations to produce solutions with the desired osmotic potential. Cotton seeds were placed in covered trays with PEG saturated germination paper which was replaced twice weekly to prevent the accumulation of PEG. Cumulative seed germination time series data were fitted similarly to the temperature study previously described. Cotton germination did not occur at -1.2 or -1.4 MPa and was completed by day 12 (Figure 3).

Lower osmotic potential inhibited seed germination by reducing the movement and imbibition of water by the germinating seed. Previous studies have also reported complete inhibition of germination at -0.1 MPa osmotic potential (Heikal and Shaddad, 1982; Meneses et al., 2011; Murungu et al., 2005). As osmotic potential decreased, the maximum seed germination decreased while the time required for the onset of germination increased with decreasing osmotic potential (Figure 3). The elongation of the hypocotyls is more sensitive to low soil moisture than the radicle elongation. At -0.3 MPa, hypocotyl length increased to 2.5 cm compared to no elongation at -1.0 MPa after 120 hours of imbibition (Wanjura and Buxton, 1972b). The radicle length at -0.03 MPa is 118.3 cm, as soil moisture stress increased to -0.3 MPa, radicle length increased to 29.7 cm and decreased to 22.5 cm at -1.0 MPa. The hypocotyl:radicle ratio decreases with increased moisture stress from 0.43, 0.21 and 0.15 at -0.03, -0.3 and -1.0 MPa, respectively indicating that in inadequate soil moisture conditions, the development of the radicle supersedes the hypocotyl development (Meneses et al., 2011; Wanjura and Buxton, 1972b). The decrease in the rate of hypocotyl elongation is attributed to the disparity between the flux of germination factors to the embryo and the reaction rates which are producing growth (Wanjura and Buxton, 1972b).

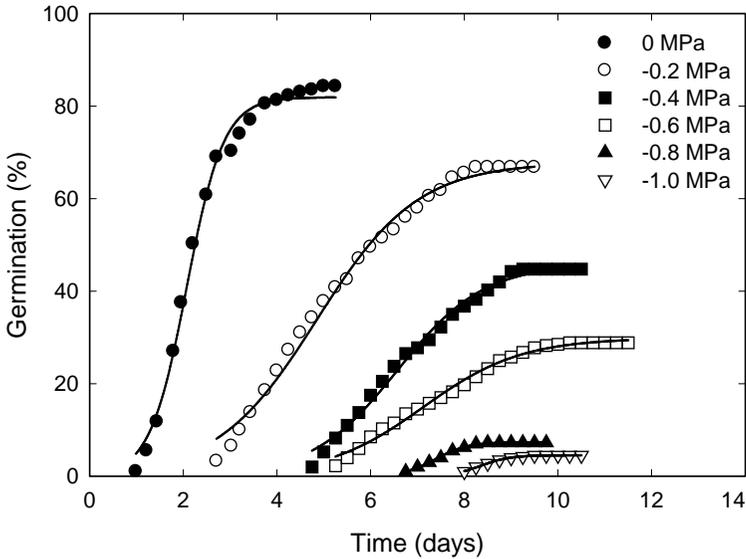


Figure 3. Observed (symbols) and predicted (lines) germination time course of cotton germinated at a range of osmotic potentials (0 to -1.4 MPa). Cotton did not germinate at -1.2 or -1.4 MPa. The symbols indicate the observed cumulative germination and the lines indicate the germination time courses fitted using a three-parameter sigmoidal function. Data are means and \pm SE of six replications.

This is related to the rate of water absorption which decreases with increased osmotic stress (Heikal and Shaddad, 1982; Wanjura and Buxton, 1972a). For example, at -0.03, -0.1, -0.3 and -1.0 MPa soil moisture, the seed-moisture percentage at 3 mm radicle emergence was 79.3, 76.1, 77.0 and 73.4% at 32.2°C, respectively. This relationship between imbibition and soil moisture was influenced by temperature (Wanjura and Buxton, 1972a). Normal seedling development also decreases with osmotic potential (Meneses et al., 2011).

Maximum seed germination was estimated from each sigmoidal curve fitted to the time series response to osmotic potential. MSG declined linearly with osmotic potential with each incremental decrease in osmotic potential decreasing the maximum seed germination by 40%, similar to Heikal and Shaddad (1982) and Murungu et al. (2005). The time to achieve 50% germination increased quadratically with osmotic potential (Figure 4). For example, at -0.4 MPa, cotton required 6.2 days to achieve 50% germination, as the osmotic potential increase to -0.6 MPa, the time to 50% germination increased to 7.3 days. Median germination at the extreme osmotic potential (-1.0 MPa) was 8 days. At optimum temperatures, germination occurred at 54, 118 and 209 hours at -0.03, -0.3 and -1.0 MPa, respectively.

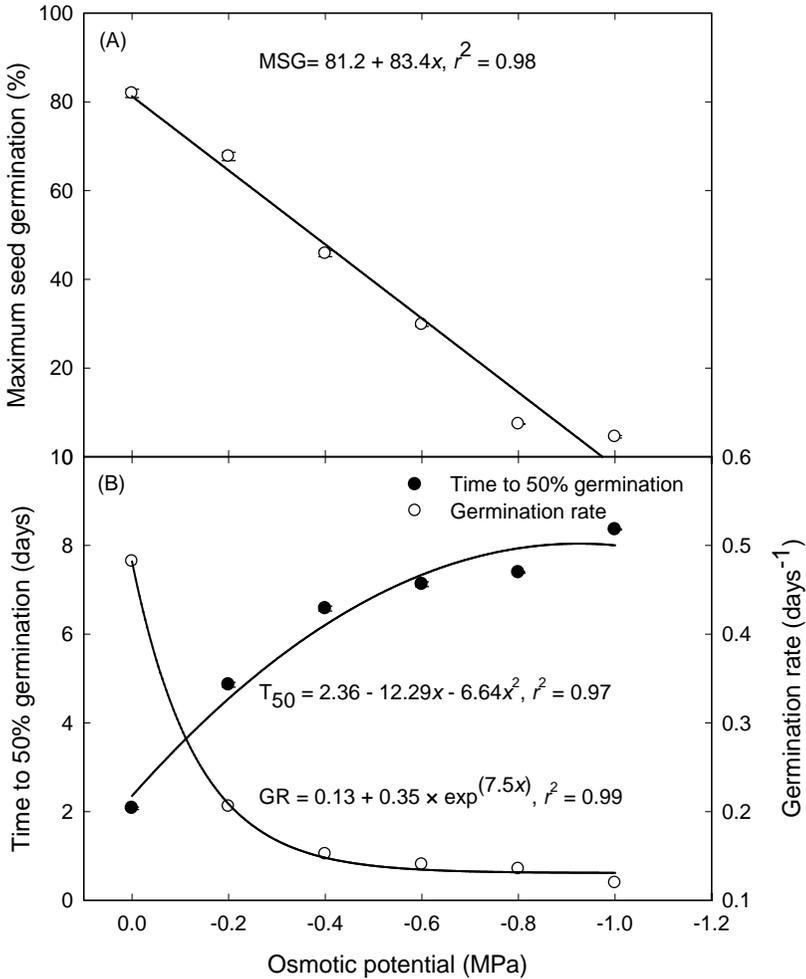


Figure 4. Osmotic potential effects on cotton (A) maximum seed germination for cotton and (B) time to 50% of maximum seed germination and germination rate. In (A), the symbols are recorded maximum seed germination and the lines are fitted using linear equation. In (B), the symbols are derived time to 50% of maximum seed germination and germination rates and the lines are fitted using quadratic and exponential equations. Data are means and \pm SE of six replications.

Germination rate is the inverse of the median germination ($GR = 1/T_{50}$) and represents the daily rate of germination. An exponential decay function best described the relationship between germination rate and osmotic potential. The germination rate at 0 MPa was 0.5 declining sharply to 0.2 at -0.2 MPa and remained relatively constant at osmotic potential beyond -0.6 MPa (Figure 4), similar to Murungu et al. (2005) results.

Under conditions of water stress, germination is delayed and this can have implications for the rate of germination and uniformity of emergence and establishment. The maximum seed germination and the germination rate decreased with decreasing osmotic potential while the time to 50% germination increased with increasing osmotic stress. The functions developed can be used in modeling cotton germination potential under variable soil moisture conditions.

PLANTING DEPTH EFFECTS ON COTTON SEED GERMINATION

In addition to temperature and water stress, the planting depth also affects the germination and emergence of cotton. Usually, cotton is seeded from 1.3 to 2.5 cm depending on the soil moisture levels and temperature. Planting deeper in crusted seed beds or early in the season with frequent rains reduces germination rates and may require replanting. Increasing seeding depth increases the heat requirement for seedling emergence (De Jong and Best, 1979), emergence time (Nasr and Selles, 1995), and the time for 50% emergence (Loeppky et al., 1989), in addition to decreasing the emergence rate (Hucl and Baker, 1990).

Cotton seeds were germinated at different planting depths from 1.3 to 6.4 cm and seedling emergence was monitored every 6 h. Using the cumulative seed emergence, sigmoidal curves and maximum emergence and time to 50% emergence were extracted similarly as described previously. The time required for the onset of seedling emergence increased with increasing planting depth (Figure 5). As seeding depth increased, the maximum seedling emergence decreased from 87% at 0.25 cm to 71% at 6.4 cm. Seedling emergence was similar for the 2.5, 3.8 and 5.1 cm and averaged 83% emergence (Figure 5). The time to 50% emergence increased linearly with planting depth from 4.9 days at 1.3 cm to 7.9 days at 6.4 cm. On the contrary, emergence rate decreased linearly from 0.21 at 1.3 cm to 0.13 at 6.4 cm (Figure 5). These trends are similar to those previously reported (Hucl and Baker, 1990; Loeppky et al., 1989; Nasr and Selles, 1995).

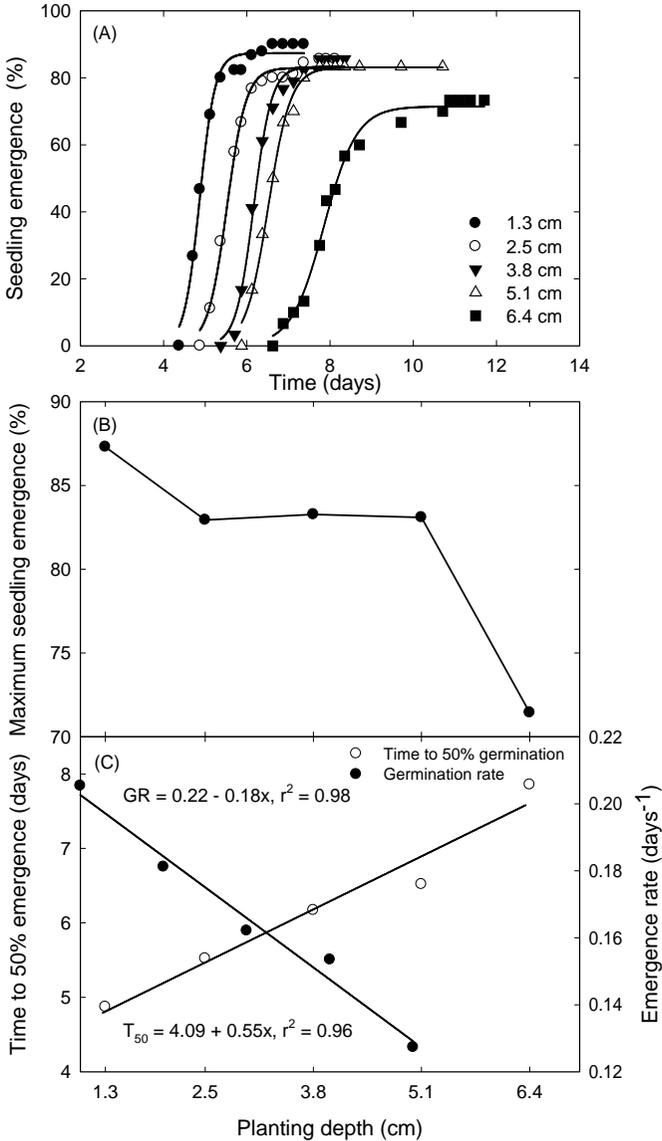


Figure 5. (A) Observed (symbols) and predicted (lines) emergence time course of cotton emerged at a range of planting depth (1.3 to 6.4 cm). The symbols indicate the observed cumulative emergence and the lines indicate the emergence time courses fitted using a three-parameter sigmoidal function. (B) Maximum seedling emergence for cotton. The symbols are recorded maximum seedling emergence. (C) Time to 50% of maximum seedling emergence and emergence rate. The lines are fitted using linear equations while the symbols are derived time to 50% of maximum seedling emergence and emergence rate. Data are means and \pm SE of six replications.

REFERENCES

- Arndt, C.H. 1945. Temperature-growth relations of the roots and hypocotyls of cotton seedlings. *Plant Physiol.* 20:200-219.
- Baskin, J.M., and C.C. Baskin. 1982. Effects of wetting and drying cycles on the germination of seeds of *Cyperus inflexus*. *Ecol.* 63:248-252.
- Berrie, A.M.M., and D.S.H. Drennan. 1971. The effect of hydration-dehydration on seed germination. *New Phytol.* 70:135-142.
- Bewley, J.D. 1997. Seed germination and dormancy. *Plant Cell* 9:1055-1066.
- Bewley, J.D., and M. Black. 1985. *Seeds- physiology of development and germination*. Springer, New York, NY.
- Bloodworth, M.E. 1960. Effect of soil temperature on water use by plants. *Transactions Seventh Int. Congr. Soil Sci.* 1:153-163.
- Boote K.J., J.W. Jones, and G. Hoogenboom. 1998. Simulation of crop growth: Cropgro model. In: R.M. Peart and R.B. Curry, editors, *Agricultural systems modeling and simulation*. Marcel Dekker, New York, NY. p. 651-692.
- Bourland, F.M., G.A. Mitchell, and B.W. White. 1985. Effects of selection for tolerance to trifluralin in cotton. p. 200-202.
- Bradford, K.J. (1990) A water relations analysis of seed germination rates. *Plant Physiol.* 94:840-849.
- Brouwer, R., and A. Hoagland. 1964. Responses of bean plants to root temperatures: anatomical aspects. *Meded. Inst. BioI. Scheik. Onderz. Land. Gewass.* 236:23-31.
- Christiansen, M. 1967. Periods of sensitivity to chilling in germinating cotton. *Plant Physiol.* 42:431-433.
- Christiansen, M.N. 1963. Influence of chilling upon seedling development of cotton. *Plant Physiol.* 38:520-522.
- Christiansen, M.N., and R.O. Thomas. 1969. Season-long effects of chilling treatments applied to germinating cottonseed. *Crop Sci.* 9:672-673.
- Cole, D.F., and M.N. Christiansen. 1975. Effect of chilling duration on germination of cotton seed. *Crop Sci.* 15:410-412.
- Cushman, J.C., and H.J. Bohnert. 2000. Genomic approaches to plant stress tolerance. *Curr. opin. plant biol.* 3:117-124.
- De Jong, R., and K. Best. 1979. The effect of soil water potential, temperature and seeding depth on seedling emergence of wheat. *Can. J. Soil Sci.* 59.
- Delmer, D.P. 2005. Agriculture in the developing world: Connecting innovations in plant research to downstream applications. *Proceedings of the National Academy of Sciences, United States of America.* 102:15739-15746.

