Chapter 20

INTEGRATED EVENTS IN THE FLOWER AND FRUIT

James McD. Stewart[1]
USDA—ARS
Knoxville, Tennessee

INTRODUCTION

The Chapters of Section I present the parameters that influence flowering and production of fruit numbers such as time of flowering, sites of production and factors involved in fruit abscission. The cotton plant is regulated in such a way that fruit load is limited by abscission of square and young bolls when there is stress upon the plant. In this way, the impact of a stress upon individual bolls that remain often is ameliorated (Eaton, 1955; also, see Chapter 12).

During the square period and subsequent boll period many sequential steps occur which lead to the open flower with all its parts and eventually to the mature fruit with the seed and lint. Each stage of development of each part of the fruit is subject to modification by the environment and by the competition for photosynthate that prevails during that stage.

The purpose of this discussion is to show how the various events in boll development are interrelated during their development. Also where appropriate, discussions of the effect of environment on the interactions will be included.

THE SQUARE PERIOD AND THE FLOWER

SQUARE

Development—The development of the cotton fruit involves a complex series of events and interactions that begins with the formation of the flower bud (square). Unfortunately, less is known of the physiology of square formation and growth than any other part of the cotton fruiting cycle. Mauney (Chapter 2) reviewed some of the factors involved in square formation. Baranov and Maltzev (1937) and Joshi *et al.* (1967) described the morphological development of the square in the four cultivated species in *Gossypium*.

The structures that make up the flower are generally recognizable during square development. The earliest structure noted is the epicalyx which becomes the bracts of the mature fruit. Enclosed within the 3-sided "square" is the developing flower bud composed of the calyx, corolla, androecium and gynoe-

[1]Presently with University of Arkansas, Fayetteville, Arkansas

cium. The last two are discussed in more detail below.

The development from just visible square to open flower generally takes 25-30 days under good growing conditions, so the actual stimulus to flower must start 10 to 15 days before this stage (Gipson and Ray, 1974; Hesketh and Low, 1968; Martin *et al.*, 1923; Quintanilha *et al.*, 1962). Elongation of the bud of *G. hirsutum* is linear until about a week before anthesis (Martin *et al.*, 1923; Quintanilha *et al.*, 1962). During the last week before bloom the rate of growth increases dramatically and the bud doubles in length. On the other hand, the diameter of the bud is strongly correlated linearly with days to anthesis (DeLanghe, 1973; Quintanilha *et al.*, 1962). DeLanghe (1973) also determined that the diameter of the preanthesis ovary was linearly related to the bud diameter, as one would expect.

The difference in correlations of age with bud length and with bud width results from the developmental sequence of the anatomical structures measured. Bud length is primarily a function of corolla development. Most likely, cell division (linear phase) ceases and cell expansion (exponential phase) begins in the corolla during the week before bloom. Cellular expansion culminates in the open petals on the morning of anthesis when full maturity of the corolla is attained. Width of the bud is related more to the growth of the carpels which continue active cell division past anthesis.

DeLanghe (1973) found that absence of the subtending leaf resulted in slow growth of the bud and early shedding of the young boll. Gibberellic acid (GA) applied to the debladed petiole replaced the growth-promoting effect of the leaf; therefore, the subtending leaf of a square probably provides the GA for growth of that bud.

Response to Environment—The response of a cotton plant in the square stage to the environment is of particular interest because of the close relationship of square and flower production to earliness of a cotton crop. Several authors (Eaton, 1955; McMichael, 1979; see also Chapters 2 and 7) discussed the tendency of young squares to abscise when the plant is subject to stress. McMichael and Guinn (personal communication, 1980), from their preliminary data and the data of others (Grimes *et al.*, 1970), suggest that the sensitivity of a flower bud to water stress stimulus is greatest during its first week after visibility. Flower numbers during the weeks subsequent to the relief of a water stress indicated that square abscission was highest in the young squares, with the rate declining more or less linearly to nil at flowering. There are no reports of flowers abscising. Clearly, the physiology of the developing fruit form changes in ways which we do not yet understand.

Square abscission is also the main plant response to other stress situations, such as nutritional stress induced by shading, prolonged cloudy weather, or competing boll load (Eaton and Rigler, 1945; Eaton and Ergle, 1954; Eaton, 1955; Ehlig and LeMert, 1973; see also Chapters 6 and 12). A high rate of abscission may also be

associated with high temperature. Data on *G. hirsutum*, presented but not discussed by Ehlig and LeMert (1973), indicated that the number of flowers per meter of row declined approximately 3 weeks after periods when the maximum temperature exceeded 42C. This effect was particularly notable during a period when the number of flowers would normally be increasing rapidly. The response to high temperature is frequently confounded by moisture stress.

Little information is available on the effects of moisture stress on squares that are retained. High temperature effects on retained squares have been noted and will be discussed below in relation to pollen development. Plants grown at a constant 29.4C in growth chambers produced abnormal flowers in which the corolla failed to open and anthers did not dehisce (Powell, 1969). Plants with an alternating high-low temperature cycle had normal flowers.

Suboptimal temperatures generally do not result in square abscission; in fact, moderately cool periods during the juvenile growth of the cotton plant may promote early square formation (Chapter 2). Square period lengthens as average (Hesketh and Low, 1968) or night temperature (Gipson and Ray, 1974) decreases. Growth of the squares generally stops when temperature falls below 15C. However, square development apparently is less sensitive to night temperature than is boll development. Hesketh and Low (1968) found that flowers, but no bolls, formed at a temperature regime of 18C/13C. The square period increased only 20 percent at a night temperature of 13C compared to 25C (33 days vs. 27.5 days) (Gipson and Ray, 1974). The day temperatures were not reported. Square periods in Pima cotton did not change with plant age or position, but some lengthening due to temperature occurred in the latter part of the season (Martin *et al.*, 1923).

OVARY

Carpel Number—The ovary is compound in cultivated cottons, but the four cultivated species differ somewhat in the average number of carpels (locks) per ovary. *Gossypium arboreum* and *G. barbadense* average between 3 and 4, whereas *G. herbaceum* and *G. hirsutum* average 4 to 5. The average number of locks is strongly influenced by genetics, but environment also plays an important roll, both on potential locks per boll (carpels per flower) and on differential rates of shedding of bolls. Since the number of carpels per flower is determined very early in square formation (ca. 3-4 weeks preanthesis), the environment and physiological state of the plant prevailing during the "squaring" period will influence the ratio of 4 to 5 carpellate flowers in upland cotton.

