

## Chapter 34

# GERMINATION AND STAND ESTABLISHMENT

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## INTRODUCTION

There is nothing more important to maximizing cotton production than a vigorous stand of seedlings. It has been repeatedly demonstrated that plants that develop from poor seed, or from seedlings that have been stressed by cold, pesticides, salt or pests, generally grow slowly, fruit poorly and produce uneconomic returns. The present paper will relate the current knowledge as to how cottonseeds germinate and how the germination environment can affect germination processes, seedling development and resistance to pests.

## COTTONSEED GERMINATION

Water imbibition is the first process of germination which triggers a multitude of metabolic and other physiological events. Early investigations by Simpson (1940) disclosed that water uptake by the dry seed is primarily through the chalazal aperture. Dye studies showed that the path of water followed the nucellar tissue around the embryo to the radicle cap, which was the initial site of embryo hydration. Various genetically controlled blocks to water entry are known to exist. Seed which are not wet by rain or dew may exhibit a moderate resistance to hydration that may persist for a few hours or more; however, genetically controlled impermeable seeds are generally endowed with chalazal blocks made up of pentosans, lignins, waxes and other water insoluble substances that exclude water for extended periods of time. Such seed may persist in soil for several years before microorganisms decompose the chalazal plug or other parts of the seed coat to permit water entry. Walhood (1958) developed a hot water (85C—1 to 2 minutes) treatment that successfully overcame impermeability in Pima S-1. Subsequent breeding efforts eliminated the problem in the Pimas. Christiansen *et al.* (1959, 1960) demonstrated that impermeable seed coats can provide long-term protection to preserve seed. Christiansen and Justice (1963) later showed that field deterioration could be greatly reduced by the heritable impermeable seed coat. Thus, impermeable seed coat can be advantageous. Little work has progressed since that time to further the idea other than an inheritance study by Lee (1964).

The rate of water entry into the embryo is initially rapid (12 hours) as a consequence of the initial change of embryo tissue from 8-10 percent to 40-80 percent water content (Dewez, 1964). Cole and Christiansen (1975) noted genetic differences in rate of water uptake among *G. hirsutum* and *G. barbadense* varieties. There is some evidence that very slow hydration (such as in high humidity) is beneficial to performance of aged or deteriorated seed, while immersion or rapid hydration can reduce subsequent seedling performance. The level of temperature during hydration can have considerable effect on the rate of water uptake and also on membrane organization in embryo cells. This will be discussed later in temperature considerations. A number of researchers have measured water uptake. The rate seems to vary widely, depending upon the genetic source, the location of production, the amount of picker or gin saw damage and the amount of field weathering. Dewez (1964) showed full hydration at 30C in 4-5 hours; Wanjura and Minton (1974) in something less than 6 hours. Cole and Christiansen (1975) showed a rapid hydration by Pima S-4 to near maximum level in 4 hours (60-70 percent) and a slow linear hydration rate for an M-8 selection to 50 percent after 8 hours. In the work by Cole and Christiansen, seed of both selections had identical cultural and storage histories.

All differences noted in rate of hydration of cottonseed most certainly reflect on field germination performance, as well as response to adverse seedbed conditions, particularly water availability and temperature. Simon (1978) suggested that unique changes in membrane configuration take place when seeds are hydrated. The major change in membranes is a change to a functional osmotic mediator or barrier that regulates germination functions. Any stress, including aging, that alters the normal course of establishment of functionality of membranes will drastically disrupt germination processes.

## ENZYMOLGY OF GERMINATION

Metabolic activity is greater and more complex during germination than at any other period in the cycle of plant life. Stored components of the seed are being mobilized and transported, new structural components and metabolic systems are developing, energy is transformed and expended at a high level. All events occur in a more or less orderly fashion unless disrupted by unfavorable external forces.

Cottonseed and most other oil-storing seed follow similar metabolic activity patterns during germination. Although detailed information is not available on all metabolic events occurring during cottonseed germination, it is possible to relate information from studies with other oil seeds to cottonseed and develop a cohesive metabolic map. Very early germination activity is supported by soluble carbohydrate reserves. The primary energy and carbon source during germination is from the stored lipid and protein. There is generally very little overall weight loss from seed to emerged seedling, indicating that most of the activity is a conversion of stored material to structural components.