- Ellis R., S. Covell, E. Roberts, and R. Summerfield. 1986. The influence of temperature on seed germination rate in grain legumes II. Intraspecific variation in chickpea (*Cicer arietinum* L.) at constant temperatures. *J. Exp. Bot.* 37:1503-1515.
- Emmerich, W.E., and S.P. Hardegree. 1990. Polyethylene glycol solution contact effects on seed germination. *Agron. J.* 82:1103-1107.
- Farooq M., S. Basra, K. Hafeez, and E. Warriach. 2004. The influence of high and low temperature treatments on the seed germination and seedling vigor of coarse and fine rice. *Int. Rice Res. Notes* 29:75-77.
- Garcia-Huidobro, J., J. Monteith, and G. Squire. 1982. Time, temperature and germination of pearl millet (*Pennisetum typhoides* s. & h.) i. Constant temperature. *J. Exp. Bot.* 33:288-296.
- Gibson, L., and R. Mullen. 1996. Soybean seed quality reductions by high day and night temperature. *Crop Sci.* 36:1615-1619.
- Giorgi, F., and P. Lionello. 2008. Climate change projections for the mediterranean region. *Global Planet Change* 63:90-104.
- Groisman, P.Y., R.W. Knight, D.R. Easterling, T.R. Karl, G.C. Hegerl, and V.N. Razuvaev. 2005. Trends in intense precipitation in the climate record. *J. Climate* 18:1326-1350.
- Guinn, G., and R.E. Hunter. 1968. Root temperature and carbohydrate status of young cotton plants. *Crop Sci.* 8:67-70.
- Hake, K., W. McCarty, N. Hooper, and G. Jividen. 1990. Seed quality and germination. *Physiology Today*, Technical Services, National Cotton Council.
- Hegarty, T. 1978. The physiology of seed hydration and dehydration, and the relation between water stress and the control of germination: A review. *Plant Cell Environ.* 1:101-119.
- Heikal, M., Shaddad M. (1982) Alleviation of osmotic stress on seed germination and seedling growth of cotton, pea and wheat by proline. *Phyton (Aust.)* 22:275-287.
- Hesketh, J.D., and J.J. Wigington. 1980. Predicting photosynthesis for ecosystem models. pp. 5-6. *Photosynthesis - Simulation methods*. CRC Press. Boca Raton. FL
- Hil, I.M.J., and R. Luck. 1991. The effect of temperature on germination and seedling growth of temperate perennial pasture legumes. *Crop Pasture Sci.* 42:175-189.
- Hsu, F., C. Nelson, and A. Matches. 1985. Temperature effects on germination of perennial warm-season forage grasses. *Crop Sci.* 25:215-220.
- Hucl, P., and R. Baker. 1990. Effect of seeding depth and temperature on tillering characteristics of four spring wheat cultivars. *Can. J. Plant Sci.* 70:409-417.
- Janssen, J. 1973. A method of recording germination curves. *Ann. Botany* 37:705-708.
- Kaspar, T.E., and W.L. Bland. 1992. Soil temperature and root growth. *Soil Sci.* 145:290-299.
- Kramer, P.J. 1942. Species differences with respect to water absorption at low soil temperatures. *Am. J. Bot.* 29:828-832.
- Krzyzanowski, F.C., and J.C. Delouche. 2011. Germination of cotton seed in relation to temperature. *Revista Brasileira de Sementes* 33:543-548.

- Larsen, S.U., and C. Andreasen. 2004. Light and heavy turfgrass seeds differ in germination percentage and mean germination thermal time. *Crop Sci.* 44:1710-1720.
- Lety, J., L.H. Stolzy, G.B. Blank, and O.R. Lunt. 1961. Effect of temperature on oxygen-diffusion rates and subsequent shoot growth and mineral content of two plant species. *Soil Sci.* 92:314-321.
- Loeppky, H., G. Lafond, and D. Fowler. 1989. Seeding depth in relation to plant development, winter survival, and yield of no-till winter wheat. *Agron. J.* 81:125-129.
- McMichael, B.L., and J.J. Burke. 1998. Soil temperature and root growth. *Hort. Sci.* 33:947-951.
- McMichael, B.L., and J.J. Burke. 1994. Metabolic activity of cotton roots in response to temperature. *Environ. Exp. Bot.* 34:201-206.
- McMichael, B.L., and Oosterhuis, D.M. 2010. Growth and development of cotton root systems. pp. 57-71. In: J.M. Stewart, D.M. Oosterhuis, J. Heitholt, and J.R. Mauney, editors, *Cotton Physiology of Cotton*. Springer. ISBN 978-90-481-34194-5.
- Meehl, G.A., T.F. Stocker, W.D. Collins, P. Friedlingstein, A.T. Gaye, et al. 2007. Global climate projections. In: S. Solomon, et al., editors, *IPCC, 2007: Climate Change 2007: the physical science basis. contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, U.K. p. 747-845.
- Meneses, C.H.S.G., R.d.L.A. Bruno, P.D. Fernandes, W.E. Pereira, L.H.G.d.M. Lima, and M.S. Vidal. 2011. Germination of cotton cultivar seeds under water stress induced by polyethylene glycol-6000. *Sci. Agric.* 68:131-138.
- Meng, T.C., S. Somani, and P. Dhar. 2004. Modeling and simulation of biological systems with stochasticity. *In silico biol.* 4:293-309.
- Michel, B.E. 1983. Evaluation of the water potentials of solutions of polyethylene glycol 8000 both in the absence and presence of other solutes. *Plant Physiol.* 72:66-70.
- Mills, N., D.M. Oosterhuis, and B.L. McMichael. 2012. Seedling root growth response to cool environmental conditions of diverse cotton genotypes. *Am. J. Agric. Sci.* 2(3):269-287.
- Moot, D., W. Scott, A. Roy, and A. Nicholls. 2000. Base temperature and thermal time requirements for germination and emergence of temperate pasture species. *New Zeal. J. Agr. Res.* 43:15-25.
- Murillo-Amador, B., R. López-Aguilar, C. Kaya, J. Larrinaga-Mayoral, and A. Flores-Hernández. 2002. Comparative effects of nacl and polyethylene glycol on germination, emergence and seedling growth of cowpea. *J. Agron. Crop Sci.* 188:235-247.
- Murungu, F., P. Nyamugafata, C. Chiduzza, L. Clark, and W. Whalley. 2005. Effects of seed priming and water potential on germination of cotton (*Gossypium hirsutum* L.) and maize (*Zea mays* L.) in laboratory assays. *S. Afr. J. Plant Soil* 22:64-70.
- Nasr, H., and F. Selles. 1995. Seedling emergence as influenced by aggregate size, bulk density, and penetration resistance of the seedbed. *Soil Till. Res.* 34:61-76.

- Nielsen, K.F. 1974. Roots and root temperature. In: E.W. Carson, editor, The Plant and its environment. Univ. Press of Virginia, Charlottesville, VA. p. 293-335.
- Nelson, L.E. 1967. Effect of root temperature variation on growth and transpiration of cotton (*Gossypium hirsutum* L.) seedlings. Agron. J. 60:296-298.
- Nepomuceno, A.L., D.M. Oosterhuis, and J.M. Stewart. 1998. Physiological responses of cotton leaves and roots to water deficit induced by polyethylene glycol. Environ. Exp. Bot. 40:29-41.
- Obroucheva, N.V. 1999. Seed germination: A guide to the early stages. Backhuys Publishers, Leiden.
- Okcu, G., M.D. Kaya, and M. Atak. 2005. Effects of salt and drought stresses on germination and seedling growth of pea (*Pisum sativum* L.). Turk. J. Agric. For 29:237-242.
- Oosterhuis, D.M. 1999. Yield response to environmental extremes in cotton. Special reports - University of Arkansas Agricultural Experiment Station 193:30-38.
- Pearson, R.W., L.F. Ratliff, and H.M. Taylor. 1970. Effect of soil temperature, strength and pH on cotton seedling root elongation. Agron. J. 62:243-246.
- Perbert, R.J. 1992. The role of temperature in germination ecophysiology. In: M. Fenner, editor, Seeds: the ecology of regeneration in plant communities. CAB International, Wallingford, UK, p. 285-325.
- Quisenberry, J.E., and B.L. McMichael. 1996. Screening cotton germplasm for root growth potential. Environ. Exp. Bot. 36:333-338
- Quisenberry, J.E., L.D. McDonald, and B.L. McMichael. 1994. Response of photosynthetic rates to genotypic differences in sink-to-source ratios in upland cotton (*Gossypium hirsutum* L.). Environ. Exp. Bot. 34:245-252.
- Quisenberry, J.E., and B.L. McMichael. 1996. Screening cotton germplasm for root growth potential. Environ. Exp. Bot. 36:333-337.
- Reddy, K.R., H.F. Hodges, and J.M. McKinion. 1997. Crop modeling and applications: A cotton example. In: L.S. Donald, editor, Advances in agronomy, Academic Press. p. 225-290.
- Roberts, E.H. 1988. Temperature and seed germination. Symp. Soc. Exp. Biol. 42:109-32.
- Roundy, B.A., and S.H. Biedenbender. 1996 Germination of warm-season grasses under constant and dynamic temperatures. J. Range Manage. 49:425-431.
- Schimpf, D., S. Flint, and I. Palmblad. 1977. Representation of germination curves with the logistic function. Ann. Bot. 41:1357-1360.
- Schrier, A.A., G. Hoffmann-Thoma, and A.J.E. van Bel. 2000. Temperature effects on symplastic and apoplastic phloem loading and loading-associated carbohydrate processing. Aust. J. Plant Physiol. 27:769-778.
- Singh, S., V. Kakani, D. Brand, B. Baldwin, and K. Reddy. 2008. Assessment of cold and heat tolerance of winter-grown canola (*Brassica napus* L.) cultivars by pollen-based parameters. J. Agron. Crop Sci. 194:225-236.

- Taylor, H.M., and B. Klepper. 1971. Water uptake by cotton roots during an irrigation cycle. *Aust. J. Biol. Sci.* 24:853-859.
- Toole, E.H., and P.L. Drummond. 1924. The germination of cottonseed. *J. Agr. Res.* 28:285-295.
- Toole, E.H., S.B. Hendricks, H.A. Borthwick, and V.K. Toole. 1956. Physiology of seed germination. *Annu. Rev. Plant Physiol.* 7:299-324.
- Ungar, I.A. 1978. Halophyte seed germination. *Bot. Rev.* 44:233-264.
- Vertucci, C.W., P. Stanwood, and M. McDonald. 1989. The kinetics of seed imbibition: controlling factors and relevance to seedling vigor. In: P.C. Stanwood and M.B. McDonald, editor, *Seed moisture*. Crop Science Society of America, Madison, WI, p. 93-115.
- Wanjura, D., and D. Buxton. 1972a. Water uptake and radicle emergence of cottonseed as affected by soil moisture and temperature. *Agron. J.* 64:427-431.
- Wanjura, D., and D. Buxton D. 1972b. Hypocotyl and radicle elongation of cotton as affected by soil environment. *Agron. J.* 64:431-434.
- Yoshida, S., and H. Eguchi. 1990. Root temperature effect on root hydraulic resistance in cucumber (*Cucumis sativus* L.) and figleaf gourd (*Cucurbita ficifolia* B.) plants. *Biotronics.* 19:121-127.

Chapter 8

THE SEEDLING DISEASE COMPLEX ON COTTON

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INTRODUCTION

Cotton seedling diseases are a major factor affecting cotton (*Gossypium hirsutum* L.) stand establishment and production worldwide (DeVay, 2001; Hillocks, 1992; Melero-Vara and Jimenez-Diaz, 1990; Ogle et al, 1993). Cotton disease loss estimates for the United States over a ten-year period from 2003-2012 averaged 2.1% and loss estimates for seedling diseases accounting for 20% of the total estimated disease losses in lint production over these years, even with the use of fungicide seed treatments (Blasingame and Patel, 2013). Seedling diseases of cotton affect the germination, emergence, and early-season growth of cotton. In the field, the most obvious symptoms of cotton seedling diseases are the absence of plants, skips, in the planting row resulting from rotted seed or preemergence damping-off, the death of the seedling prior to emergence. In addition, seedlings may die within the first one to four weeks after planting from seedling diseases, postemergence damping-off. Poor stand uniformity has been shown to negatively affect yield. In the Rio Grande Valley of Texas, in single-drilled cotton, skips reducing plant populations by 25% lowered yields 13% and skips reducing plant populations by 40% lowered yields 26% (Heilman et al., 1976). Seedling diseases also may delay early-season crop growth and result in additional management problems. In severe disease situations replanting may be required.

A number of soilborne or seed-borne pathogens can affect cotton seedlings. These pathogens can act individually or in association with other pathogens as a part of the cotton seedling disease complex (Johnson, et al., 1969; Minton, et al., 1982; Rothrock et al., 2012). There have been more than 40 fungi isolated from diseased cotton seedlings, but only a few are considered primary pathogens (Hillocks, 1992). *Pythium* spp., *Rhizoctonia solani* Kühn, teleomorph *Thanatephorus cucumeris* (A.B. Frank) Donk, *Thielaviopsis basicola* (Berk. & Broome) Ferraris (syn. *Chalara elegans* Nag Raj & Kendrick) and *Fusarium* spp. are considered to be the primary pathogens of cotton seedlings (Colyer et al., 1991; Davis, 1975; DeVay, 2001; Fulton and Bollenbacher, 1959; Johnson et al., 1978; Melero-Vara and Jimenez-Diaz, 1990; Roy and Bourland, 1972; Rude, 1984). These pathogens cause a range of symptoms on roots and hypocotyls (DeVay, 2001; Fulton and Bollenbacher, 1959; Johnson et al., 1978; Roy and Bourland, 1972). All

of these groups of pathogens are ubiquitous in cotton soils, except *T. basicola*. Limited research has examined the importance of soil pathogen populations on disease development. DeVay et al. (1982) observed that survival of cotton seedlings from nontreated seed was negatively correlated with populations of *P. ultimum* in field soils. However, Davis et al. (1997) in California did not find an association between *R. solani* or *Pythium* soil populations and stands. In Tennessee, Johnson and Doyle (1986) also found no relationship between *Pythium* soil populations and isolation frequency or disease on cotton seedlings. The multi-year data from that National Cottonseed Treatment Trials found a low but significant negative correlation between *Pythium* soil populations and stand, but not other pathogens examined (Rothrock et al., 2012). However, soil population explained only a small portion of the variability in stands observed across trials. This information suggests soil pathogen populations are not the primary factor in determining seedling disease severity on cotton.