Figure 1 shows the ratio of flowers with 4 and 5 carpels and the relative flowering rate of 'Acala' grown with and without adequate moisture (Beckett and Hubbard, 1932). The percentage of 5-carpel flowers increased to peak bloom and then declined. The decrease in carpel number was probably associated with the onset of flowering and accumulation of boll load that occurred during the fourth week prior to the declines. Surprisingly, the water stress conditions seemed to

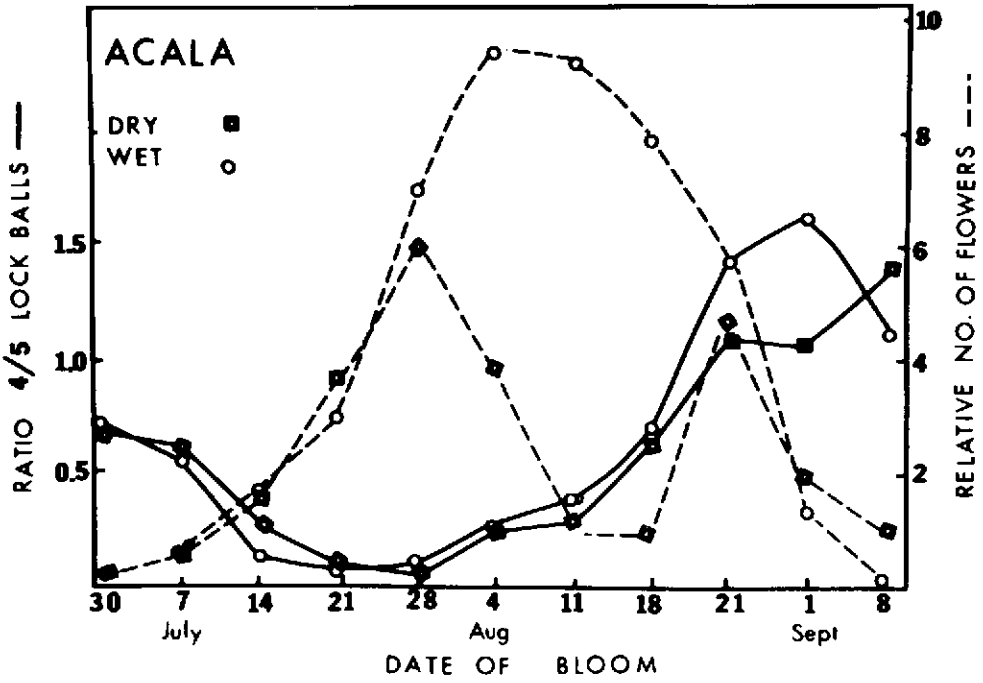

Figure 1. Ratio between Acala flowers with 4 and 5 carpels per ovary and the relative flowering rate during the bloom season as affected by water stress. Results are calculated from data presented by Beckett and Hubbard (1932).

have had only minor influences on the average number of carpels per flower (Beckett and Hubbard, 1932). In other experiments by the same authors (Beckett and Hubbard, 1932) Lone Star was grown at Greenville, Texas; although the flowering pattern differed from that of Acala in California, there were similar trends in the average number of carpels per flower with stage of development.

Leding and Lytton (1933), working with an unspecified variety of upland cotton in New Mexico, determined carpels per flower from peak bloom (late July) to late September under close spacing and moisture stress. In all cases, their results showed the same trend as shown in Figure 1 for corresponding periods. Moisture stress decreased the average number of carpels per flower slightly, but close spacing of plants had a major effect in reducing the average.

"Locks per boll" is primarily determined by carpels per flower, but differential shedding of 4 and 5 lock bolls has some influence. Beckett and Hubbard (1932) found that a great percentage of 5-lock bolls set under favorable growing conditions, but under moisture stress, the 5-lock bolls were consistently shed more often than 4-lock bolls. Johnson and Addicott (1967) found more 5-lock bolls after, than before, August 1 and speculated that 5-lock bolls were retained perferential-

ly. However, they assumed that the ratio of 4 to 5 carpellate flowers did not change, so their interpretation, which is contrary to the previous reports, may be invalid.

The tendency for the average lock number to decrease with increasing boll load, and the strongly negative response to close spacing (shading) suggest that the carbohydrate status of the plant influences the relative number of 4 and 5 carpellate ovaries produced. The effects of temperature on carpel number have not been definitively determined.

Ovules—The morphological development of cotton ovules has been described (Baranov and Maltzev, 1937; Joshi *et al.*, 1967; Stewart, 1975), but the physiology of ovule development before flowering has received only limited attention. Powell (1969) found that *G. hirsutum* plants maintained at a constant high temperature (29.4C) had very low fruit set, even when pollinated with pollen of known viability. An alternating high-low temperature regime resulted in normal fertility. A possible explanation is that the egg is inviable under constant temperature; however, failure of the stigma and style to support pollen germination and tube growth cannot be ruled out.

Hughes (1966) found seasonal and positional effects on the number of ovules per carpel in *G. barbadense* in Sudan. Flowers tended to have fewer ovules per lock the more distant they were from the main stem. The first sympodial node averaged the most ovules. Flowers produced very early and very late in the season averaged fewer ovules than those produced mid-season. The seasonal trend occurred on defruited as well as fruited plants, hence was independent of boll load. In general, flowers that have bloomed at the same time, regardless of position on the plant, tended to average the same number of ovules. Hughes (1966) also found that nitrogen fertilization produced a sizable increase in the average number of ovules/lock. Potassium gave a slight positive response, but phosphorus showed no effect.

Turner *et al.* (1977) found that as the bloom season progressed, the number of ovules per flower of *G. hirsutum* increased. Our unpublished data from these experiments showed that all of the 8 cultivars tested had an increase in ovules per carpel over the 5-week bloom-period observed. Thus, most of the increase in ovules per flower was due to an increase in ovules per carpel rather than carpels per flower (Figure 2). Others (Porter, 1936; Johnson, 1966) also observed an increase in ovules or seeds per boll (S/B) from early season to late season.

Pearson (1949a) noted vestigial structures which resulted in "false" motes in mature cotton. From my observations (unpublished), these structures, located at the base of the carpel, are incompletely developed ovules. Most likely, these structures reach full development in mid- and late-season squares of *G. hirsutum* and account for the increase in ovules per lock. Pearson (1949a) noted that false mote number declined from July 12 to August 9.