Most of the early research on metabolic activity in cottonseed centered on lipid.

Of particular interest was the state of lipases in the seed. Olcott and Fontaine (1941) found no lipase activity in preparations from dormant seed but that activity increased rapidly with the onset of germination. Bamann and Ullmann (1940) and McIlrath (1956) agreed that little activity was present in dormant seed. Others (Ramakrishnon and Nevgi, 1951) noted considerable activity. Activity in dormant seed may be a consequence of field wetting and activation of a lipase precursor that remains active after redrying (Christiansen, 1960).

The hydrolysis of lipid during germination was studied under light and dark conditions by White (1958). Storage lipid was found to be depleted after 14 days. Contrary to reports by other researchers, White found no marked increase in free fatty acids during germination, indicating a continuous metabolism of fatty acids to organic acids and sugars via the glyoxylate pathway.

Isocitratase activity in germinating cottonseed has been of considerable interest as a method of monitoring the glyoxylate pathway. Scholl (1974, 1976) related level of activity with seedling vigor. It is also more chilling sensitive than other enzymes of the pathway (Smith *et al.*, 1971). A discussion of temperature-isocitratase interaction will be covered in a later section.

Germination-related protein hydrolysis, transport of amino acids and resynthesis into other structural and metabolically active entities has received less attention than lipids. Some early work with dormant seeds demonstrated the presence of active proteolytic systems (Rossi-Fanelli *et al.*, 1965) and active ribosomes (Phillips, 1964). Leffler (1976a) reported four-fold increases in ribonuclease over seven days germination which was paralleled in part by a large increase in polyribosomes. Patterson and Flint (1979) showed chilling at 13C reduced stomate conductance and reduced photosynthesis in seedling cotton. Parallel reductions in dry weight accumulation were noted.

## ENVIRONMENTAL EFFECTS ON GERMINATION

### TEMPERATURE

Cotton has been the subject of more chilling stress research than all other crop species. Yet, many questions remain, and the problem of low temperature induced loss of seedling stands continues.

The research has progressed along three avenues: 1. The kinds of temperature regimes that cause injury; 2. The nature of injury (physical, metabolic, biotic interaction); and 3. Methods of preventing or ameliorating injury (genetic, cultural and chemical). These are discussed below.

Cottonseed and seedlings are not uniformly sensitive to chilling at all stages of germination. Incidence of chilling during initial hydration can be extremely damaging. As little as four hours of chilling at the onset of hydration can kill all seeds or cause high incidence of aborted root tips; however, if seed are hydrated to 12-13 percent moisture, little injury occurs (Christiansen, 1967). Chilling after the radicle has elongated 2-3 cm causes cortex sloughing, slowing of early growth,

sensitivity to pathogens, membrane leakage of sugars and amino acids and long-term growth reduction and flowering delay (Christiansen, 1963, 1967, 1970).

Emerged seedlings also are sensitive to cold below 12C. The primary impact seems to be on water relations through inactivation of root water uptake and a continued high rate of leaf water loss (J.R. McWilliams, personal communication), which causes seedling desiccation (Kramer, 1940; Guinn, 1971a; Christiansen, 1978). Reports of injury to mature plants are also noted in the literature including the early work of Sellschop and Salmon (1928). Later work by Gipson and co-workers (1968b, 1970) related cool night temperature to effects on fiber quality and yield, and seed properties (see Chapter 5).

Early studies by Arndt (1937), Ludwig (1932) and Camp and Walker (1927) established minimum temperatures for germination under controlled conditions. These experiments indicated that temperatures below 15C are deleterious to germinating seed. A number of field studies are reported as guides to time of planting. These were primarily date of planting studies to ascertain optimal periods for maximizing stand and yield (Holekamp *et al.*, 1960; McQuigg and Calvert, 1966; Riley *et al.*, 1964). Some of the most definitive work on field stand performance is that of Wanjura and Minton (1974).

Symptomology studies have included both physical and chemical descriptions of cold injury. The early work of Arndt (1937), Camp and Walker (1927) and Ludwig (1932) was primarily in terms of germination. Later work by Christiansen and Thomas (1969) gave evidence of season-long expression of the effects of cold occurring during seed germination. These results, which show the linear cumulative effect of increments of chilling on final plant height, date of flowering and earliness, suggest that cold stress during germination affects a very basic control system in the seedling.