The importance of the environment and seedling diseases on cotton stand establishment have been examined as part of National Cottonseed Treatment Trials, a standardized fungicide seed treatment trial conducted by the Cotton Disease Council with cooperators across the cotton belt in the United States (Rothrock et al., 2012). The average minimal daily soil temperature and total rainfall the first three days after planting had a dramatic effect on mean stand across treatments, sites, and years for the 146 trials examined (Figure 1). Stand decreased as the average minimal soil temperature decreased from 24 to 12°C. As temperature decreased, increasing rainfall after planting had an increasing impact on limiting stands. The importance of seedling diseases on the stand reductions observed is evident in these same studies by examining the difference in percent stand between seed treatments having the combination of a triazole and either metalaxyl or mefenoxam fungicides and seed not receiving a fungicide treatment (Figure 2). Average difference in percent stand was 13.2 across all environments and stand difference increased dramatically as temperature after planting decreased and at lower temperatures as rainfall increased after planting. For these trials seedling diseases accounted for most of the stand differences observed across planting environments.

SEEDLING PATHOGENS

Pythium species

Seedling diseases caused by *Pythium* spp. are widespread in cotton-growing areas. The genus *Pythium* is in the Oomycetes which are now in the kingdom Stramenopila and are no longer considered fungi and include saprophytic and facultative parasitic species. Many species of *Pythium* contribute to the cotton seedling disease complex (DeVay et al., 1982; Howell, 2001; Johnson et al., 1978; Johnson and Chambers, 1973). The most common symptoms associated with *Pythium* spp. are seed rot and preemergence damping-off (Arndt, 1943; Howell, 2001; Howell, 2002; Spencer and Cooper, 1967). Younger seedlings (6 days old) were more susceptible to *Pythium* spp. than older seedlings (12 days old) (Arndt, 1943; Spencer and Cooper, 1967). *Pythium* spp. also cause root rotting and hypocotyl lesions

(Arndt, 1943; Howell, 2001). *Pythium* spp. can cause severe stand loss in cotton (DeVay et al., 1982; Fulton and Bollenbacher, 1959; Howell, 2001; Johnson et al., 1978; Ogle et al., 1993). Johnson et al. (1978) found that *Pythium* spp. were the most frequently isolated pathogen from diseased cotton seedlings in Tennessee. A significant negative correlation was obtained between percentage of seedlings from which *Pythium* spp. were isolated and percent emergence (Johnson and Doyle, 1986). The use of the fungicide seed treatment Metalaxyl, with selective activity against *Pythium* spp., in the National Cottonseed Treatment Trials examined the importance of *Pythium* spp. in the seedling disease complex (Rothrock et al., 2012). Of the 119 trials that had a fungicide response, metalaxyl increased stand significantly in 40 trials demonstrating the widespread importance of this group of pathogens in stand establishment.

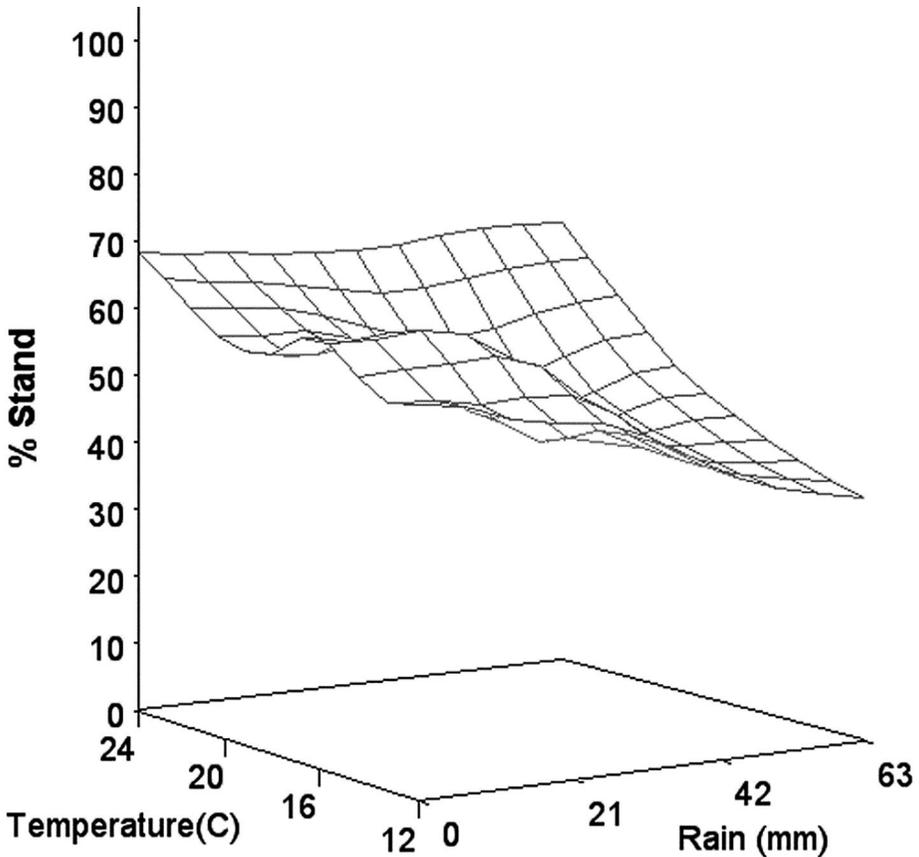


Figure 1. Percent stand for the mean of trials from 1999 to 2004 in relation to rainfall and average minimal soil temperature for the first 3 days after planting (Rothrock et al., 2012).

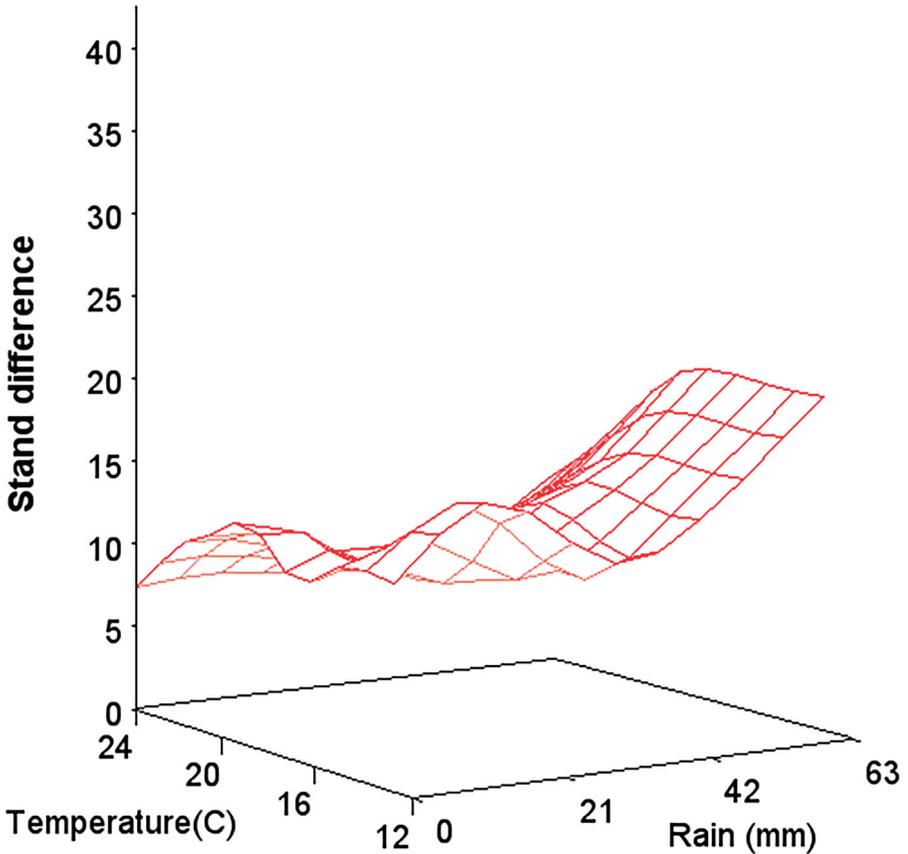


Figure 2. Difference in mean stand percentages between seed treated a triazole and either metaxyl or mfenoxam and seed with no fungicide treatment for trials from 1999 to 2004 in relation to rainfall and average minimal soil temperature for the first 3 days after planting (Rothrock et al., 2012).

The primary survival structures for *Pythium* spp. are oospores in the soil or in plant residue. The saprophytic nature of the pathogen also allows colonization of organic matter in the soil. A study by Nelson and Craft (1989) showed root and seed exudates stimulated germination of almost 100% of oospores of *P. ultimum* within three hours of initial exposure.

Losses from *Pythium* spp. increase as soil temperature decrease from a minimal soil temperature of 24 to 12°C and with increasing rainfall the first 3 days after planting (Figure 3) (Rothrock et al., 2012). These results are supported by earlier research from Tennessee that found in 7 years of field studies, soil moisture and temperature at planting directly affected isolation frequency of *Pythium*, with isolation being negatively correlated with minimal soil temperature and positively correlated with soil moisture content (Johnson et al., 1969). These observations on the role of soil temperature and moisture

on the severity of seedling disease caused by different *Pythium* spp. also are supported by controlled studies. Optimal temperature for root rot in cotton caused by *P. irregulare* was 15°C, over a range of temperatures from 15 to 31°C (Roncadori and McCarter, 1972). Arndt (1943) found *P. ultimum* caused the most damage at 18 and 21°C over a temperature range of 18 to 30°C. McCarter and Roncadori (1971) found that germinated seed exposed to temperatures of 10°C for 3 or 5 days, which were planted in soil infested with *Pythium* spp., had reduced emergence compared to germinated seed not exposed to low temperatures. *Pythium* species have a range of temperature preferences, with *P. ultimum* growing well at low to moderate temperatures and *P. aphanidermatum* having an optimal temperature of 37°C (Howell, 2002). Growth of *Pythium* spp. is increased at higher soil water contents (Griffin, 1963; Hillocks, 1992). In addition, soil texture has been reported to affect *Pythium* spp. In Tennessee, *Pythium* was a greater problem in clay soils than in sandy soils (Johnson and Doyle, 1986).

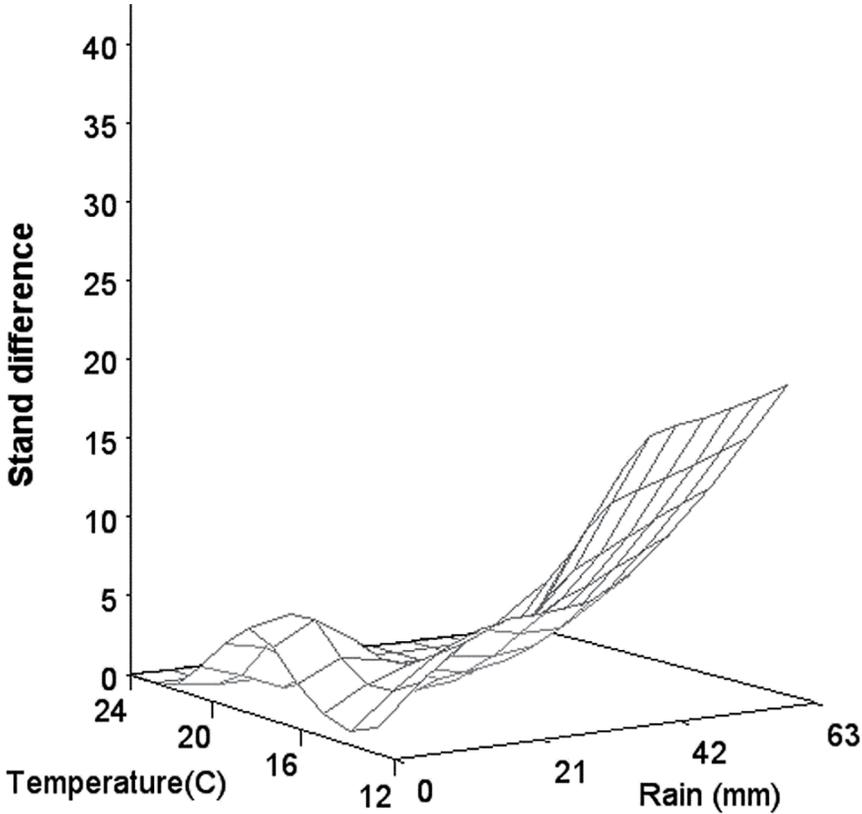


Figure 3. Difference in mean stand percentages between seed with a metalaxyl fungicide treatment and no fungicide treatment for trials from 1999 to 2004 in relation to rainfall and average minimal soil temperature for the first 3 days after planting (Rothrock et al., 2012).

Rhizoctonia solani

R. solani is a common and important pathogen in the seedling disease complex of cotton. *R. solani* causes seed rot, preemergence damping-off, postemergence damping-off, as well as boll rots and leaf spots (Garber and Leach, 1971). Fulton and Bollenbacher (1959) and Ogle et al. (1993) reported preemergence death of cotton due to *R. solani*. However, the symptoms associated with seed rot and preemergence damping-off of *R. solani* are often hard to distinguish from symptoms of other seedling pathogens. The symptom most often associated with *R. solani* is postemergence damping-off (Garber and Leach, 1971; Rothrock, 1996; Rothrock, 2001; Rude, 1984) and include brown to reddish brown lesions found on the hypocotyl near or below the soil line. The lesions are usually sunken, referred to as "sore shin" or "wire-stem," and girdle the hypocotyl killing the seedling. Studies have shown that cotton becomes increasingly resistant to *R. solani* with age (Hunter et al., 1978; Neal, 1942). *R. solani* was reported to be the most important pathogen associated with diseased cotton seedlings in Mississippi (Davis, 1975; Ranney, 1962) and Oklahoma (Ray and McLaughlin (1942). The use of the fungicide treatment PCNB in the National Cottonseed Treatment Trials, which is selective for control of *R. solani*, increased plant stands over nontreated seed in 44 of 119 trials where a significant fungicide seed treatment response was found, demonstrating the widespread importance of *R. solani* in stand establishment.

R. solani reproduces and exists primarily as vegetative mycelium and/or sclerotia. It is a facultative parasite, which can grow saprophytically through the soil or on organic matter. *R. solani* is a very diverse species which is grouped below the species level into anastomosis groups (AG groups). This grouping is based on the ability of the hyphae of two isolates of the fungus to recognize and fuse with one another (anastomose). Isolates in an AG generally have similar host ranges and cause similar symptoms. Although the pathogen diversity on cotton has not been critically examined, Weinhold (1977) found that AG 4 was connected with soils historically used for cotton production. Rothrock et al. (1995) also found only AG 4 at two sites in Arkansas, both sites having a history of cotton production. Other AGs have been isolated from cotton and can cause symptoms on cotton under controlled conditions (Carling et al., 1994; Carling et al., 2002; Wrather et al., 2002).

Seedling disease in cotton caused by *R. solani* has been reported to be affected by temperature. Germinated seeds planted in soil infested with *R. solani* and exposed to a 10°C temperature for 3-5 days, had reduced emergence compared to germinated seeds not exposed to the low temperatures (McCarter and Roncadori, 1971; Shao and Christianson, 1982). There was a negative correlation between temperature and exudates and a positive correlation between exudates and *R. solani* growth (Hayman, 1969). However, the results from the National Cottonseed Treatment Trials, which showed an average stand difference of 3.6 for PCNB treated seed compared to nontreated seed did not find much difference in fungicide response over minimal soil temperatures from 12 to 24°C and rainfall from none to 62 mm for the first 3 days after planting in 146 trials (Rothrock et al., 2012). Soil moisture has been shown to have little effect on the colonization of cotton plants by *R. solani* (Huisman, 1988; Johnson et al., 1969).