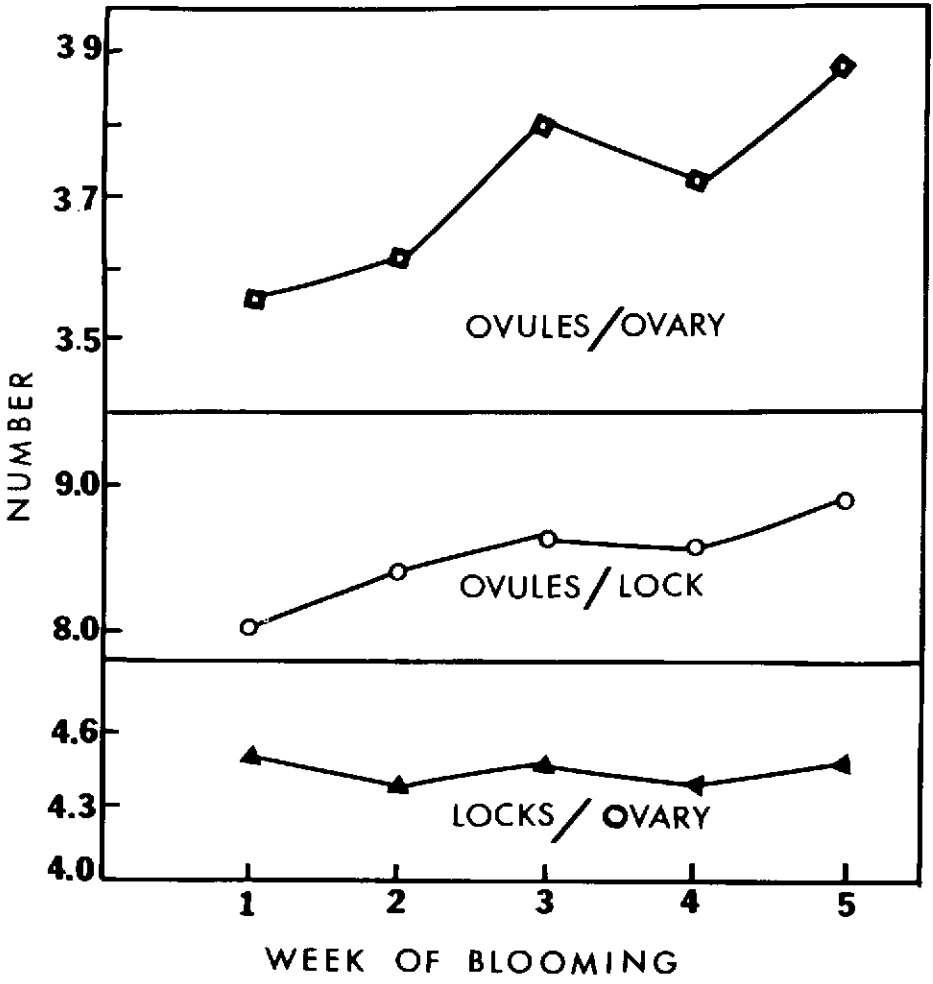

Figure 2. Influence of the week of blooming on the number of locks per ovary, ovules per lock and ovules/ovary. Average of 8 cultivars.

The influence of temperature on the number of ovules per flower has not been determined directly. However, the trends observed by Hughes (1966) in the Sudan and Turner *et al.* (1977) in the USA indicate that both cool temperatures and excessively hot temperatures during the formation of the square result in a lower number of ovules per lock.

STAMEN

Information on cotton stamen development is generally limited to morphology and microsporogensis (Beal, 1928; Baranov and Maltzev, 1937; Joshi *et al.*, 1967) rather than physiology, but some work has been done on the time of critical events. The microsporangia (anthers) develop very early in the square period and are established before the ovary obtains its complement of ovules (Quintanilha *et al.*, 1962). Meyer (1966) found that the number of anthers in *G. hirsutum* cv. M-8 was significantly and positively correlated with the mean relative humidity 22-23 days before anthesis. There was no correlation between anther number and temperature or absolute humidity. The 22-23-day-period corresponds approximately with the time of meiosis according to the time scale of Quintanilha *et al.* (1962). Meyer's (1966) results may indicate that anthers abort, since anther number is certainly determined considerably in advance of meiosis.

Continuous high or low humidity may have a negative influence in cotton production. Hoffman & Rawlins (1970) grew *G. hirsutum* plants in growth chambers which differed in relative humidities (R.H.). At constant low (25 percent) or high (90 percent) atmospheric relative humidity the anthers of the flower failed to dehisce. Also, the filaments were shorter at the extremes of R.H. compared to 40 or 60 percent R.H. The cause of indehiscence was not explored. Most likely, at high R.H. the anthers were normal, but could not dry sufficiently to dehisce, whereas at low R.H. the anthers were probably abnormal.

Structural variations within the developing anthers may influence subsequent events. Joshi *et al.* (1967) noted that anthers which failed to produce fibrous thickening in the endothecial layer did not dehisce, even though they contained mature viable pollen grains. Conditions responsible for the aberration were not explored. Also, genetic mutants occur where viable pollen is produced, but the anthers fail to dehisce (Murthi and Weaver, 1974).

Microsporogenesis—A detailed description of microsporogenesis is beyond the scope of this review; however, mention should be made of environmental influences during critical periods of development of the pollen. A number of reports (Sarvella, 1966; Meyer, 1969; Powell, 1969; McDonald and Stith, 1972) indicate that high temperature (32C+) 15-17 days before anthesis increases the sterility of pollen in temperature-sensitive male sterile stocks. Even fertile lines begin to show sterile anthers above 38C (Meyer, 1969). McDonald and Stith (1972) found simple correlations between maximum temperature at 17 days preanthesis and sterility. Multiple correlation analysis indicated an interaction at 19 days preanthesis also. In addition, these authors found that correlations existed between sterility and maximum humidity at 19 days (simple correlation) and 15 days (multiple correlation). Powell (1969) found that *G. hirsutum* grown at a constant temperature of 29.4C or above failed to produce viable pollen. Humidity was maintained at 70 percent. When the plants were grown with an alternating high-low temperature regime, the pollen was fertile. Fisher (1975) examined a