Several researchers including Kramer (1940) and Guinn (1971b) observed that emerged seedlings suffer water stress under chilling conditions. Christiansen and Ashworth (1978) later demonstrated that chilling injury at 8C did not occur, if the aerial parts were in high humidity. They suggested and demonstrated the ability of a chemical anti-transpirant to prevent cold injury in growth chamber studies.

Morphological and anatomical changes are induced by chilling. Change in size and shape of the first and second true leaves are caused by chilling during germination, and abortion of the root terminal is a common symptom of chilling during initial seed hydration (Christiansen, 1963). As indicated earlier, collapse and disintegration of radicle cortical cells are induced by cold during germination.

Many researchers have searched for the key site of cold impact on germinating seeds and seedlings. Definition of the major site of injury is desirable in order to intelligently seek solutions to the problems of chilling injury. Most of the studies involved comparative chemistry of constituents. Guinn (1971b) researched the effect of cold on carbohydrates, amino acids, proteins and nucleic acids in

emerged seedlings. Reducing sugars and amino acids accumulated in chilled roots, and protein and nucleic acid syntheses were retarded. DNA of both roots and leaves was reduced when roots were chilled. Guinn, therefore, suggested that cotton roots are a major source of the building blocks necessary for shoot development and that root metabolism is upset by chilling. Christiansen (1970) reported similar studies on pre-emerged seedlings, noting a general disarray of metabolic systems after chilling. Chilling retardation of isocitratase activity, lipid catabolism and lower soluble sugars were reported by Mohapatra *et al.* (1970). Leffler (1976a) noted chilling alteration of RNAase activity and cytoplasmic protein synthesis.

The functionality of cells, organelles within cells and the compartmentation of biochemical pathways is dependent upon membrane integrity. A number of reports implicated environmental stress with disruption of membrane form and function (Lyons and Raison, 1970). Christiansen *et al.* (1970) reported greatly increased exudation of sugars and amino acids from radicles following chilling, low pH and O<sub>2</sub> deficiency. Guinn (1971a) reported similar results with cotyledonary tissue, with the exception that membrane leakage of cotyledonary tissue continued after chilling was stopped, while it ceased in radicles, if cold exposure was less than 48 hours. Smith and Fites (1973) found permeability of glyoxysomes to succinate was reduced by chilling, which suggests cold-blockage of a metabolically active carrier system. By contrast, Christiansen (1970) reported that cold-induced (5C) radicle exudation can be quickly blocked by post-facto addition of calcium to the system. The latter situation suggests a physical function of calcium in stabilizing membrane structure, particularly since little metabolism goes on at 5C in cotton. St. John and Christiansen (1976) implicated the level of linolenic acid in radicle membranes with tolerance to chilling stress by using a specific chemical inhibitor of linolenic acid synthesis (Hilton *et al.*, 1971) to prevent chill hardening. The work firmly implicates fatty acid quality with functionality and survival under chilling conditions.

## OXYGEN REQUIREMENTS

Germination of seeds with lipid reserve requires a large supply of oxygen to support conversion of CH<sub>2</sub> units to Krebs cycle acids and to other metabolic entities. Restriction of free air to germinating cottonseed will inhibit germination, and waterlogging of seedbeds will cause stand failure. Tackett and Pearson (1964) studied the interaction of oxygen and soil compaction on root growth. They found that O<sub>2</sub> levels below 10 percent depressed seedling root growth at low soil bulk densities (1.5 g/cc); at higher densities, soil compaction became the factor controlling root extension; at very low density (1.3 g/cc) root growth was near normal at 5 percent oxygen levels. In earlier work Leonard and Pinckard (1946) reported that root growth ceased at 0.5 percent O<sub>2</sub>. Camp and Lund (1964) also noted depression of root growth at levels below 10 percent in low density soils and found no beneficial effects of increasing O<sub>2</sub> in compacted soil.

Bowen (1961) found that seedling emergence was markedly inhibited, if physical impedance of the emerging seedling exceeded 15 psi (see Chapter 36).