Thielaviopsis basicola

T. basicola, the causal agent of black root rot of cotton, has become an important seedling disease on cotton throughout the world (Allen, 2001; Johnson and Doyle, 1986; Mathre et al., 1966; Nehl et al., 2004; Roy and Bourland, 1982). *T. basicola* has a wide host range and is found in both agricultural and non-agricultural soils (Yarwood, 1974). Symptoms associated with *T. basicola* include blackening of the roots and the belowground portions of hypocotyls and stunting of cotton plants (Allen, 2001; Melero-Vara and Jimenez-Diaz, 1990; Rothrock, 1992). Generally, the damage is limited to the cortical layer and does not progress into the endodermis and the vascular cylinder (Allen, 2001; Mathre et al., 1966; Mauk and Hine, 1988; Walker et al., 1999). In severe cases, the development of the lateral roots is restricted or prevented (Allen, 2001). In Mississippi, *T. basicola* was detected in 18 of 36 locations and observed on 100% of cotton seedlings for four locations (Roy and Bourland, 1982). Surveys in Arkansas and Texas found *T. basicola* in over 70% of cotton fields (Rothrock, 1997; Wheeler et al., 2000). For surveys in Mississippi, incidence of *T. basicola* was negatively correlated with cotton stand and positively correlated with root and hypocotyl disease indices (Roy and Bourland, 1982). In microplot studies, soil infested with moderate levels of *T. basicola*, 100 chlamydospores/g soil, had lower yields in two of three years, reducing seed cotton yields 15 and 21% (Jaraba et al., 2013).

T. basicola is a hemibiotroph, functioning ecologically as an obligate parasite (Hood and Shew, 1997; Nan et al., 1992). The fungus produces two types of single-celled asexual spores, chlamydospores and endoconidia. The chlamydospores are thick-walled, dark, and produced in chains. The chlamydospores are the survival structures and primary inoculum of *T. basicola*. The fungus can survive as chlamydospores in the soil for more than 3 years and also may persist as endoconidia for up to 8 months (Allen, 2001). Under favorable conditions, the spores germinate and produce germ tubes in response to exudates from the plant roots. The pathogen directly infects the host plant and hyphae grow both intracellularly and intercellularly throughout the cortical cells (Allen, 2001; Mathre et al., 1966; Mauk and Hine, 1988).

Black root rot of cotton caused by *T. basicola* is more severe under cool environments after planting, less than 24°C (Allen, 2001; Blank et al, 1953; Maier, 1966; Rothrock, 1992). Black root rot is more severe in wet soils than in well-drained soils (King and Presley, 1942). At a temperature of 24°C, soils with a higher matric potential (-10 J/kg), had 32% colonization of root tissue by *T. basicola*, compared to 12% at a lower matric potential (-30 J/kg) (Rothrock, 1992). Soil texture also has been suggested as important in the development of black root rot. Disease symptoms are often more severe in crops grown in clay soils as opposed to crops grown in sandy soils (Hillocks, 1992). Microplot studies comparing soil textures, found soil with 87% sand had less root colonization and reproduction and survival of the pathogen than soils with a range of sand contents from 48 to 74% (Jaraba et al., 2013).

Fusarium species

Fusarium spp. are commonly isolated from diseased seedlings and are frequently the most isolated genus of fungi from cotton seedlings (Colyer, 1988; Davis, 1975; Fulton and Bollenbacher, 1959; Johnson et al., 1978; Johnson and Doyle, 1986; Melero-Vara and Jimenez-Diaz,

1990; Ray and McLaughlin, 1942; Roy and Bourland, 1982). A number *Fusarium* spp. are isolated from cotton seedlings (Abd-El Salam et al., 2006; Colyer, 1988; Palmateer et al., 2004; Roy and Bourland, 1982). Roy and Bourland (1982) found a positive correlation between incidence of *Fusarium oxysporum* Schlechtend.: Fr. and disease severity on seedlings. However, Johnson and Doyle (1986) found a negative correlation between disease severity and isolation frequency of *Fusarium* spp. Pathogenicity for isolates of several species, most frequently *F. oxysporum* and *F. solani* (Mart.) Sacc. have been demonstrated (Abd-El Salam et al., 2006, Colyer, 1988; Colyer, 2001; Melero-Vara and Jimenez-Diaz, 1990; Roy and Bourland, 1982). Symptoms caused by *Fusarium* spp. include decayed seed, brown or black lesions on the hypocotyls and roots, and death of seedlings. Affected seedlings that emerge from the soil, often exhibit stunting, chlorosis, and reduced root systems. In the field, the effects of *Fusarium* spp. are similar to the other pathogens in the disease complex, in that they result in poor plant stands or skips (Colyer, 2001).

Species of *Fusarium* survive in the soil as chlamydo spores or as hyphae. They can grow saprophytically in nature colonizing organic matter in the soil and can parasitize non-host plants, including weeds (Colyer, 2001). Species within the genus are distinguished based on the occurrence and appearance of macroconidia, microconidia, conidiophores, and chlamydo spores.

SEEDLING PATHOGEN INTERACTIONS ON COTTON

In addition to seedling disease pathogens interacting to form a complex, these pathogens also may interact with other pathogens of cotton. Cotton seedling disease caused by *R. solani* was more severe in soils infested with the nematodes *Rotylenchulus reniformis*, *Hoplolairnus tylenchiformis*, *Meloidogyne incognita*, *M. arenaria*, or *M. hapla* (Brodie and Cooper, 1964). Susceptibility of cotton seedlings to *R. solani* also may be for a longer duration in plants previously infected by one of these nematodes. A synergistic interaction between *M. incognita* and *T. basicola* has been described in cotton (Walker et al., 1998). Microplots infested with both *T. basicola* and *M. incognita* showed an increase in seedling death and a decrease in plant growth and yield compared with microplots infested with either pathogen alone. Co-infection of cotton by both *T. basicola* and *M. incognita* appeared to increase the effective range of temperatures at which the pathogens caused damage (Walker et al., 1999). Histological studies showed the presence of *T. basicola* in the vascular root tissue in plants grown in soils where the fungus and *M. incognita* were present, suggesting the nematode allows *T. basicola* to colonize vascular tissues that are not normally colonized in the absence of the nematode (Walker et al. 1999). Early-season insect pressure from thrips also may increase seedling disease damage. Micinski et al. (1990) found that yields in Louisiana were increased across planting dates by the in-furrow application of Temik (aldicarb) plus Terraclor Super X (PCNB + etridiazole) compared to no in-furrow treatment suggesting early-season damage from thrips and seedling disease reduced yields. Yields across planting dates also were more stable by apply the in-furrow application compared to no in-furrow treatment suggesting early-season damage from thrips and seedling diseases were responsible for some of the yield variability over planting dates.

The interaction between *F. oxysporum* f.sp. *vasinfectum* and *M. incognita* is the most well-known nematode fungal interaction, being first reported by Atkinson in 1892. Atkinson (1982) observed that severity of Fusarium wilt of cotton increased in the presence of root-knot nematodes. *F. oxysporum* f. sp. *vasinfectum* also can interact with the reniform (*R. reniformis*) (Hillocks, 1992; Neal, 1954), sting (*Belonolaimus gracilis* and *B. longicaudatus*) (Holdeman and Graham, 1954; Minton and Minton, 1966), lance (*Hoplolaimus seinhorsti*) (Brodie and Cooper, 1964, Lewis and Smith, 1976; Rajaram, 1979), and lesion (*Pratylenchus brachyurus*) (Maggi, 1981) nematodes. The interactions between the fungus and these nematodes increase the severity of Fusarium wilt of cotton, however, what role they play in seedling disease severity caused by *F. oxysporum* is unknown.

SEEDLING DISEASE CONTROL

Cotton seedling disease control relies on avoidance. Control practices used to limit seedling disease severity include planting high quality seed, planting in well drained soils on raised seedbeds, and delaying planting until soil temperatures and weather forecasts are favorable for rapid germination and emergence (Chambers, 1995; DeVay and Rothrock, 2001; Hillocks, 1992; Kerby et al., 1989; Minton and Garber, 1983, Minton, et al., 1982; Rude, 1984; Wheeler, et al., 1997). These practices should favor emerging plants and have been reported to be effective in controlling seedling disease (Chambers, 1995; Colyer and Vernon, 2005; Minton and Garber, 1983, Minton, et al., 1982). In addition, these practices decrease abiotic stresses, including soil crusting, soil anoxia, and chilling injury.

Early planting is a common practice for growers, in order to maximize the growing season, limit late-season insect pressure, and allow for favorable weather at harvest. Research in Louisiana has shown that poor stands and increased seedling diseases are often associated with early planting dates; with early April plantings resulting in low plant populations, late April and early May plantings resulting in intermediate plant populations, and mid-May plantings resulting in high plant populations (Colyer et al., 1991). Early planting is often associated with lower soil temperatures and increased rainfall, which are conditions favorable for cotton seedling diseases and delay plant emergence and development as shown earlier in the chapter for stand establishment (Figure 1). Soil temperatures, as well as soil moisture, affect both the host and the pathogens (Johnson et al., 1969; Minton et al., 1982; Riley et al., 1969). Johnson et al. (1969) found poor stands at minimal soil temperatures of 10°C or lower, with good stands at minimal soil temperatures of 19°C or higher. In Georgia, the efficacy of fungicide treatments was reduced when planting before March 29 or after May 2 (Guthrie, 1991).

Cottonseed in the United States is universally treated with combinations of fungicides prior to sale to protect the crop from a range of seedling disease pathogens, but also as a result of centralized seed production and sales due to the delinting process and the use of transgenic cultivars (Davis et al, 1997; Hillocks, 1992; Minton and Garber, 1983). It is common to use multiple fungicides to target the range of seedling disease pathogens that affect cotton. Davis et al. (1997) in California observed a stand improvement for the fungicides myclobutanil + metalaxyl or myclobutanil in 22 of 25 trials. Metalaxyl alone improved stands only in trials in 1995, but not

the 18 trials in 1993 or 1994. In these studies, a fungicide response occurred over environments with mean soil temperatures that ranged from 19.7 to 22.2°C the first 5 days after planting, suggesting that seedling diseases control for adequate stands occurs over a range of planting environments. Kaufman et al. (1998) in Texas found seed treatments containing triazole fungicides improved cotton stands, reduced root necrosis, and increased yields. Wheeler et al. (2000) also demonstrated yield increases from seed treatment fungicides. The National Cottonseed Treatment Trials found improved stands from the use of fungicide seed treatment in 119 of 211 trials (Rothrock et al., 2012). New seed treatment chemistries combinations continue to be introduced and new products registered. For the control of *R. solani* and other pathogens, azoxystrobin and triazole fungicides, were shown to significantly improve stands when used in combination with mefenoxam or metalaxyl compared to the historical standard carboxin + PCNB + metalaxyl (Rothrock et al., 2012). Fichtner et al. (2005) observed that triadimenol with thiram and metalaxyl was more effective in reducing root necrosis and hypocotyl discoloration compared to seed treated with carboxin + PCNB + mefenoxam. Newer chemistries such as the triazoles are also increasing the spectrum of seedling diseases being controlled, with the triazoles providing some control of *T. basicola* (Kaufman et al., 1998; Toksoz et al., 2009). Additional control of seedling diseases may be achieved by the application additional fungicides either on the seed before planting (custom seed treatments) or in the planting furrow (in-furrow treatments).

SUMMARY

Seedling diseases are one the major factors limiting stands and stand uniformity in cotton early in the season. In addition to poor stands, vigor of surviving seedling may be poor delaying plant development. A number of pathogens cause seedling diseases including *Rhizoctonia solani* *Pythium* spp. *Thielaviopsis basicola* and *Fusarium* spp. These seedling pathogens are common in fields and thus the important factor in determine seedling disease severity in a given year and field are soil environment within the first few days after planting. Low soil temperatures and rainfall after planting increase seedling disease severity and reduce stands and stand uniformity. Control relies on avoiding disease pressure by creating a soil environment less favorable for disease. In addition, seed treatment fungicides have been used universally for years and the efficacy of fungicide seed treatments continue to improve in efficacy and the spectrum of pathogens they control.

REFERENCES

- Abd-Elsalam, K.A., A.M. Asran-Amal, M.R. Omar, and A.A. Aly. 2006. Frequency and diversity of *Fusarium* spp. colonizing roots of Egyptian cottons. Arch. Phytopathol. Plant Prot. 39:165-177.
- Allen, S.J. 2001. Black Root Rot. In: T.L. Kirkpatrick, and C. S. Rothrock, editors, Compendium of cotton seedling diseases. APS Press, St. Paul, MN. pp. 16-17.
- Arndt, C.H. 1943. *Pythium ultimum* and the damping-off of cotton seedlings. Phytopathol. 33:607-611.

- Atkinson, G.F. 1892. Some diseases of cotton. Agricultural Experiment Station of the Agricultural and Mechanical College Bulletin 41. The Brown Printing Co., State Printers, Montgomery, AL.
- Blank, L.M., P.J. Leyendecker, and R.M. Nakayama. 1953. Observations on the black root rot symptoms on cotton seedlings at different soil temperatures. *Plant Dis. Rep.* 47:473-476.
- Blasingame, D., and M.V. Patel. 2013. Cotton disease loss estimate committee report. Proceedings of Beltwide Cotton Production Conference. National Cotton Council of America, Memphis, TN. pp. 1242-1246.
- Brodie, B.B., and W.E. Cooper. 1964. Relation of parasitic nematodes to postemergence damping-off of cotton. *Phytopathol.* 54:1023-27.
- Carling, D.E., R.E. Baird, R.D. Gitaitis, K.A. Brainard, and S. Kuninaga. 2002. Characterization of AG-13, a newly reported anastomosis group of *Rhizoctonia solani*. *Phytopathol.* 92:893-899.
- Carling, D.E., C.S. Rothrock, G.C. MacNish, M.W. Sweetingham, K.A. Brainard, and S.A. Winters. 1994. Characterization of anastomosis group 11 (AG 11) of *Rhizoctonia solani*. *Phytopathol.* 84:1387-1393.
- Chambers, A.Y. 1995. Comparative effects of no-till and conventional tillage on severity of three major cotton diseases in Tennessee. In: M.R. McClelland et al., editors, Conservation-tillage systems for cotton: A review of research and demonstrations results from across the Cotton Belt. Arkansas Agric. Exp. Stn. Bull.
- Colyer, P.D. 1988. Frequency and pathogenicity of *Fusarium* spp. associated with seedling diseases of cotton in Louisiana. *Plant Dis.* 72:400-402.
- Colyer, P.D. 2001. Diseases caused by *Fusarium* Species. In: T.L. Kirkpatrick, and C.S. Rothrock, editors, Compendium of Cotton Seedling Diseases. APS Press, St. Paul, MN. pp. 18-19.
- Colyer, P.D., S. Micinski, and K.T. Nguyen. 1991. Effects of planting date on the efficiency of an in-furrow pesticide and the development of cotton seedling disease. *Plant Dis.* 75:739-742.
- Colyer, P.D., and P.R. Vernon. 2005. Impact of stale seedbed production on seedling diseases in cotton. *Plant Dis.* 89:744-748.
- Davis, R.G. 1975. Microorganisms associated with diseased cotton seedlings in Mississippi. *Plant Dis. Rep.* 59:277-280.
- Davis, R.M., J.J. Nunez, and K.V. Subbarao. 1997. Benefits of cotton seed treatments for the control of seedling diseases in relation to inoculum densities of *Pythium* species and *Rhizoctonia solani*. *Plant Dis.* 81:766-768.
- DeVay, J.E. 2001. Seedling diseases. In: T.L. Kirkpatrick, and C.S. Rothrock, editors, Compendium of cotton seedling diseases. APS Press, St. Paul, MN. pp. 13-14.
- DeVay, J.E., R.H. Garber, and D. Matheron. 1982. Role of *Pythium* species in the seedling disease complex of cotton in California. *Plant Dis.* 66:151-154.