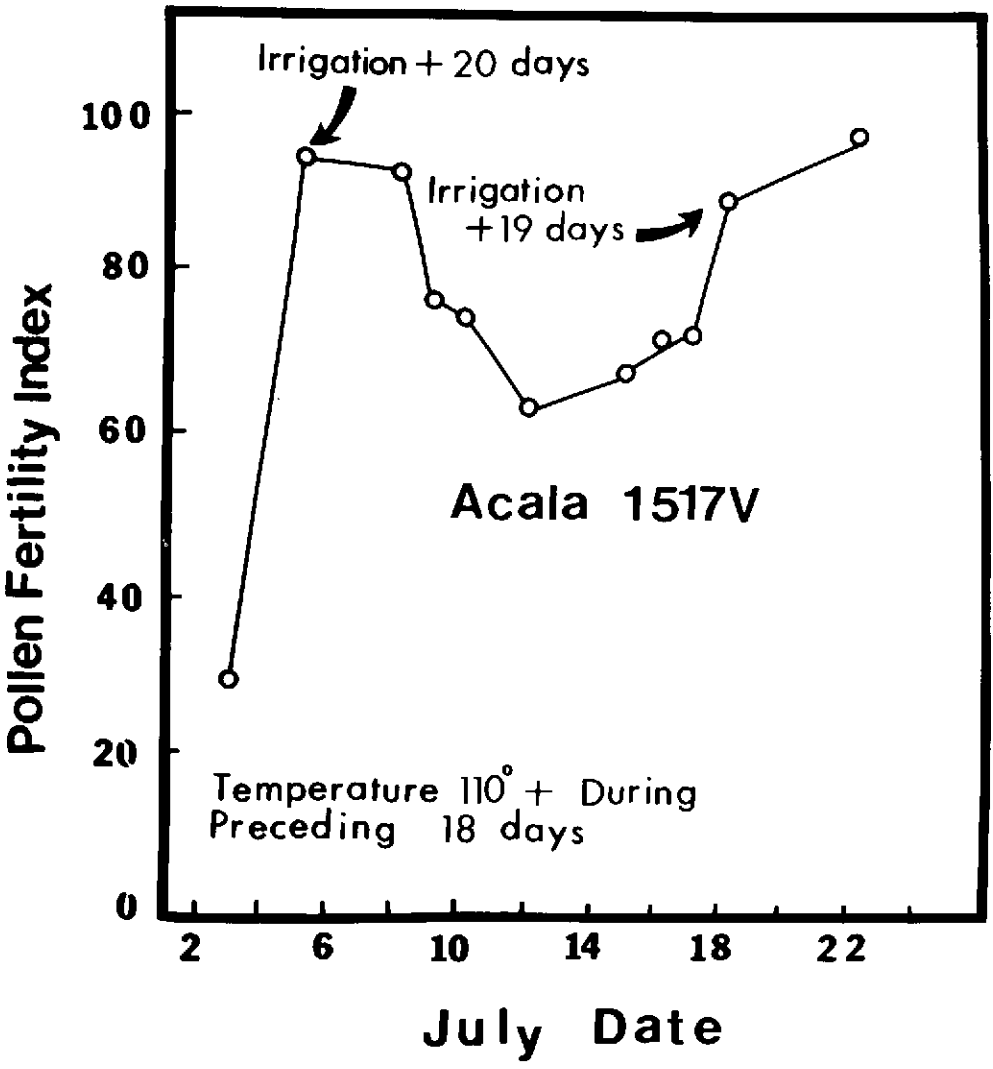

Figure 3. Influence of temperature on pollen fertility under production conditions. The temperature exceeded 43C each day during the 18 days prior to July 3. Irrigations, which reduce field temperature, were approximately 3 weeks before the days indicated by the arrows. Data from Fisher (1975).

number of cultivars under field conditions in Arizona and found that there were genetic differences in heat-sensitivity. Figure 3 is a graph of pollen fertility for one of the most heat-sensitive cultivars observed by Fisher (1975). The decreased level of pollen fertility could be attributed to high temperatures during the

preceding 3 weeks. The role of humidity could not be determined from the data; however, the cooling effect of irrigation (Stockton and Walhood, 1960) was probably sufficient to restore fertility.

Environmental factors such as temperature and humidity during critical periods of microsporogenesis apparently can induce pollen sterility and anther indehiscence. The exact stage of development at which the sensitivity occurs is not known; however, based on the time scale of Sarvella (1964) or Quintanilha *et al.*, (1962) it occurs after, rather than during, meiosis. Generally, in field plantings, the conditions must be extreme before a significant impact is noted and even then the genetic component is a major factor. If one considers the results of Powell (1969), it may be that pollen sterility is related to minimum temperatures rather than maximum temperature. That is, if during the critical period the night temperature does not drop low enough after a hot day, sterility results. This idea is supported by the results of Fisher (1973) who compared temperature and boll set over 7 years.

ANTHESIS

Flower Opening—The culmination of the square period occurs with the opening of the flower. The cells of the petals expand rapidly during the 24-hours preceding anthesis, and by early- to mid-morning the corolla is fully expanded. Simultaneous with petal expansion is the elongation of the stigma-style and of the filaments of the stamens. Less evident, but well documented (Anderson and Kerr, 1938; Lang, 1938; Stewart, 1975; Ramsey and Berlin, 1976a,b), is the fact that cotton fiber expansion begins early on the day of anthesis. This phenomenon occurs independently of fertilization; hence, it is related to the hormonal balance associated with the developmental progression of the flower bud rather than the pollination event. The hormonal or biological control of cotton flower opening has not been studied, but it is reasonable to assume that the simultaneously expanding tissues of the flower (petals, style, filaments, fibers) are all under the same temporal hormonal stimulus. Work by Beasley (1973), DeLanghe *et al.* (1978), Dhindsa (1978a) and Kosmidou (Chapter 25) strongly indicate that the stimulus to fiber initiation is gibberellic acid, but auxins cannot be ruled out.

The actual expansion process is probably mediated by active transport of sugars and potassium and synthesis of malate. This is indicated indirectly by the fact that the opening of the flower is strongly related to temperature; cool temperatures may delay expansion by several hours. Moderate drought seems to have less influence on opening than low temperature (unpublished observations).

The opening of the flower is the stage of sexual maturity of the cotton reproductive system. The embryo sac within the ovule is fully developed and receptive to fertilization. Pollen is shed from the anthers and will germinate on appropriately receptive stigmas.

***In Vitro* Germination and Tube Growth**—Studies on cotton pollen have been limited by the extreme sensitivity of the pollen to moisture. Whenever the grains or tubes contact freely available water, they rupture. Approaches to circumvent this problem involve both non-aqueous methods and aqueous gels or solutions of high osmolarity. The non-aqueous method of Klyukvina (as reported by Miravalle, 1965) involved grains coated with refined castor oil and maintained at 100 percent R.H. Although the original author reported nearly complete germination, Miravalle (1965) obtained no germination by the method. Bronkers (1961) developed a method involving exposure of the pollen to acenaphthene vapors in a humid atmosphere. Miravalle (1965) confirmed that cotton pollen has a high percent germination under these conditions; however, the tube growth reported by both authors was limited to no more than 3 times the grain diameter.

Aqueous methods using high osmolar solutions have received more attention than non-aqueous methods. Hancock (1949) got high percent germination but no tube growth on 35 percent sucrose solidified with 1½ percent agar. Vasil (1958) used 40 percent sucrose with 0.01 percent boric acid without agar and obtained lower germination but longer tubes (up to 0.78 mm). Taylor (1972) used 25 percent sucrose, and in addition to boric acid, he included manganous sulfate and calcium nitrate. Also, he solidified his medium with 3.5 percent agar and then aged the germination plates for 2 days under refrigeration before applying pollen grains. Although germination averaged only 30 percent, tube length averaged 15 pollen grain diameters (1.65 mm) with some tubes reaching 30 diameters. Wauford (1979) used Taylor's medium as a starting control and made subsequent additions and modifications in a number of parameters. The changes most noticeably improving germination or tube growth were the addition of $MgSO_4$ and a pH of 7.6. When $MgSO_4$ and KNO_3 were present, Ca was found to be nonessential or inhibitory. Govila and Roa (1969) also found beneficial effects on germination with the addition of magnesium and potassium. Wauford's (1979) best medium consisted of 25 percent sucrose, 3.5 percent agar, pH 7.6, 5.9 mM $MnSO_4$, 1.6 mM H_3BO_4, 1.0mM KNO_3, 0.8 mM $MgSO_4$, 1 μM GA and 0.1 mM IAA. With this, he averaged 47 percent germination and 2.60 mm tube length.

Wauford (1979) also examined the influence of different sugars on germination and tube growth. No germination occurred on fructose and only about 12 percent occurred on raffinose. Glucose and sucrose each supported about 33 percent germination under the conditions used. Tube growth was greatest on sucrose (1.6 mm), less on raffinose (1.2mm) and very low on glucose (0.2mm). In most cases, tube growth ceased due to rupture of the tube.

Investigations concerning the influences of growth regulators on cotton pollen germination and tube growth are limited and conflicting. Taylor (1972) reported that IAA and GA did not help or were inhibitory, whereas Wauford (1979) found them to be beneficial. The latter author found also that the growth retardant, succinic acid-2, 2-dimethylhydrazide (SADH), inhibited both germination and tube growth. Tube growth was almost 3 times as sensitive to SADH as was

germination. IAA or GA could not relieve the inhibition. In a preliminary experiment (unpublished), we noted that pollen tube growth was insensitive to 10^{-5} M 2-chloroethylphosphonic acid in the medium. Lipe and Morgan (1973a) found that anther and stigma-style tissues produce exceptionally high levels of ethylene. These data suggest that ethylene, although present, does not inhibit germination and tube growth. Whether it may serve as a promoter should be investigated.