### MINERAL DEFICIENCIES AND TOXICITIES

Cottonseed germination requirements for exogenous minerals have not been thoroughly researched. Christiansen (unpublished) found no beneficial effects during 5 days of germination of additions of N, P, K, Mg, Mn, Bo or Fe to germination media at favorable temperature. Many researchers have observed favorable effects of calcium, particularly under stress. Presley and Leonard (1948) found cotton seedlings were more sensitive to calcium deficiency at low water levels. Wilcs (1959) noted beneficial effects of calcium in improvement of resistance to damping off organisms. Puente (1966) investigated the interaction of chilling and available calcium and observed improved survival and growth, if Ca was added to soil at levels of 1.4 and 3.8 g Ca/100 g soil. He noted that higher phosphorus levels caused increases of chilling injury to tap roots. Hood and Ensminger (1964) also found increased phosphorus in the form of ammonium phosphate was detrimental to seedling emergence.

Accumulation of salts at the soil surface due to upward movement of water and surface evaporation can be a serious problem, particularly in areas of the world where soils are sodic and irrigation water is high in salinity. Acid soils may also present root development problems with aluminum and manganese toxicity.

### CHEMICAL AIDS TO GERMINATION AND STAND ESTABLISHMENT

Use of chemicals to influence germination, emergence or stand persistence has been researched in several ways including modification of the seed bed environment, alteration of the chemistry of germination and functions of the emerged seedling. Most of the synthetic growth regulators were applied as seed or seedling treatment. Gibberellins induce more rapid emergence and taller seedlings (Ergle, 1958; Ergle and Bird, 1958; Bird and Ergle, 1961) but do not provide any salutary effect on stand survival or agronomic performance, particularly under stress. Cycocel, 2-4-D, naphthalene acetic acid and other growth active substances have provided little benefit (Bhardwaj *et al.*, 1963; Coats, 1966; Walhood, 1958). Reddy *et al.* (1979) reported that Cycocel prevented root rot in seedlings.

Cole and Wheeler (1974) compared hot water treatment (85C), seed soaking at 30C, cyclic AMP and gibberellic acid and noted some advantage in germination and seedling growth for AMP and GA and benefits in germination at low temperature for AMP, GA and soaking at 30C. McDaniel and Taylor (Agronomy Abst. 1979, p. 115) reported similar results.

As stated earlier, much of the injury to emerged seedlings is due to cold inhibition of root water uptake and resultant water stressing of seedlings. If highly effective antitranspirants can be developed, they offer a chemical method

to reduce stand losses (Christiansen and Ashworth, 1978).

Modification of the germination environment has been studied to prevent soil crusting, improve internal soil structure for aeration and drainage and to increase soil temperature. Bennett *et al.* (1964) tried various calcium compounds, Krilium (a soil conditioner), asphalt mulch and black plastic to improve stand establishment. The treatments all improved emergence, with black plastic the most effective. Ranney and Wooten (1966) reported similar studies with spray-applied petroleum mulch.

A considerable volume of literature exists concerned with seed protectants, including systemic fungicides and insecticides as well as topical seed protectants. Likewise, in-furrow fungicide treatment has been researched extensively. It is not within the scope of this review to discuss these topics.

## STAND IMPROVEMENT

What are the possibilities for progress toward solution of the problem? Several researchers demonstrated genetic differences in response to environmental adversity. Marani and Dag (1962b) noted maternal influence on germination at low temperature. Similar genetic variance in response to hydration-chilling sensitivity occurs between Acala and DPL selections. Reciprocal crosses of sensitive and tolerant lines indicated maternal control (Christiansen and Lewis, 1973). Bird and co-workers in Texas produced a number of lines which are superior in tolerance to the interacting effects of stress and seedling disease (see Chapter 35). Buxton and Sprenger (1976) reported genetic differences in field emergence from cold soils in Arizona.

The present knowledge of the effect of adverse environment and interacting seedling disease suggest that major progress can be made in improving stand production through breeding efforts. This statement is based on the assumption that genetic variability exists within available *G. hirsutum* and *G. barbadense* stocks. If this assumption is not true, the road to improvement may be much longer and dependent upon gene introgression from wild species or by other genetic engineering.

Chemical improvement of seedling resistance should not be ruled out. It is just a matter of time until we find ways to chemically alter stress tolerance.