- DeVay, J. E., and C.S. Rothrock. 2001. Control of seedling diseases. In: T.L. Kirkpatrick, and C. S. Rothrock, editors, Compendium of cotton diseases. American Phytopathological Society, St. Paul, MN. pp. 19-20.
- Fichtner, S.M., T. Isakeit, T.A. Wheeler, H.W. Kaufman, and J.R. Gannaway. 2005. Evaluation of several approaches to manage *Meloidogyne incognita* and cotton seedling disease complexes in the high plains of Texas. *J. Nematol.* 37:66-70.
- Fulton, N.D., and K. Bollenbacher. 1959. Pathogenicity of fungi isolated from diseased cotton seedlings. *Phytopathol.* 49:684-689.
- Garber, R.H., and L.D. Leach. 1971. Cottonseed treatment in the control of disease research in the San Joaquin Valley of California. *Univ. Calif. Div. Agric. Sci. Prog. Rep.* 4-7.
- Griffin, D.M. 1963. Soil factors and the ecology of fungi. 2. Behaviour of *Pythium ultimum* at all soil-water suctions. *Trans. Brit. Mycol. Soc.* 46:368-372.
- Guthrie, D.S. 1991. Cotton Response to fertilizer placement and planting depth. *Agron. J.* 83:836-839.
- Hayman, D.S. 1969. The influence of temperature on exudation of nutrients from cottonseeds and on preemergence damping-off by *Rhizoctonia solani*. *Can. J. Bot.* 47:1663-1669.
- Heilman, M.D., Namken, L.N., and Cantu, R.V. 1976. Effects of stand loss on lint cotton yields in single and double-drilled rows. *Proceedings of Beltwide Cotton Prod. Conf. National Cotton Council of America, Memphis, TN.* pp. 87-91.
- Hillocks, R.J. 1992. *Cotton Diseases.* CAB International, Oxon.
- Holdeman, Q.L., and T.W. Graham. 1954. Effect of the sting nematode on the expression of Fusarium wilt in cotton. *Phytopathol.* 44:683-85
- Hood, M.E., and H.D. Shew. 1997. Reassessment of the role of saprophytic activity in the ecology of *Thielaviopsis basicola*. *Phytopathol.* 87:1214-1219.
- Howell, C.R. 2001. Diseases caused by *Pythium* species. In: T.L. Kirkpatrick, and C.S. Rothrock, editors, Compendium of cotton seedling diseases. APS Press, St. Paul, MN. Pp. 14-15.
- Howell, C.R. 2002. Cotton seedling preemergence damping-off incited by *Rhizopus oryza* and *Pythium* spp. and its biological control with *Trichoderma* spp. *Phytopathol.* 92:177-180.
- Huisman, O.C. 1988. Colonization of field-grown cotton roots by pathogenic soil borne fungi. *Phytopathol.* 78:716-722.
- Hunter, R.E., J.M. Halloin, J.A. Veech, and W.W. Carter. 1978. Terpenoid accumulation in hypocotyls of cotton seedlings during aging and after infection by *Rhizoctonia solani*. *Phytopathol.* 68:347-350.
- Jaraba, J., C.S. Rothrock, T.L. Kirkpatrick, and K.R. Brye. 2013. Soil texture influence on *Meloidogyne incognita* and *Thielaviopsis basicola* and their interaction on cotton. *Plant Dis.* 97 (In press).
- Johnson, L.F., D.D. Baird, A.Y. Chambers, and N.B. Shamiyeh. 1978. Fungi associated with postemergence seedling disease of cotton in three soils. *Phytopathol.* 68:917-920.

- Johnson, L.F., and A.Y. Chambers. 1973. Isolation and identity of three species of *Pythium* that cause cotton seedling blight. *Plant Dis. Rep.* 57:848-852.
- Johnson, L.F., A.Y. Chambers, and J.W. Measells. 1969. Influence of soil moisture, temperature, and planting date on severity of cotton seedling blight. *Univ. Tenn. Agri. Exp. Stn. Bull.* 461.
- Johnson, L.F., and J.H. Doyle. 1986. Relationship of seedling disease of cotton to characteristics of loessial soils in Tennessee. *Phytopathol.* 76:286-290.
- Kaufman, H., T. Wheeler, R. Graves, G. Schuster, P. Kidd, and K. Siders. 1998. Large plot performance of seedling disease seed treatment fungicides. Vol. 1. *Proc. Beltwide Cotton Prod. Conf. National Cotton Council of America, Memphis, TN.* pp. 149-152.
- Kerby, T.A., M. Keeley, and S. Johnson. 1989. Weather and seed quality variables to predict cotton seedling emergence. *Agron. J.* 81:415-419.
- King, C.J., and J.T. Presley. 1942. A root rot of cotton caused by *Thielaviopsis basicola*. *Phytopathol.* 32:752-761.
- Lewis, S.A., and F.H. Smith. 1976. Host plant distribution and ecological associations of *Hoplolaimus columbus*. *J. Nematol.* 8:264-270.
- Maggenti, A. 1981. *General Nematology.* Springer-Verlag, New York, N.Y.
- Maier, C.R. 1966. Implication in chemical control presented by the variability of cotton seedling disease pathogens. *Plant Dis. Rep.* 50:301-305.
- Mathre, D.E., A.V. Ravenscroft., and R.H. Garber. 1966. The role of *Thielaviopsis basicola* as a primary cause of field reduction in cotton in California. *Phytopathol.* 56:1213-1216.
- Mauk, P.A., and R.B. Hine. 1988. Infection, colonization, of *Gossypium hirsutum* and *G. barbadense*, and development of black root rot caused by *Thielaviopsis basicola*. *Phytopathol.* 78:1662-1667.
- McCarter, S.M., and R.W. Roncadori. 1971. Influence of temperature during cottonseed germination on growth and disease susceptibility. *Phytopathol.* 61:126-1429.
- Melero-Vara, J.M., and R.M. Jimenez-Diaz. 1990. Etiology, incidence, and distribution of cotton seedling damping off in southern Spain. *Plant Dis.* 74:597-600.
- Micinski, S., P.D. Colyer, K.T. Nguyen, and K.L. Koonce. 1990. Effects of planting date and early-season pest control on yield in cotton. *J. Prod. Agric* 3:597-602.
- Minton, E.B., and R.H. Garber. 1983. Controlling the seedling disease complex of cotton. *Plant Dis.* 67:115-118.
- Minton, N.A., and E.B. Minton. 1966. Effect of root knot and sting nematodes on expression of Fusarium wilt of cotton in three soils. *Phytopathol.* 56:319-22
- Minton, E.B., G.C. Papavizas, and J.A. Lewis. 1982. Effect of fungicide seed treatments and seed quality on seedling disease and yield of cotton. *Plant Dis.* 66:832-835.
- Nan, Z.B., P.G. Long, R.A. Skipp, and D.H. Hopcroft. 1992. Microscopy of invasion of red clover root by *Trichocladium basicola*, and effects of benomyl and prochloraz. *Plant Pathol.* 41:449-461.

- Neal, D.C. 1942. *Rhizoctonia* infection of cotton and symptoms accompanying the disease in plants beyond the seedling stage. *Phytopathol.* 32:641-642.
- Neal, D.C. 1954. The reniform nematode and its relationship to the incidence of Fusarium wilt of cotton at Baton Rouge, Louisiana. *Phytopathol.* 44:447-450.
- Nehl, D.B., S.J. Allen, A.H. Mondal, and P.A. Lonergan. 2004. Black root rot: a pandemic in Australian cotton. *Australasian Plant Pathol.* 33:87-95.
- Nelson, E.B., and C.M. Craft. 1989. Comparative germination of culture-produced and plant-produced sporangia of *Pythium ultimum* in response to soluble seed exudates components. *Phytopathology* 79:1009-1013.
- Ogle, H.J., S.A. Marcelle, and P.J. Dart. 1993. Pathogenicity of fungi associated with seedling disease of cotton. *Aus. J. Exp. Agric.* 33:923-929.
- Palmateer, A.J., K.S. McLean, G. Morgan-Jones, and E. van Santen. 2004. Frequency and diversity of fungi colonizing tissues of upland cotton. *Mycopathologia* 157:303-316.
- Rajaram, B. 1979. Biochemical association between infection of *Hoplostaimus seinhorsti* Luc, 1958 and the development of *Fusarium oxysporum* f. sp. vasinfectum (Atk.) Snyder & Hansen on cotton. *Indian Zoologist* 3:135-138.
- Ranney, C.D. 1962. Fungi involved in the seedling disease complex of cotton in the Yazoo-Mississippi Delta. *Plant Dis. Rep.* 46:122-123.
- Ray, W.W. and J.H. McLaughlin. 1942. Isolation and infection tests with seed- and soil-borne pathogens. *Phytopathology* 32:233-238.
- Riley, J.A., O.H. Newton, J.W. Measells, D.A. Downey, and L. Leonard. 1969. Soil temperatures and cotton planting in the mid-South. *Miss Agric. Exp. Stn. Bull.* 678.
- Roncadori, R.W., and S.W. McCarter. 1972. Effects of soil temperature and plant age on *Pythium* root rot of cotton. *Phytopathology* 62:373-376.
- Rothrock, C.S. 1992. Influence of soil temperature, water, and texture on *Thielaviopsis basicola* and black root rot on cotton. *Phytopathology* 82:1202-1206.
- Rothrock, C.S. 1996. Cotton diseases incited by *Rhizoctonia solani*. In: B. Sneh, S. Jabaji-Hare, S. Neate, and G. Dijst, editors, *Rhizoctonia* species: taxonomy, molecular, biology, ecology, pathology, and disease control. Kluwer Academic Publishers, Boston. p. 269-277.
- Rothrock, C.S. 1997. Prevalence and distribution of *Thielaviopsis basicola*. *Proceedings Belt-wide Cotton Prod. Conf. National Cotton Council of America, Memphis, TN.* p. 75-76.
- Rothrock, C.S. 2001. Diseases caused by *Rhizoctonia solani*. Page 15-16 In: T.L. Kirkpatrick and C.S. Rothrock, editors, *Compendium of cotton seedling diseases*. APS Press, St. Paul, MN. p. 15-16.
- Rothrock, C.S., T.L. Kirkpatrick, R.E. Frans, and H.D. Scott. 1995. The influence of winter legume cover crops on soil borne plant pathogens and cotton seedling diseases. *Plant Dis.* 79:167-171.

- Rothrock, C.S., S.A. Winters, P.K. Miller, E. Gbur, L.M. Verhalen, et al. 2012. Importance of fungicide seed treatment and environment on seedling diseases of cotton. *Plant Dis.* 96:1805-1817.
- Roy, K.W., and F.B. Bourland. 1982. Epidemiological and mycofloral relationships in cotton seedling disease in Mississippi. *Phytopathology* 72:868-872.
- Rude, P.A. 1984. Integrated Pest Management for Cotton in the Western Region of the United States. University of California, Division of Agriculture and Natural Resources Pub. 3305.
- Shao, F.M., and M.N. Christianson. 1982. Cotton seedling radicle exudates in relation to susceptibility to *Verticillium* wilt and *Rhizoctonia* root rot. *Phytopathol.* 105:351-359.
- Spencer, J.A., and W.E. Cooper. 1967. Pathogenesis of cotton (*Gossypium hirsutum*) by *Pythium* species: zoospore and mycelium attraction and infectivity. *Phytopathology* 57:1332-1338.
- Toksoz, H., C.S. Rothrock, and T.L. Kirkpatrick. 2009. Efficacy of seed treatment chemicals for black root rot, caused by *Thielaviopsis basicola*, on cotton. *Plant Dis.* 93:354-362.
- Walker, N.R., T.L. Kirkpatrick, and C.S. Rothrock. 1998. Interaction between *Meloidogyne incognita* and *Thielaviopsis basicola* on cotton (*Gossypium hirsutum*). *J. Nematol.* 30:415-422.
- Walker, N.R., T.L. Kirkpatrick, and C.S. Rothrock. 1999. Effects of temperature on histopathology of the interaction between *Meloidogyne incognita* and *Thielaviopsis basicola* on cotton. *Phytopathol.* 89:613-617.
- Weinhold, A.R. 1977. Populations of *Rhizoctonia solani* in agriculture soil determined by a screening procedure. *Phytopathol.* 67:566-569.
- Wheeler, T.A., J.R. Gannaway, H.W. Kaufman, J.K. Dever, J.C. Mertley, and J.W. Keeling. 1997. Influence of tillage, seed quality, and fungicide seed treatments on cotton emergence and yield. *J. Prod. Agric.* 10:394-400.
- Wheeler, T.A., K.D. Hake, and J.K. Dever. 2000. Survey of *Meloidogyne incognita* and *Thielaviopsis basicola*: Their impact on cotton fruiting and producers' management choices in infested fields. *J. Nematol.* 32(4S):576-583.
- Wrather, J.A., B. Phipps, and C.S. Rothrock. 2002. Fungi associated with postemergence cotton seedling disease in Missouri. Online. *Plant Health Progress*. doi: 10.1094/PHP-2002-0722-01-RS
- Yarwood, C.E. 1974. Habitats of *Thielaviopsis basicola* in California. *Plant Dis. Rep.* 58:54-56.

Chapter 9

COTTON SEEDLING GROWTH AND DEVELOPMENT RESPONSES TO TEMPERATURE AND DROUGHT STRESS

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INTRODUCTION

The United States is the third leading cotton (*Gossypium hirsutum* L.) producing country in the world with its production mainly concentrated in the southern states of Texas, Georgia, Arkansas, Louisiana, and Mississippi (Meyer, 2018). Cotton farmers in the southern US region favor early planting of cotton (Wrather et al., 2008), with a peak planting window from April 27 to the first week of May. Early planting provides the advantage of early season rainfall, minimizes late-season pest problems, and minimizes the risk of premature termination of the cotton crop by freezing temperatures in the fall (Bradow, 1991; Pettigrew, 2002). In addition, cotton planted in the early spring receives maximum solar radiation from emergence to squaring which promotes early vegetative growth and development and finally, cotton yield (Pettigrew, 2002). However, the early growth of cotton, seed germination and seedling development, is influenced by genotype x environment interactions. Temperature and soil moisture are the predominant abiotic factors that affect the early growth of cotton (Singh et al., 2018). Cotton is a warm temperature-requiring crop that needs a temperature of about 30°C for optimal seed germination and seedling development (Barpete et al., 2015). Early season planting always carries a risk of uncertainty in weather forecasting, potentially increasing the chance of chilling injury during the early growth stages. Low soil temperatures (<20°C) cause radicle abortion and necrosis of root tips, potentially leading to uneven stand establishment, poor seedling development, and decreased fiber yields (Bradow and Bauer, 2010). The average farm size for operations categorized as small and large in the USA ranges from 200 acres to 1500 acres, respectively. Farmers using a single cotton variety consider staggering planting dates to plant large acreage farms that can potentially expose the early growth stages of cotton to low temperatures. Also, above or below optimum soil moisture conditions combined with cool temperatures more negatively impact seedling development than either stress in isolation (Wanjura et al., 1971; Wanjura and Buxton, 1972a, b). Therefore, the objective of this chapter is to provide an overview of the effects of temperature and moisture stress on early growth and development of cotton seedlings.