Bronkers *et al.* (1972) used the method of Bronkers (1961) in their search for correlations between atmospheric conditions during growth of the parent plant and pollen germination percentage. No correlations were evident within the ranges of their climatic conditions. We noticed (unpublished data) that pollen germination and tube growth on artificial medium (Wauford, 1979) were influenced by the mineral nutrition of the parent plant.

The ultrastructure and some of the histochemistry of pollen cytoplasm were examined by Jensen and coworkers (Jensen *et al.*, 1968; Fisher *et al.*, 1968). They used Bronkers (1961) method for germinating pollen in their comparison of germinated and ungerminated pollen cytoplasm. The results of those studies indicate that mature pollen contains the reserves necessary for germination and early tube growth.

Pollen Storage—Cotton pollen generally cannot be stored for long periods (Govila & Rao, 1969). Wauford (unpublished data) obtained less than 5 percent germination of *G. hirsutum* pollen collected at 1:00 pm and then stored at room temperature for 24 hours. J.R. Barrow (personal communication) found that pollen collected at 8:00 am had only 1 percent viability at the end of the next day. However, buds collected the day before anthesis and stored under moderate refrigeration retain viability of the pollen for a few days. Harrison and Fulton (1934) reported that Pima pollen stored 4 days in a household refrigerator, where the lowest temperature was 4C, allowed 60 percent retention of bolls, but seed per boll (S/B) dropped from 13.2 to 11.6. I (unpublished data) and Barrow (personal communication) both observed that pollen will not survive freezing for 24 or 48 hours at -5C. It is likely that freezing for any length of time will kill cotton pollen.

***In Vivo* Pollen Tube Growth**—Iyengar (1938), in the first systematic study of pollen tube behavior in *Gossypium*, found that pollen on the basal part of the style germinated much less readily than pollen placed at the top of the style. The pollen of both American and Asiatic cottons germinate within 30 minutes after contacting a receptive stigma (Pundir, 1972). The tubes extend between the papillate hairs of the stigma and penetrate into the conducting tissue. After grain germination, the generative cell divides to form the two sperm cells. These cells and the tube cytoplasm are described in detail by Jensen and Fisher (1968).

The growth rate of the pollen tubes is slow for the first 2 hours as they traverse the stigmatic tissue to the conducting tissue. The rate in *G. hirsutum* then

increases to a maximum of about 3 mm/hr. as the tubes pass through the style. As the tubes approach the base of the style, the rate declines. The lowest growth rate is in the ovary (Iyengar, 1938; Pundir, 1972). *G. arboreum* pollen tube growth follows the same pattern except at a slower rate. When *G. hirsutum* pollen is placed on *G. arboreum* flowers, a slower rate of tube growth results. Conversely, the rate of tube growth of *G. arboreum* in the style of *G. hirsutum* is greatly increased (Pundir, 1972). These results indicate that the nutritive-hormonal balance of the conducting tissue is important to tube growth.

Suy (1979) examined some of the effects of environment on pollen tube growth of *G. hirsutum*. Light intensity did not influence the rate of growth, but red light (6,500 A) increased slightly (13 percent) the length obtained in 4 hours compared to white light. Relative humidity at 55 and 80 percent did not affect tube growth but 30 percent R.H. decreased growth. The most important factor was temperature. During the 4-hour test period adopted by Suy (1979), the rate of elongation was near zero below 19C and above 45C. Rate was linearly related to temperature up to 37C, but growth declined rapidly above that temperature. These results indicate that hot, dry conditions will inhibit pollen tube growth. If such conditions persisted, fertilization and seed set would be adversely affected.

At the opposite extreme, Pearson (1949b) observed that rain during morning hours decreased boll set and increased the number of unfertilized ovules in those bolls which did set. Her observations are, no doubt, a direct result of the moisture sensitivity of pollen grains.

THE BOLL PERIOD

SEED AND BOLL SET

Fertilization—The boll period of cotton traditionally is measured from the day of anthesis. Pollination occurs on the day the flower opens, but fertilization does not occur until 12 or more hours later. Many (Baranov and Maltzev, 1937; Constantine, 1964; Iyengar, 1938; Joshi *et al.*, 1967; Pundir, 1972) have described fertilization in cotton, but the work of Jensen and coworkers (Fisher *et al.*, 1968a,b; Jensen, 1965, 1968a, 1968b; Jensen and Fisher, 1967, 1968; Jensen *et al.*, 1968; Jensen *et al.*, 1977; Schulz and Jensen, 1977) has made cotton a model system for double fertilization. Only a brief summary will be given here. For details and ultrastructure the reader should refer to the above citations.

To accomplish fertilization, the pollen tube, by some unknown mechanism, grows toward and into the micropyle. Often, several tubes may enter a micropyle, but only one succeeds in penetrating one of the two synergids. This receiving cell begins to degenerate as a response to pollination and can be identified before the pollen tube reaches it. The sperm cells are discharged into the synergid through a lateral pore. The nucleus of one of the sperm cells enters the egg cell and begins fusion with the nucleus of that cell. The other sperm nucleus enters the polar cell

and fuses with one of the polar nuclei. This 2x nucleus then fuses with the remaining polar nucleus to form the 3x primary endosperm nucleus. Apparently, the nuclear fusions in the polar cell occur before the zygote is formed. The primary endosperm nucleus divides immediately and repeatedly, whereas the newly formed zygote shrinks in size and does not divide for about 72 hours.

Seed Setting Efficiency—The fertilization process is of primary importance to the production of the cotton crop. Any adverse factor during square development (discussed previously) which decreases egg or pollen viability or tube growth may adversely affect yield. Walhood and McMeans (1964) determined fruit retention as a function of seed number. To limit fertilization, they removed 50 percent or 90 percent of the stigma before pollination. Boll retention dropped from 68 percent in the control to 3.8 percent in the 10 percent stigma treatment. Within the bolls that set, seed number was reduced from 32 in the control to 10 in the 10 percent stigma. They concluded (Walhood and McMeans, 1974) that a high number of ovules must be fertilized to assure retention of a boll. The same conclusion was implied by Pearson (1949b).

The number of seeds in a boll is a function of ovules per flower and fertilization efficiency. The former is primarily a function of genetics but with an environmental component as discussed earlier. The reverse is true for fertilization efficiency. Turner *et al.* (1977) used the term "seed setting efficiency" (SSE) to designate the number of seeds produced compared to the number of ovules available for fertilization. When they determined SSE over a 5-weeks bloom period, they found that efficiency of fertilization declined late in the season one year but did not change the second year. In the 8 cultivars examined, the apparent SSE was approximately 90 percent. In reality, SSE was probably lower since more of the bolls with fewer seed would shed (Walhood & McMeans, 1964).