TEMPERATURE

Shoot Growth and Development

Crop growth describes an irreversible increase in dry mass or volume, and development involves differentiation of cells into specific tissue types or organs, and both are controlled by environmental and genetic factors. Sub or supra-optimal temperature has been considered as a most damaging stress factor, especially during reproductive development of cotton (Reddy et al., 1992b; Reddy et al., 1992c; Oosterhuis, 2002). Cultivar-specific optimum temperatures are different for different growth stages, including seed germination, seedling establishment, leaf area and canopy development, flowering, boll development, and maturation (Pearson et al., 1970; Reddy et al., 1992b; Reddy et al., 1992c; Reddy et al., 1992a; Barpete et al., 2015; Singh et al., 2018), and are determined by ecotypes. Cotton cultivars grown in tropical parts of the world are well adapted to higher temperatures especially in south Asia, where summer temperatures can potentially rise above the optimum and reach around 48-50°C during the cotton growing season. Recent studies have reported the effect of day/night temperatures on late vegetative and reproductive growth in cotton (Oosterhuis, 2002; Kakani et al., 2005; Singh et al., 2007; Snider et al., 2011). Morphologically, higher temperatures (>30°C) significantly reduce leaf area, stem elongation, and biomass accumulation during early growth stages (Reddy et al., 1992a). Declines in pollen viability and germination (Saini et al., 1983; Kakani et al., 2005; Jain et al., 2007; Snider et al., 2011), and square and boll retention (Reddy et al., 1991; Reddy et al., 1992c) in response to heat stress during reproductive growth have been reported in Upland cotton. Similar but more pronounced responses were observed in Pima cotton (*Gossypium barbadense* L.) with increasing temperature (from 30/22°C to 40/32°C) during reproductive growth (Reddy et al., 1992b; Reddy et al., 1995). Upland cotton developed a significantly higher number of fruiting branches than Pima cotton at higher temperatures (Reddy et al., 1995). Reddy et al. (1995) also observed that Pima cotton required more heat units than Upland cotton to produce the first square at 30°C, based on which Pima cotton was concluded to be less heat tolerant during reproductive growth. Various physiological changes have been observed in response to varying temperatures in cotton at late vegetative and reproductive growth stages. These include a decline in photosynthesis due to an increase in photorespiration (Perry et al., 1983), primarily caused by reduced chlorophyll contents (Reddy et al., 2004; Snider et al., 2009; Snider et al., 2010), decrease in actual quantum yield of photosystem II (Φ_{PSII}) and maximum photochemical efficiency (F_v/F_m) (Bibi et al., 2008; Snider et al., 2009; Snider et al., 2010), inhibition of Rubisco activase, and increased membrane leakage (Bibi et al., 2008). Also, the rapid increase in dark respiration at higher temperatures can limit the accumulation of carbohydrates, especially fructose and glucose in the source (leaves) and their translocation to the final sink (bolls) (Arevalo et al., 2008). This trend of decreasing photosynthesis with increasing temperatures could substantially lower cotton yields.

As evident from the above review, there is extensive information on heat stress effects during the reproductive phase of growth, but few studies have focused on heat tolerance at germination and early seedling growth in cotton. Ashraf et al. (1994) found significant genotypic differences

for seedling growth among cotton cultivars when subjected to 48°C for a 21-day period after incubating the seeds at 30°C. The authors further identified cultivars B-557 and MNH-93 had higher percent germination, dry biomass, leaf soluble sugars, proline sugars, and lower relative injury compared to cultivars CIM-70, NIAB-78, and S-12. The study, however, found no germination of seeds incubated at 50°C. Reddy et al. (1992a) observed plant height, leaf area, and main stem elongation rate in Upland cotton were relatively less sensitive to temperature regime (20/12 - 40/32 °C day/night) during seedling growth for the first two weeks after emergence than after that time. Similar responses were observed in Pima cotton to the temperature regime were during seedling growth (Reddy et al., 1992b).

Brand et al. (2016) identified low temperatures as the most damaging to seedling growth and development while studying root and shoot morphological responses to multiple stresses (carbon dioxide, ultraviolet-B radiation, and temperature). Low temperatures have also been associated strongly with seedling diseases like damping off, which are the primary reasons for root deterioration under chilling conditions (Brown and McCarter, 1976). Brown and McCarter (1976) also reported low temperature as the major factor that impacts the degree of damage by pathogens like *Rhizoctonia* and *Pythium* in cotton seedlings. Significant increases in the number of vegetative branches, especially in Pima cotton were found at low temperatures (Reddy et al., 1992a; Reddy et al., 1992b). Fully emerged seedlings of cotton, when exposed to suboptimal temperatures in the late spring, showed a significant reduction in seedling development and subsequent yield (Bradow and Bauer, 2010). Seedling emergence and development rates can determine cultivar sensitivity to cool temperatures under controlled conditions (Arndt, 1945; Pearson et al., 1970; Reddy et al., 1992a; Reddy et al., 1992b; Singh et al., 2018) and relative performance under field conditions (Steiner and Jacobsen, 1992). Moreover, leaf or cotyledon area, leaf dry weight, shoot and root dry weight, and plant height can substantially determine early vigor in modern cultivars and advanced cotton breeding lines (Reddy et al., 1992a; Reddy et al., 1992b; Liu et al., 2015; Brand et al., 2016; Singh et al., 2018). The genotypic difference in early vigor recovery from post-emergence chilling stress often informs the selection of cultivars for early planting when soil temperatures are cool. Singh et al. (2018) observed the seedling growth of nine elite cotton lines obtained from different breeding programs was more sensitive to low temperature (22°C) than drought stress (50% of optimum moisture). Further, the study reported significant differences in the response and degree of tolerance to low temperature among the cotton lines during seedling growth. The identified tolerant lines might, however, yield similar to other lines under late planting conditions when temperatures are optimal.

Physiological indicators of heat tolerance in a plant include membrane leakage, chlorophyll fluorescence, and antioxidant enzyme activity (Oosterhuis et al., 2008). Consequently, a thermo-tolerant plant shows relatively less leakage, higher photochemical efficiency of PS II and greater antioxidant enzyme activity under heat stress than a thermo-sensitive plant (Oosterhuis et al., 2008). For rapid screening purposes, fluorescence and membrane leakage assays, are heavily applied in previous studies determining cotton responses to high and low temperatures (Murphy and Noland, 1982; Cottee et al., 2007; Bibi et al., 2008, BÖLEK et al., 2013). These techniques aid in the selection of germplasm for improvement in cold or heat tolerance among commercial cultivars and advanced breeding lines (Oosterhuis et al., 2008).

Root Growth and Development

One of the major problems during early season planting of cotton is lack of uniform stand establishment because cool soil temperatures can substantially reduce root elongation rate, which determines root length (Pearson et al., 1970). Because of the difficulty associated with root phenotyping, most of the previous studies were only concentrated on studying root length (Newman, 1966; Reicosky, 1970). The studies concluded that suboptimal temperatures could substantially reduce root growth and simultaneous hydraulic conductance during early growth stages, even if soil moisture was optimum (Pearson et al., 1970, Radin, 1990, Bolger et al., 1992). Bradow (1991) observed a significant decline in root and shoot growth of 10-day old seedlings at low temperatures (10 to 25°C) in cotton. Christiansen et al. (1970) reported a significant loss of amino acid and sugars like glucose, fructose, and sucrose from the roots under chilling temperature (5°C) and the amount of loss increased linearly with increasing duration of chilling temperature. The study also identified marked differences among cotton cultivars in their capacity to recover from post-emergence chilling stress. The cultivar-specific differences in cotton were also observed for shoot and root water content under moderate chilling conditions (>15°C) (Bradow, 1991). Bauer and Bradow (1996) found greater emergence rate and root length in early maturing varieties than late maturing varieties at suboptimal temperatures. However, slower root growth in the late maturing varieties may be advantageous when low soil moisture and cool conditions prevail in the field (Bauer and Bradow, 1996).

DROUGHT

Drought can be defined as a prolonged period of below-average precipitation in a given region that can be measured by collecting historical data on precipitation and other weather parameters. It can severely limit agricultural productivity by providing insufficient soil moisture for proper growth and development at a given time. Agricultural drought is typically determined by plant available soil water in the root zone area, which is the amount of water that a typical soil retains between field capacity and permanent wilting point. Furthermore, researchers have imposed water deficit conditions either based on volumetric soil moisture content ($\text{m}^3 \cdot \text{m}^{-3}$), soil water tension, or evapotranspiration. In general, when soil moisture tension exceeds 30 to 50 centibars, depending on soil type, drought stress can occur (Perry et al., 2012).

Shoot Growth and Development

Cotton is mainly (about 65%) grown under dryland conditions particularly in the southeastern regions of the U.S., while irrigated cotton (about 35%) is concentrated in the arid southwestern U.S. (Perry et al., 2012). However, where dryland cotton is produced, the crop can receive less than the optimum average precipitation over the cotton growing season which significantly reduces the region-specific yield potential, and necessitates identifying cultivars that perform well under drought conditions. Cotton is most sensitive to water stress from a period of late squaring to early bloom when the water requirement ranges from 0.5 to 0.7 cm of water per day in the southeastern US (Perry et al., 2012). Low water availability during this period may cause a reduced number of bolls per plant, fiber quality, and seed cotton yield. Because the reproductive

stage is considered the most sensitive to drought in cotton, most of the shoot morphological and physiological growth characteristics in response to drought stress have been studied during late vegetative and reproductive growth stages (Ackerson et al., 1977; Saini and Lalonde, 1997; Pettigrew, 2004a; Pettigrew, 2004b; Chastain et al., 2014; Chastain et al., 2016). These studies have used various growth traits such as leaf folding, plant height, leaf area, leaf size and stem elongation (Singh et al., 2018; Jordan et al., 1975; Turner et al., 1986; Ball et al., 1994; Pettigrew, 1994; Pace et al., 1999), node number and plant dry weights (Pace et al., 1999), and squaring and boll production (Grimes and Yamada, 1982; McMichael and Hesketh, 1982; Turner et al., 1986; Gerik et al., 1996; Pettigrew, 2004a) to quantify the effects of drought on cotton seedlings.

Certain changes in response to drought stress may occur at cellular levels like reduced cell expansion, changes in grana, thylakoid membrane, mitochondria, chloroplast membrane integrity, and also modification in chemical compositions like formation of long-chain alkanes, decreased glycolipids and phospholipids, and increased triacylglycerol levels in epicuticular wax of water-stressed leaves (Loka et al., 2011). Though these above changes may not objectively specify drought tolerance. Singh et al. (2018) classified nine elite cotton lines developed from nine different breeding groups into different degrees of drought tolerances, ranging from very low to very high tolerance, based on the morphological growth of seedlings. Secondly, they observed differential responses of seedling growth to cold and drought stresses among the cotton lines, with low temperatures were more damaging than drought stress. The adverse effect of drought stress on photosynthesis (Jordan and Ritchie, 1971; Turner et al., 1986; Ephrath et al., 1994; Pettigrew, 2004b) may be caused by decreasing relative water content (Lawlor and Cornic, 2002) and leaf water potential (Lawlor and Cornic, 2002; Chastain et al., 2014). However, results for decreasing photosynthetic rates under water deficit conditions are contradictory in that some studies have concluded stomatal conductance to be a major limiting factor for reduced leaf photosynthetic rates under mild water stress, while others concluded non-stomatal limitations are the primary cause for downregulating leaf photosynthesis under severe water stresses (Ennahli and Earl, 2005). Many past studies found that decreasing leaf water potential may cause stomatal closure that limits carbon dioxide diffusion into the mesophyll cells, and thus reduces carbon fixation and photosynthetic rates (Chaves, 1991; Flexas et al., 2004; Chastain et al., 2014). Jordan (1970) observed a low level of stomatal resistance in field-grown cotton cultivar Lankart 57 even when leaf water potential was reduced to -30 bars. Others have recognized that non-stomatal limitations such as inhibition of metabolic processes like ribulose-1,5-bisphosphate carboxylase synthesis, Rubisco activity, and adenosine triphosphate synthesis can also play a role in reducing photosynthetic rates under drought stress (Gimenez et al., 1992; Medrano et al., 1997; Tezara et al., 1999). Ennahli and Earl (2005) observed that lowered CO₂ concentrations in leaves at moderate water stress (15% relative soil water content) were recovered back to normal levels upon hydration, but photosynthetic rates remained low even after a drought recovery period, which was caused due to non-stomatal inhibition. Also, the results obtained under field conditions for changes in PSII quantum efficiency (Φ_{PSII}) and PSII electron transport in response to water stress were contradictory to greenhouse studies. Certain field experiments showed little change in PSII quantum efficiency (Φ_{PSII}) and PSII electron transport under moderate stress levels than what was observed by the greenhouse and other field studies (Chastain et

al., 2014; Kitao and Lei. 2007; Zhang et al., 2011). Pettigrew (2004b) explained the variability in physiological response to drought stress based on the time of the day. The CO₂ exchange rates (CER) and light-adapted photosystem II quantum efficiency in dryland leaves were greater in the morning than in the afternoon, and these results differed from what was observed in irrigated plants. Photosynthetic tolerance to moisture stress strongly correlates to leaf development and growth stage in cotton (Karami et al., 1980; Chastain et al., 2016). Karami et al. (1980) observed cotton was more photosynthetically tolerant to drought during reproductive stages than during the vegetative stage. Also, Chastain et al. (2016) identified younger leaves were more photosynthetically tolerant to drought stress than mature leaves in cotton. The authors also found that net photosynthesis remained unchanged in younger leaves but had declined in mature leaves later in the season. Significant declines in the photosynthetic capacity and carbon fixation under drought stress could result in increased photorespiration and reactive oxygen species production (Foyer et al., 1997; Faria et al., 1999) that can cause oxidative damage to lipids, proteins, carbohydrates, and nucleic acids (Monk et al., 1987; Gigon et al., 2004; Deeba et al., 2012).