Small motes in mature cotton are ovules which were not fertilized (Pearson, 1949b). As such, the number of small motes in a boll can be used as an indication of fertilization efficiency. Rea (1929) found that mote content varied from 14 to 47 percent in 16 cultivars of upland cotton. High mote count was related to drought conditions. Pearson (1949b) examined several cultivars at 8 locations for 3 years and found that the influence of environment was much more important in the occurrence of motes than the influence of cultivar. Cultivars tended to retain their same rank regardless of when and where grown Locational factors seemed to be more important in the occurrence of small motes than seasonal factors. On a day-to-day basis, the number of motes could be correlated with increases and decreases in maximum temperature or with rainfall during the morning hours. Pearson (1949b) concluded that high temperature was probably the most important determiner of mote number in a crop since few bolls set on rainy days. Hughes (1968) found that about 5 percent of the ovules of *G. barbadense* became motes. Under Sudan conditions, early bolls had more unfertilized ovules than later bolls, but the number increased again toward the end of the season. He found

no relationship between number of motes and weather or irrigation cycle.

There is disagreement concerning the ovule positions most likely not to be fertilized. Walhood and McMeans (1964) found that bolls with low S/B had the seed at the apex. Iyengar (1938) observed that the earliest pollen tubes reaching the ovary did not necessarily enter the top ovules. Order of entry did not depend on position. Rea (1928) and Porter (1936) reported a progressive increase in motes from the apex to the base of the boll. Hughes (1968) found 20 percent of the motes in the basal position with no differences between the other positions. On the contrary, Pearson (1949b) found that the apex position failed to get fertilized just as often as the basal position, if only one mote per locule occurred. When there were two or more motes per locule, or when the number of ovules per locule increased, the basal position was less likely to be fertilized. Taken together, the reports indicate that the basal position is least likely to be fertilized.

Consequences of Fertilization—Assuming adequate nutrition and moisture within the plant to support additional bolls, failure of the ovules to be fertilized results in abscission of the young boll. Lipe and Morgan (1972, 1973a,b) showed that ethylene production by young fruiting forms was sufficient to induce abscission. The process of fertilization supplies an additional stimulus that counters the action of ethylene and prevents the shed of the young boll. Cognee (1975), Walhood (1957) and Walhood and McMeans (1964) demonstrated that GA was nearly as effective as fertilization in promoting boll retention (see Chapter 23).

An interesting observation related to fertilization was made by Jensen *et al.* (1977). When unfertilized ovules were cultured with GA and IAA, the two polar nuclei of the embryo sac fused and divided several times. In addition, one of the synergids degenerated. Since these events occurred without actual fertilization, the question of hormone source under natural conditions is open. Must actual penetration by the sperm cells occur, or does the process of tube growth produce sufficient hormone to trigger the observed changes?

Some phenomena occur independently of fertilization but are accelerated, or will continue, only if fertilization is successful. For example, the decline and separation of the corolla, staminal column and style occur without fertilization, but growth of the pollen tube in the style accelerates the evolution of ethylene (Lipe and Morgan, 1973a) and the senescence of these structures. As noted earlier, the fiber initials begin to elongate on the day of anthesis and will continue elongation for a few days without fertilization occurring (Cognee, 1975; Quintanilha *et al.*, 1962; see Chapter 23); however, the stimulus associated with fertilization is necessary for the grand elongation of the fiber.

In practical terms, the subsequent processes and events of boll development are dependent upon the fertilization event. The various aspects of boll development which are discussed in detail in other chapters need not be repeated here. In subsequent discussion, I will attempt to point out the aspects of boll development that may be competitive or interrelated in some way. Also, the influence of environmental factors on some aspects of development will be included.

DRY MATTER DISTRIBUTION

The development of a cotton boll with all of its components is an integrated process in which the various events occurring at any given time are under similar environmental, nutritional and hormonal influences. The distribution of available carbohydrate is controlled by the relative strength of the various competing sinks (Chapter 22). There are indications that some of the developing seed parts and constituents respond differentially to environmental changes; however, these phenomena have received only limited attention.

In *G. hirsutum* the ovary (bur) reaches full size and weight in 3-4 weeks (Leffler, 1976c) from anthesis. The seed reach their full volume and the fibers attain their maximum length (Schubert *et al.*, 1973) during this period also. The enlarging phase in *G. barbadense* takes somewhat longer (Schubert *et al.*, 1976). That most of the tissues expand simultaneously and then cease expansion at the same time seems more than coincidental. Conceivably, these tissues are under the same hormonal control to expand during the first 3-4 weeks postanthesis. During the third week the hormonal balance influencing the boll probably shifts to one which promotes accumulation rather than expansion.

Typical Weight Distribution—Figure 4 illustrates dry weight changes in various tissues of seeds of *G. hirsutum* cv. Coker 310. The seed were divided into the anatomical structures: fibers, outer integument, palisade, endosperm and embryo. Depending on age of the seed, the endosperm fraction included also the inner integument and nucellus, or in general, those tissues which support embryo growth. Fibers included fuzz fibers. For each of the two years, fiber weight increased up to about 16 days, at which time a decline occurred in the rate of increase. Thereafter, there was a resumption of rapid weight increase. The outer integument (not shown in Figure 4) increased in weight slowly for the first 2 weeks, but tripled in weight between 15 and 20 days postanthesis (DPA). The weight was constant or declined slightly (from 8 mg to 7 mg) for the next 2 weeks (36 DPA) then increased in weight slowly (to 10 mg) until opening. The palisade, which is formed from the outer epidermis of the inner integument, was evident and separable at about 14 DPA. The rate of weight increase in this structure was greatest between 20 and 30 DPA but a very slow increase in weight continued until opening. The endosperm tissue weight increased steadily up to 20 DPA and then declined in weight equivalent to the weight increase of the embryo. The weight of the embryo exceeded the weight of its supporting tissue at about 30-32 DPA. Beyond that, the rate of embryo weight increase was greatest. After 30 DPA, essentially all dry matter increase was in the fibers and embryo with the former accumulating cellulose and the latter accumulating oil and protein.

RELATIVE WEIGHT DISTRIBUTION AND DEVELOPMENTAL EVENTS

External vs. Internal Weight—The relative distribution of dry weight during seed development is best illustrated by the ratios of weights versus time and by direct

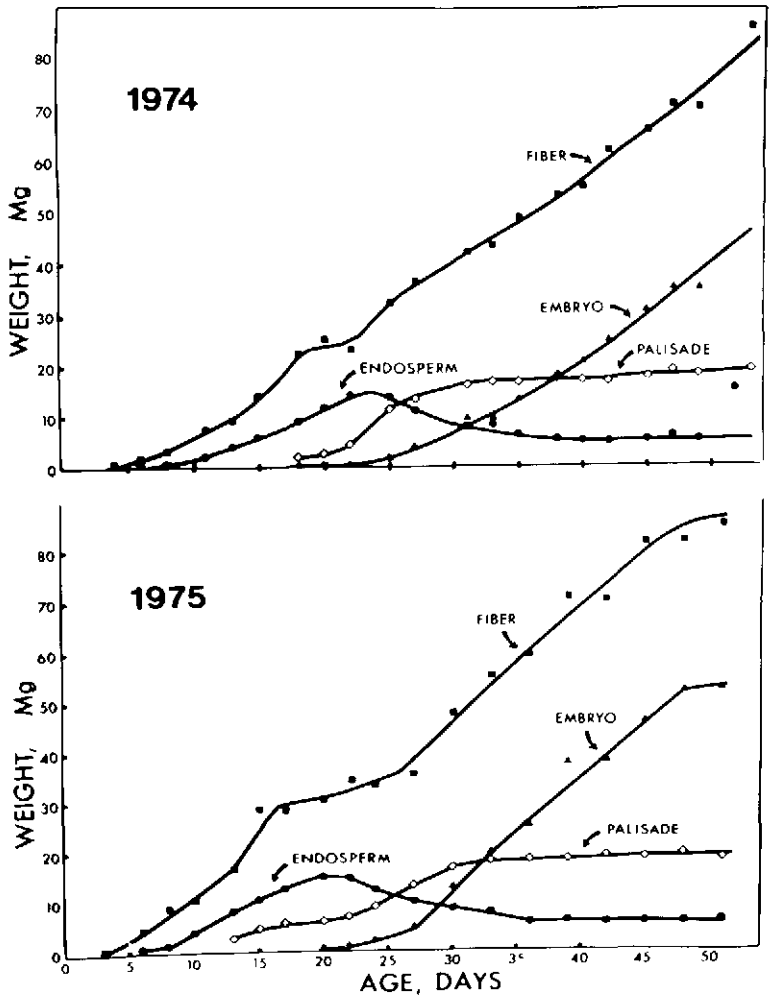

Figure 4. Distribution of mass into the various parts of Coker 310 seed during development. Fiber includes fuzz fiber and endosperm includes inner integument and nucellus. Each point is the average of the contents of 15 bolls.

comparison of the growth of seed parts. Figure 5 presents the ratios of external weight (fiber, fuzz and outer integument) and internal weight (remainder of seed) as a function of DPA. The external part of the seed receives the greatest portion of photosynthate during the first few DPA. This is indicated by the rapid increase in the ratio of external to internal weight during that period. After 4 to 6 DPA, depending on cultivar and environment, the internal weight increases

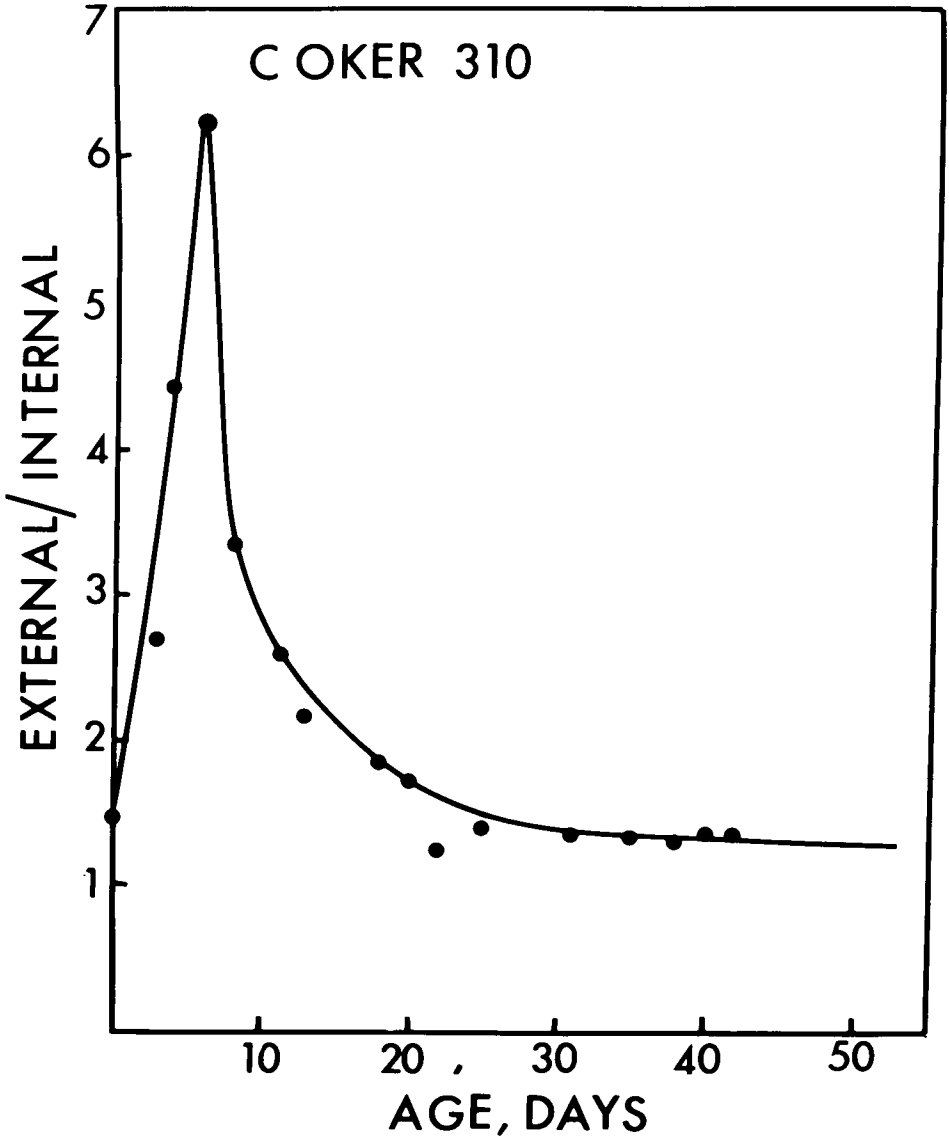

Figure 5. Ratio of external weight (fiber and outer integument) and internal weight (remainder of seed) from anthesis to boll opening in Coker 310.

somewhat faster up to about 20 DPA. From that time until maturity, the distribution of dry matter between external and internal seed parts is about equal, or perhaps internal weight gain is favored slightly, as will be seen later. Thus, three

distinct phases in the distribution of mass occur during the development of the seed. Each of these is discussed in more detail below.

Differentiation and Potentiation of the Outer Integument—As indicated earlier, several events, including fiber initiation, are related more to the flowering event than to fertilization. Unfertilized seeds and bolls will enlarge at the same rate as fertilized seeds and bolls until the third day after anthesis (Baert *et al.*, 1975; Cognee, 1975; see also Chapter 25). The stimulus of pollen tube growth (independent of fertilization) probably promotes and prolongs this growth.

Sufficient observations have been made on the early growth of the cotton seed to recognize that its development from -1 to 4-6 DPA is a unique stage which is almost independent of, and in no way secondary to, fertilization and the start of the embryo-endosperm complex. From Figure 5 it is evident that nearly all of the dry matter distributed to the seed during this period goes into the outer integument with its newly forming fibers. Thus, it is almost exclusively this tissue that is responding to changes in nutrition, hormonal balance and environment.