Dark respiration rates do vary with water stress, but the results of field studies differed from environmentally controlled studies (Loka et al., 2011). Genotypic differences have been reported for drought tolerance based on morphological parameters (Hatfield et al., 1987; McMichael and Quisenberry, 1991; Lopez et al., 1995; Singh et al., 2017b; Singh et al., 2018) and physiological parameters (Nepomuceno et al., 1998; Leidi et al., 1999; Burke, 2007; Rahman et al., 2008). Identifying variability in photosynthetic rate (Pn), stomatal conductance (gs), transpiration rate (E), and PS II thermostability among cotton genotypes grown in drought conditions could aid in selection of the genes for drought tolerance (Rahman et al., 2008; Snider et al., 2013). Thus, water efficient cotton genotypes selected based on morphological and physiological responses to water-deficit regimes would have stable lint yields in the environments having variable soil moisture contents (Turner et al., 1986). Cotton response to water stress greatly depends upon plant age, leaf size and position, time of day, growth stage, and genotype. Therefore, understanding of such parameters is a prerequisite while conducting drought tolerance studies at different growth stages of cotton. The interaction of agronomic practices, environments and MG are well studied in the past. Planting early to medium maturing varieties of cotton crop is a common practice to obtain quite stable yields under dryland conditions (Rosenow et al., 1983). To study the response of the plant in later stages of the life is very complex because of overlapping detrimental effects of drought stress and underground competition for space with neighboring plants (Blum, 1996). Thus, observing the response in the early growth stages for drought stress is a convenient way to avoid such errors, especially for field experiments of early planting.

Root Growth and Development

Despite a broad investigation on cotton responses to drought stress, few have focused on seedling growth characteristics (Jordan, 1970; Jordan et al., 1975; Kawakami et al., 2010; Singh et al., 2018). However, cotton sensitivity to drought stress at the time of seedling growth was closely associated with dry biomass and root morphology (Pace et al., 1999; Basal et al., 2005; Singh et al., 2018). Early growth stages of cotton primarily consist of a tap root system unless

an injury to the primary root has increased the number of secondary and tertiary roots. Although it has been quite difficult to evaluate the performance of cultivars based on root growth and development (McMichael, 1986), a significant amount of data has been reported in the past for an impact of drought stress on root growth in the seedling stage. This involved the use of various techniques and methods with advancement in technology over time to accurately measure root morphology (Newman, 1966; Reicosky et al., 1970; Reicosky et al., 1972; Taylor and Klepper, 1979; Bohm, 1979; Heen, 1980). At present, the use of a WinRhizo root scanner has been described as one of the most convenient methods to analyze the root characteristics during early growth stages in various crops (Narayanan et al., 2014; Wijewardana et al., 2015; Singh et al., 2017b, Singh et al., 2018). Steudle (2000) explained that the transport of water in the roots occurs through apoplastic and/or symplastic pathways, regulated by hydraulic and osmotic modes of action, respectively, depending upon the presence or absence of transpiration. Plants under drought stress show slowed root growth and symplastic flow of water characterizing roots hydraulic resistance that limits water loss from the roots to the soil. (Steudle, 2000).

Root growth has been found to be less sensitive to water deficits than shoot growth which is primarily due to osmoregulation that allow cells in root hairs to maintain lower osmotic potential (Oosterhuis and Wullschleger, 1987). The lowered osmotic potential allows consistent turgor pressure in the cells despite decreasing soil water potential which maintains root growth under water stress. This also allows partitioning of total biomass more towards roots than shoots and thus, results in higher root-shoot ratio under drought stress conditions (Oosterhuis and Wullschleger, 1987; McMichael and Quisenberry, 1991; Ball et al., 1994; Singh et al., 2017b; Singh et al., 2018). Ball et al. (1994) identified the significance of the position of roots within the soil profile while assessing any root traits against water stress conditions. The authors observed a greater number of medium roots (0.62 mm mean diameter) than small sized roots (0.30 mm mean diameter) in the upper zone, while the lower zone contained an equal number of both types of roots under drought as well as during the recovery period. The study explained the reason behind such a phenomenon was that the lower zone consistently held more water than the upper zone during stress periods. The study also provided the significance of studying variability in root size in response to drought stress.

Certain plant growth regulators, PGR-IV and Mepiquat Chloride, have been recognized as alleviating the damaging impact of drought stress through altered root growth characteristics in cotton. Zhao and Oosterhuis (1997) observed significantly higher stomatal conductance (g_s), leaf net photosynthetic rate, and root dry weight in water-stressed plants treated with PGR-IV compared to untreated plants. Furrow application of PGR-IV also showed an enhanced seedling growth regarding root length, root dry weight, lateral root numbers per plant, and final yields in cotton (Oosterhuis, 1995). Iqbal et al. (2005) observed accelerated root growth in cotton seedlings after treating the seeds with Mepiquat Chloride. Pace et al. (1999) observed a significant increase in root length and root thickness in drought-treated cotton seedlings compared to untreated seedlings at the end of drought recovery period. The use of polyethylene glycol (PEG) is another method to identify drought tolerance among genotypes during seed germination and seedling growth in different crops (Nepomuceno et al., 1998; Seepaul et al., 2012; Singh et al., 2017a). Nepomuceno et al. (1998) identified the difference in photosynthetic rate, stomatal

conductance, transpiration, and dry weights using PEG 6000 for drought tolerance among cotton cultivars. Past studies have reported differential growth responses between root and shoot growth to drought stress, and concluded root growth was more sensitive to drought and cold than the shoot (Pace et al., 1999; Singh et al., 2018). Thus, it is essential to study root patterns either independently or in combination for drought tolerance in cotton. Cotton genotypes or breeding lines exhibited significant variability for seedling root-shoot ratio, root length, root dry weights, root diameters, root numbers, root surface area, root length per unit volume, and other root parameters under drought stress (Quisenberry et al., 1981; Basal et al., 2005; Singh et al., 2018). Therefore, studying root morphological characteristics of cotton seedlings in response to drought conditions has been identified as an important method in breeding programs for improving drought tolerance in cotton.

MOLECULAR STUDIES

Studies that characterize cotton cultivars for cold tolerance using molecular approaches are limited in the literature. Phospholipases (PLDa) have been found to play a significant role in the cold stress response in cold tolerant species (Kargiotidou et al., 2010). Kargiotidou et al. (2010) observed the induction of two PLDa genes, PLDa1 and PLDa2, under cold stress (10°C or less) in cotton. However, the levels of two isoforms significantly changed under varying temperature and light conditions. Glycine betaine, an effective osmoprotectant, has been exploited for improving drought tolerance in several crops like cotton, wheat, barley, and corn. Naidu et al. (1998) identified increased amounts of glycine betaine in all cotton cultivars under water deficit conditions. The author further identified genotypic variations for glycine betaine levels, where the highest levels were found in the cultivar Tamcot Sphinx, adapted to dryland conditions, and the lowest levels were observed in the cultivar Siokra S101, adapted to irrigated conditions. Also, seed treatment with glycinebetaine along with foliar application after emergence and at the squaring increased cotton yield under rainfed conditions (Naidu et al., 1998). Transgenic *GF14λ*-expressing cotton plants displayed the “stay green” phenotype and improved tolerance to water deficit conditions because of increased photosynthesis and stomatal conductance regulated by GF14λ (Yan et al., 2004).

SUMMARY

Cotton researchers are interested in studying the early growth of cotton in response to suboptimal temperatures and soil moisture conditions. Low temperatures and variable soil moisture contents are the major abiotic factors influencing seed germination and early seedling growth. Genotype x environment interactions do exist among cotton cultivars that aid in selecting cultivars for regions with variable temperature and moisture conditions. Screening techniques based on growth and physiology can successfully screen commercial cotton cultivars for early season cold and drought tolerance. Further, the early maturing cultivars have shown greater shoot and root growth at low temperatures and drought conditions compared to late maturing cultivars. Therefore, early maturing and cold tolerant cultivars can be selected by cotton breeding programs for developing cold stress tolerance. However, there is also a need to focus on molecular

mechanisms contributing to stress tolerance in diverse cotton germplasm that could provide long-term benefits. Thus, developing resistant commercial varieties could ensure uniform and even stand establishment, and subsequent vigorous shoot and root growth under varying temperatures and moisture conditions when cotton is planted early in the season.

REFERENCES

- Ackerson, R., D. Krieg, T. Miller, and R. Zartman. 1977. Water relations of field grown cotton and sorghum: Temporal and diurnal changes in leaf water, osmotic, and turgor potentials. *Crop Sci.* 17:76-80.
- Arevalo, L., D.M. Oosterhuis, D. Coker, and R. Brown. 2008. Physiological response of cotton to high night temperature. *Amer. J. Plant Sci. Biotechnol.* 2:63-68.
- Arndt, C. 1945. Temperature-growth relations of the roots and hypocotyls of cotton seedlings. *Plant Physiol.* 20:200-220.
- Ashraf, M., M. Saeed, and M. Qureshi. 1994. Tolerance to high temperature in cotton (*Gossypium hirsutum* L.) at initial growth stages. *Environ. Exper. Bot.* 34:275-283.
- Ball, R.A., D.M. Oosterhuis, and A. Mauromoustakos. 1994. Growth dynamics of the cotton plant during water-deficit stress. *Agron. J.* 86:788-795.
- Barpete, S., M.C. Oğuz, S. Özcan, E. Anayol, H.A. Ahmed, K.M. Khawar, and S. Özcan. 2015. Effect of temperature on germination, seed vigor index and seedling growth of five Turkish cotton (*Gossypium hirsutum* L.) cultivars. *Fresenius Environ. Bull.* 24:1-7.
- Basal, H., C. Smith, P. Thaxton, and J. Hemphill. 2005. Seedling drought tolerance in upland cotton. *Crop Sci.* 45:766-771.
- Bauer, P.J., and J.M. Bradow. 1996. Cotton genotype response to early-season cold temperatures. *Crop Sci.* 36:1602-1606.
- Bibi, A., D. Oosterhuis, and E. Gonias. 2008. Photosynthesis, the quantum yield of photosystem II and membrane leakage as affected by high temperatures in cotton genotypes. *Cotton Sci.* 12:150-159.
- Blum, A. 1996. Crop responses to drought and the interpretation of adaptation. *Plant Growth Regul.* 20:135-148.
- Böhm, W. 1979. Root parameters and their measurement. In: W. Böhm, editor, *Methods of studying root systems*. Springer, Berlin, Heidelberg. p. 125-138.
- BÖLEK, Y., M.N. Nas, and H. Cokkizgin. 2013. Hydropriming and hot water-induced heat shock increase cotton seed germination and seedling emergence at low temperature. *Turk. J. Agric. For.* 37: 300-306.
- Bradow, J.M. 1991. Cotton cultivar responses to suboptimal postemergent temperatures. *Crop Sci.* 31:1595-1599.
- Bradow, J.M., and P.J. Bauer. 2010. Germination and seedling development. In: J.M. Stewart, D.M. Oosterhuis, J.J. Heitholt, and J.R. Mauney, editors, *Physiology of Cotton*. Springer, Netherlands. p. 48-56.

- Brand, D., C. Wijewardana, W. Gao, and K.R. Reddy. 2016. Interactive effects of carbon dioxide, low temperature, and ultraviolet-B radiation on cotton seedling root and shoot morphology and growth. *Front. Earth Sci.* 10:607-620.
- Brown, E., and S. McCarter. 1976. Effect of a seedling disease caused by *Rhizoctonia solani* on subsequent growth and yield of cotton. *Phytopathology* 66:111-115.
- Burke, J.J. 2007. Evaluation of source leaf responses to water-deficit stresses in cotton using a novel stress bioassay. *Plant Physiol.* 143:108-121.
- Chastain, D., J. Snider, G. Collins, C. Perry, J. Whitaker, and S. Byrd. 2014. Water deficit in field-grown *Gossypium hirsutum* primarily limits net photosynthesis by decreasing stomatal conductance, increasing photorespiration, and increasing the ratio of dark respiration to gross photosynthesis. *J. Plant Physiol.* 171:1576-1585.
- Chastain, D.R., J.L. Snider, J.S. Choinski, G.D. Collins, C.D. Perry, J. Whitaker, T.L. Grey, R.B. Sorensen, M. van Iersel, and S.A. Byrd. 2016. Leaf ontogeny strongly influences photosynthetic tolerance to drought and high temperature in *Gossypium hirsutum*. *J. Plant Physiol.* 199:18-28.
- Chaves, M. 1991. Effects of water deficits on carbon assimilation. *J. Exp. Bot.* 42:1-16.
- Christiansen, M., H. Carns, and D.J. Slyter. 1970. Stimulation of solute loss from radicles of *Gossypium hirsutum* L. by chilling, anaerobiosis, and low pH. *Plant Physiol.* 46:53-56.
- Cottee, N.S., D.K.Y Tan, J.T. Cothren, M.P. Bange, and L.C. Campbell. 2007. Screening cotton cultivars for thermotolerance under field conditions. In: Proc. 4th World Cotton Research Conference. Lubbock, TX, USA, 10-14th September, 2007.
- Deeba, F., A.K. Pandey, S. Ranjan, A. Mishra, R. Singh, Y. Sharma, P.A. Shirke, and V. Pandey. 2012. Physiological and proteomic responses of cotton (*Gossypium herbaceum* L.) to drought stress. *Plant Physiol. Biochem.* 53:6-18.
- Ennahli, S., and H.J. Earl. 2005. Physiological limitations to photosynthetic carbon assimilation in cotton under water stress. *Crop Sci.* 45:2374-2382.
- Ephrath, J., A. Marani, and B. Bravdo. 1994. Photosynthetic rate, stomatal resistance and leaf water potential in cotton (*Gossypium hirsutum*) as affected by soil moisture and irradiance. *Photosynthetica* 29:63-71.
- Faria, T., M. Vaz, P. Schwanz, A. Polle, J. Pereira, and M. Chaves. 1999. Responses of photosynthetic and defence systems to high temperature stress in *Quercus suber* L. seedlings grown under elevated CO₂. *Plant Biol.* 1:365-371.
- Flexas, J., J. Bota, F. Loreto, G. Cornic, and T. Sharkey. 2004. Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants. *Plant Biol.* 6:269-279.
- Foyer, C.H., H. Lopez-Delgado, J.F. Dat, and I.M. Scott. 1997. Hydrogen peroxide-and glutathione-associated mechanisms of acclimatory stress tolerance and signalling. *Physiol. Plant.* 100:241-254.
- Gerik, T., K. Faver, P. Thaxton, and K. El-Zik. 1996. Late season water stress in cotton: I. Plant growth, water use, and yield. *Crop Sci.* 36:914-921.

- Gigon, A., A.R. Matos, D. Laffray, Y. Zuily-Fodil, and A.T. Pham-Thi. 2004. Effect of drought stress on lipid metabolism in the leaves of *Arabidopsis thaliana* (ecotype Columbia). *Ann. Bot.* 94:345-351.
- Gimenez, C., V.J. Mitchell, and D.W. Lawlor. 1992. Regulation of photosynthetic rate of two sunflower hybrids under water stress. *Plant Physiol.* 98:516-524.
- Grimes, D., and H. Yamada. 1982. Relation of cotton growth and yield to minimum leaf water potential. *Crop Sci.* 22:134-139.
- Hatfield, J., J. Quisenberry, and R. Dilbeck. 1987. Use of canopy temperatures of identify water conservation in cotton germplasm. *Crop Sci.* 27:269-273.
- Heen, A. 1980. Methods for root studies of annual plants. *Meldinger fra Norges Landbrukshogskole (Agricultural University of Norway)* 59:2-17.
- Iqbal, M., N. Nisar, R.S.A. Khan, and K. Hayat. 2005. Contribution of mepiquat chloride in drought tolerance in cotton seedlings. *Asian J. Plant Sci.* 4:530-532.
- Jain, M., P.V. Prasad, K.J. Boote, A.L. Hartwell Jr, and P.S. Chourey. 2007. Effects of season-long high temperature growth conditions on sugar-to-starch metabolism in developing microspores of grain sorghum (*Sorghum bicolor* L. Moench). *Planta* 227:67-79.
- Jordan, W. 1970. Growth of cotton seedlings in relation to maximum daily plant-water potential. *Agron. J.* 62:699-701.
- Jordan, W., and J. Ritchie. 1971. Influence of soil water stress on evaporation, root absorption, and internal water status of cotton. *Plant Physiol.* 48:783-788.
- Jordan, W.R. 1970. Growth of cotton seedlings in relation to maximum daily plant-water potential. *Agron. J.* 62:699-701.
- Jordan, W.R., K.W. Brown, and J.C. Thomas. 1975. Leaf age as a determinant in stomatal control of water loss from cotton during water stress. *Plant Physiol.* 56:595-599.
- Kakani, V., K. Reddy, S. Koti, T. Wallace, P.V. Prasad, V. Reddy, and D. Zhao. 2005. Differences in in vitro pollen germination and pollen tube growth of cotton cultivars in response to high temperature. *Ann. Bot.* 96:59-67.
- Karami, E., D. Krieg, and J. Quisenberry. 1980. Water relations and carbon-14 assimilation of cotton with different leaf morphology. *Crop Sci.* 20:421-426.
- Kargiotidou, A., I. Kappas, A. Tsaftaris, D. Galanopoulou, and T. Farmaki. 2010. Cold acclimation and low temperature resistance in cotton: *Gossypium hirsutum* phospholipase D α isoforms are differentially regulated by temperature and light. *J. Exp. Bot.* 61:2991-3002.
- Kawakami, E.M., D.M. Oosterhuis, and J.L. Snider. 2010. Physiological effects of 1-methylcyclopropene on well-watered and water-stressed cotton plants. *J. Plant Growth Regul.* 29:280-288.
- Kitao, M., and T.T. Lei. 2007. Circumvention of over-excitation of PSII by maintaining electron transport rate in leaves of four cotton genotypes developed under long-term drought. *Plant Biol.* 9:69-76.

- Lawlor, D., and G. Cornic. 2002. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant Cell Environ.* 25:275-294.
- Leidi, E.O., M. Lopez, J. Gorham, and J. Gutierrez. 1999. Variation in carbon isotope discrimination and other traits related to drought tolerance in upland cotton cultivars under dryland conditions. *Field Crops Res.* 61:109-123.
- Liu, S., M. Remley, F. Bourland, R. Nichols, W. Stevens, A.P. Jones, and F. Fritschi. 2015. Early vigor of advanced breeding lines and modern cotton cultivars. *Crop Sci.* 55:1729-1740.
- Loka, D.A., D.M. Oosterhuis, and G.L. Ritchie. 2011. Water-deficit stress in cotton. In: D.M. Oosterhuis, editor, *Stress Physiology in Cotton*. The Cotton Foundation, Cordova, TN. p. 37-72.
- Lopez, M., J. Gutierrez, and E.O. Leidi. 1995. Selection and characterization of cotton cultivars for dryland production in the south-west of Spain. *Eur. J. Agron.* 4:119-126.
- McMichael, B. 1986. Growth of roots. In: J.R. Mauney, and J.M. Stewart, editors, *Cotton physiology*. The Cotton Foundation, Memphis, TN. p. 29-38.
- McMichael, B., and J. Hesketh. 1982. Field investigations of the response of cotton to water deficits. *Field Crops Res.* 5:319-333.
- McMichael, B., and J. Quisenberry. 1991. Genetic variation for root-shoot relationships among cotton germplasm. *Environ. Exp. Bot.* 31:461-470.
- Medrano, H., M. Parry, X. Socias, and D. Lawlor. 1997. Long term water stress inactivates Rubisco in subterranean clover. *Ann. Appl. Biol.* 131:491-501.
- Meyer, L. 2018. *Cotton and Wool Outlook / CWS-18b*. Economic Research Service, USDA, US. 11p.
- Monk, L.S., K.V. Fagerstedt, and R.M. Crawford. 1987. Superoxide dismutase as an anaerobic polypeptide a key factor in recovery from oxygen deprivation in iris pseudacorus? *Plant Physiol.* 85:1016-1020.
- Murphy, J.B., and T.L. Noland. 1982. Temperature effects on seed imbibition and leakage mediated by viscosity and membranes. *Plant Physiol.* 69:428-431.
- Naim, A.H., and F.E.G. Ahmed. 2016. Interactive effect of temperature and water stress induced by PEG on germination and early seedling growth of two chickpea (*Cicer arietinum* L.) cultivars. *Open Access Library Journal* 10:1-7.
- Narayanan, S., A. Mohan, K.S. Gill, and P. Prasad. 2014. Variability of root traits in spring wheat germplasm. *PLoS ONE* 9:0317.
- Nepomuceno, A., D. Oosterhuis, and J. Stewart. 1998. Physiological responses of cotton leaves and roots to water deficit induced by polyethylene glycol. *Environ. Exp. Bot.* 40:29-41.
- Newman, E. 1966. A method of estimating the total length of root in a sample. *J. Appl. Ecol.* 3:139-145.
- Oosterhuis, D. 2002. Day or night high temperatures: A major cause of yield variability. *Cotton Grower* 46:8-9.

- Oosterhuis, D.M. 1995. Effects of PGR-IV on the growth and yield of cotton: A review. Proceedings of First World Cotton Research Conf. Brisbane, Australia. CSIRO, Canberra. p. 29-39.
- Oosterhuis, D.M., and S.D. Wulschleger. 1987. Osmotic adjustment in cotton (*Gossypium hirsutum* L.) leaves and roots in response to water stress. *Plant Physiol.* 84:1154-1157.
- Oosterhuis, D.M., F.M. Bourland, A.C. Bibi, E.D. Gonias, D. Loka, and D. Storch. 2008. Screening for temperature tolerance in cotton. *Summaries of Arkansas cotton research.* p. 37-41.
- Pace, P., H.T. Cralle, S.H. El-Halawany, J.T. Cothren, and S.A. Senseman. 1999. Drought-induced changes in shoot and root growth of young cotton plants. *J. Cotton Sci.* 3:183-187.
- Pearson, R., L. Ratliff, and H. Taylor. 1970. Effect of soil temperature, strength, and pH on cotton seedling root elongation. *Agron. J.* 62:243-246.
- Perry, C., E. Barnes, D. Munk, K. Fisher, and P. Bauer. 2012. Cotton irrigation management for humid regions. Cotton Incorporated, Cary, NC.
- Perry, S.W., D.R. Krieg, and R.B. Hutmacher. 1983. Photosynthetic rate control in cotton photorespiration. *Plant Physiol.* 73:662-665.
- Pettigrew, W. 2004a. Moisture deficit effects on cotton lint yield, yield components, and boll distribution. *Agron. J.* 96:377-383.
- Pettigrew, W. 2004b. Physiological consequences of moisture deficit stress in cotton. *Crop Sci.* 44:1265-1272.
- Pettigrew, W.T. 1994. Source-to-sink manipulation effects on cotton lint yield and yield components. *Agron. J.* 86:731-735.
- Pettigrew, W.T. 2002. Improved yield potential with an early planting cotton production system. *Agron. J.* 94:997-1003.
- Quisenberry, J., W. Jordan, B. Roark, and D. Fryrear. 1981. Exotic cottons as genetic sources for drought resistance. *Crop Sci.* 21:889-895.
- Radin, J.W. 1990. Responses of transpiration and hydraulic conductance to root temperature in nitrogen-and phosphorus-deficient cotton seedlings. *Plant Physiol.* 92:855-857.
- Rahman, M., I. Ullah, M. Ahsraf, J. Stewart, and Y. Zafar. 2008. Genotypic variation for drought tolerance in cotton. *Agron. Sustain. Dev.* 28:439-447.
- Reddy, K., H. Hodges, and V. Reddy. 1992c. Temperature effects on cotton fruit retention. *Agron. J.* 84:26-30.
- Reddy, K., H. Hodges, J. McKinion, and G. Wall. 1992b. Temperature effects on Pima cotton growth and development. *Agron. J.* 84:237-243.
- Reddy, K., V. Reddy, and H. Hodges. 1992a. Temperature effects on early season cotton growth and development. *Agron. J.* 84:229-237.
- Reddy, K.R., H.F. Hodges, and J.M. McKinion. 1995. Carbon dioxide and temperature effects on pima cotton development. *Agron. J.* 87:820-826.

- Reddy, K.R., V.G. Kakanl, D. Zhao, S. Kotl, and W. Gao. 2004. Interactive effects of ultraviolet-B radiation and temperature on cotton physiology, growth, development and hyperspectral reflectance. *Photochem. Photobiol.* 79:416-427.
- Reddy, V., D. Baker, and H. Hodges. 1991. Temperature effects on cotton canopy growth, photosynthesis, and respiration. *Agron. J.* 83:699-704.
- Reicosky, D., R. Millington, A. Klute, and D. Peters. 1972. Patterns of water uptake and root distribution of soybeans (*Glycine max.*) in the presence of a water table. *Agron. J.* 64:292-297.
- Reicosky, D., R. Millington, and D. Peters. 1970. A comparison of three methods for estimating root length. *Agron. J.* 62:451-453.
- Rosenow, D.T., J.E. Quisenberry, C.W. Wendt, and L.E. Clark. 1983. Drought tolerant sorghum and cotton germplasm. *Agric. Water Manag.* 7:207-222.
- Saini, H., M. Sedgley, and D. Aspinall. 1983. Effect of heat stress during floral development on pollen tube growth and ovary anatomy in wheat (*Triticum aestivum* L.). *Functional Plant Biol.* 10:137-144.
- Saini, H.S., and S. Lalonde. 1997. Injuries to reproductive development under water stress, and their consequences for crop productivity. *J. Crop Prod.* 1:223-248.
- Seepaul, R., B. Macoon, and K.R. Reddy. 2012. Ecotypic differences in switchgrass seed germination responses to in vitro osmotic stress. *Seed Technol.* 34:173-182.
- Singh, B., E. Norvell, C. Wijewardana, T. Wallace, D. Chastain, and K.R. Reddy. 2018. Assessing morphological characteristics of elite cotton lines from different breeding programs for low temperature and drought tolerance. *J. Agron. Crop. Sci.* 4:467-476.
- Singh, B., K.R. Reddy, E.D. Redoña, and T. Walker. 2017a. Developing a screening tool for osmotic stress tolerance classification of rice cultivars based on in vitro seed germination. *Crop Sci.* 57:387-394.
- Singh, B., K.R. Reddy, E.D. Redoña, and T. Walker. 2017b. Screening of rice cultivars for morpho-physiological responses to early-season soil moisture stress. *Rice Sci.* 24:322-335.
- Singh, R.P., P.V. Prasad, K. Sunita, S. Giri, and K.R. Reddy. 2007. Influence of high temperature and breeding for heat tolerance in cotton: a review. *Adv. Agron.* 93:313-385.
- Snider, J. L., D.M. Oosterhuis, G.D. Collins, C. Pilon, and T.R. FitzSimons. 2013. Field-acclimated *Gossypium hirsutum* cultivars exhibit genotypic and seasonal differences in photosystem II thermostability. *J. Plant Physiol.* 170:489-496.
- Snider, J., D. Oosterhuis, and E. Kawakami. 2010. Genotypic differences in thermotolerance are dependent upon prestress capacity for antioxidant protection of the photosynthetic apparatus in *Gossypium hirsutum*. *Physiol. Plant.* 138:268-277.
- Snider, J., D. Oosterhuis, B. Skulman, and E. Kawakami. 2009. Heat stress-induced limitations to reproductive success in *Gossypium hirsutum*. *Physiol. Plant.* 137:125-138.

- Snider, J.L., D.M. Oosterhuis, and E.M. Kawakami. 2011. Diurnal pollen tube growth rate is slowed by high temperature in field-grown *Gossypium hirsutum* pistils. *J. Plant Physiol.* 168:441-448.
- Steiner, J., and T. Jacobsen. 1992. Time of planting and diurnal soil temperature effects on cotton seedling field emergence and rate of development. *Crop Sci.* 32:238-244.
- Steudle, E. 2000. Water uptake by roots: Effects of water deficit. *J. Exp. Bot.* 51:1531-1542.
- Taylor, H., and B. Klepper. 1979. The role of rooting characteristics in the supply of water to plants. *Adv. Agron.* 30:99-128.
- Tezara, W., V. Mitchel, S. Driscoll, and D. Lawlor. 1999. Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. *Nature* 401:914-917.
- Turner, N., A. Hearn, J. Begg, and G. Constable. 1986. Cotton (*Gossypium hirsutum* L.): Physiological and morphological responses to water deficits and their relationship to yield. *Field Crops Res.* 14:153-170.
- Wanjura, D., and D. Buxton. 1972a. Water uptake and radicle emergence of cottonseed as affected by soil moisture and temperature. *Agron. J.* 64:427-431.
- Wanjura, D.F., D.R. Buxton, and H.N. Stapleton. 1971. The Effect of temperature, soil moisture, and physical impedance. *Cotton: A College of Agriculture, Univ. of Arizona, Tucson.*
- Wanjura, D.F., and D.R. Buxton. 1972b. Hypocotyl and radicle elongation of cotton as affected by soil environment. *Agron. J.* 64:431-434.
- Wijewardana, C., M. Hock, B. Henry, and K.R. Reddy. 2015. Screening corn hybrids for cold tolerance using morphological traits for early-season seeding. *Crop Sci.* 55:851-867.
- Wrather, J.A., B.J. Phipps, W.E. Stevens, A.S. Phillips, and E.D. Vories. 2008. Cotton planting date and plant population effects on yield and fiber quality in the Mississippi Delta. *J. Cotton Sci.* 12:1-7.
- Yan, J., C. He, J. Wang, Z. Mao, S.A. Holaday, R.D. Allen, and H. Zhang. 2004. Overexpression of the Arabidopsis 14-3-3 protein GF14 λ in cotton leads to a "stay-green" phenotype and improves stress tolerance under moderate drought conditions. *Plant Cell Physiol.* 45:1007-1014.
- Zhao, D., and D. Oosterhuis. 1997. Physiological response of growth chamber-grown cotton plants to the plant growth regulator PGR-IV under water-deficit stress. *Environ. Exp. Bot.* 38:7-14.
- Zhang, Y.L., Y.Y. Hu, H.H. Luo, W.S. Chow, and W.F. Zhang. 2011. Two distinct strategies of cotton and soybean differing in leaf movement to perform photosynthesis under drought in the field. *Funct. Plant Biol.* 38: 567-575.

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