Associated with this period of development are a number of phenomena that determine the fiber (and perhaps seed) quantity and quality at maturity. Ramsey and Berlin (1976b) showed that the process of fiber differentiation can be recognized 16 hours before anthesis (see Chapter 26). Most of the fibers on the ovule, except near the micropyle, begin to expand on the day of anthesis (Anderson and Kerr, 1938; Lang, 1938; Ramsey and Berlin, 1976a; Stewart, 1975); thus, the environmental conditions preceding and during this time, no doubt, exert a strong influence on the number of fibers per unit area of seed and per seed. Worley *et al.* (1976) showed fibers per seed (F/S) to be a basic unit in the yield model of cotton. I calculated fibers per seed (F/S= lint per seed/micronaire x mean length) for four cultivars grown in 25 locations across the U.S. Cotton Belt and found a range of 13,000 to 21,000 among locations for a given cultivar (unpublished data). Although the number of fibers per seed were not measured directly, the large variation in calculated values does indicate that environment (location) can significantly influence the number of fibers produced. This is an area of fiber physiology that has not been examined.

A second parameter that may be strongly influenced during the period immediately following anthesis is fiber length. The information currently available on the response of fibers to hormones suggests that the potential for length is established during this period. Specifically, Beasley (1977b) demonstrated that GA greatly stimulates elongation of fibers on *in vitro*-grown ovules, but that its presence is essential only during the first few DPA. Likewise, ABA is effective in the inhibition of fiber growth only during the first 4 days of culture (Dhindsa *et al.*, 1976). IAA was effective in promotion of fiber development throughout the culture period (Beasley *et al.*, 1974). Others (Baert *et al.*, 1975; Singh and Singh, 1975) report results that substantiate those responses. In a study involving inhibitors of the phytohormones, Dhindsa (1978a) concluded that GA mainly promotes ovule

growth while IAA is mainly responsible for fiber growth. DeLanghe and coworkers (DeLanghe *et al.*, 1978) found that the morphology and size of the nucleolus within the nucleus of the young fiber were strongly influenced by GA, auxin and ABA. There was a correlation between nucleolar size at 8 DPA and final fiber length (however, maximum nucleolar size was attained at 5 DPA). They suggested that GA is the potentiating stimulus (production of ribosomes) and that IAA is the actuating stimulus (use or output of ribosomes).

Additional support for this is found in the report of Berlin and Smutzer (1976). Fiber could incorporate ^{14}C-uridine into RNA up to but not beyond 6 DPA. Also, Dhindsa (1978) found that 5-bromo-2-deoxyuridine, a thymidine analogue, inhibited fiber production, but only during the first 6 days of culture. Since GA is effective only during the few days after anthesis, this strongly indicates that the total potential for fiber length is determined during this period. (More detailed accounts of fiber development are covered in Chapters 23, 25 and 26). Cytokinins apparently do not have a major influence on the growth of the external part of the seed at this time (Beasley, 1977b; Beasley *et al.*, 1974), but may be related to internal events (see below).

Other phenomena concerning the external ovule parts are associated with this period before the endosperm-embryo complex is well established. Stomata develop on the ovules in large numbers, especially on the chalazal end, before the fibers begin to enlarge (Ayyangar, 1948; Stewart, 1975). Although no direct relationship has been demonstrated, it is an attractive hypothesis that these stomata function in the uptake of CO_2 for incorporation by the PEP-carboxylase pathway into malate. This pathway and organic acid are apparently important in the expansion of the fiber (Dhindsa *et al.*, 1975). Also, there is a high concentration of malate in the liquid endosperm which develops up to about 12 DPA. Mauney *et al.* (1967) found that ammonium malate was beneficial to the *in vitro* culture of very young embryos. Whether the stomata function for the exchange of CO_2 in the synthesis of these malate pools (fiber, endosperm) should be investigated.

Cell division in the epidermis (and probably the entire integument) accounts for the increase in surface area up to 6 DPA. Thereafter, very few divisions occur, and any increase in ovule surface area is accomplished by cell expansions (Berlin, 1977). Information presented by Berlin (1977) indicates that the non-fiber cells of the epidermis undergo between 2 and 3 cycles of division during the 6-day period. Cessation of division corresponds closely with the initiation of fuzz fibers (Lang, 1938).

Endosperm—An interesting feature of reserve storage occurs in the outer integument at anthesis. The preanthesis ovule contains very little starch; however, during the 24 hours preceding expansion of the fibers, starch begins to accumulate in the outer integument (Baranov and Maltzev, 1937; Stewart, unpublished). One could say teleologically that the plant has placed a reserve there for the growth of the fibers that soon start to expand. In reality, the starch does not

appear to be metabolized during elongation but continues to be accumulated and stored up to 20 DPA. The ultimate distribution of this reserve is unclear, but the integument during the latter part of development contains no starch. Since there is a very rapid accumulation of the starch during the 24-hour period before anthesis, a plausible method to determine the use of this reserve would be to pulse label it with ^{14}C at that time. Assuming a low level of turnover, the redistribution of the label could be followed after the 3-week period.

As can be seen, most of the activity of the cotton ovule during the first few days after anthesis is associated with the external part. Internally significant events occur which initially do not alter photosynthate distribution, but later have profound effects. Some of the changes have already been mentioned. Briefly, the free nuclear endosperm begins to develop almost immediately after triple fusion (1 DPA). As the endosperm develops, the nucellar material is consumed. The fertilized egg contracts and does not divide until 3 to 4 DPA. Continued divisions during the globular stage result in small cells with little increase in embryo size (Jensen, 1963; Pollock and Jensen, 1964).

Although a role for cytokinins in early ovule growth has not been demonstrated unequivocally by exogenous application, Sandstedt (1971) detected cytokinin activity in 1 DPA boll contents. This activity peaked at 4 DPA, was level to 8 DPA, then declined to an undetectable level by 15 DPA. He suggested that there was a causal relationship between the activity he detected and the observations of Pollock and Jensen (1964) concerning zygote division. Another possible correlation not considered by Sandstedt (1971) is cytokinin activity and endosperm activity. Free nuclear divisions occur rapidly from 1 DPA and continue in a pattern similar to the activity level reported for cytokinin. By 15 DPA, the endosperm is largely cellular. Whether cytokinins are produced within the ovule by the endosperm-embryo complex or transported into the ovule is unclear. The former suggestion is supported (but not proven) by the fact that the unfertilized polar nuclei can be induced to fuse and divide by ovule culture in GA and IAA without cytokinin (Jensen *et al.*, 1977).

The development of the endosperm-embryo complex signals the end of the differentiation and potentiation stage of the integument. Marked changes occur in the growth patterns of the ovule. Evidence indicates that IAA is the actuating hormone and that it is produced by the new complex (see Chapter 23). The new pattern of growth can properly be called the enlarging phase.

Enlarging Phase—Figure 5 shows that the ratio of external to internal weight begins to decline during the second week of development. This corresponds to the period in which rapid expansion of the ovule and fiber occurs. Stewart and Kerr (1974) showed that the increase in fiber length (L) during this period was allometrically related to the increase in ovule volume (V); $L = \beta V^{\alpha}$ or $\log L = \log \beta + \alpha \log V$ (Figure 6). The α and β are the growth parameters. The slope (α) of the lines for eight cultivars over three environments varied more among

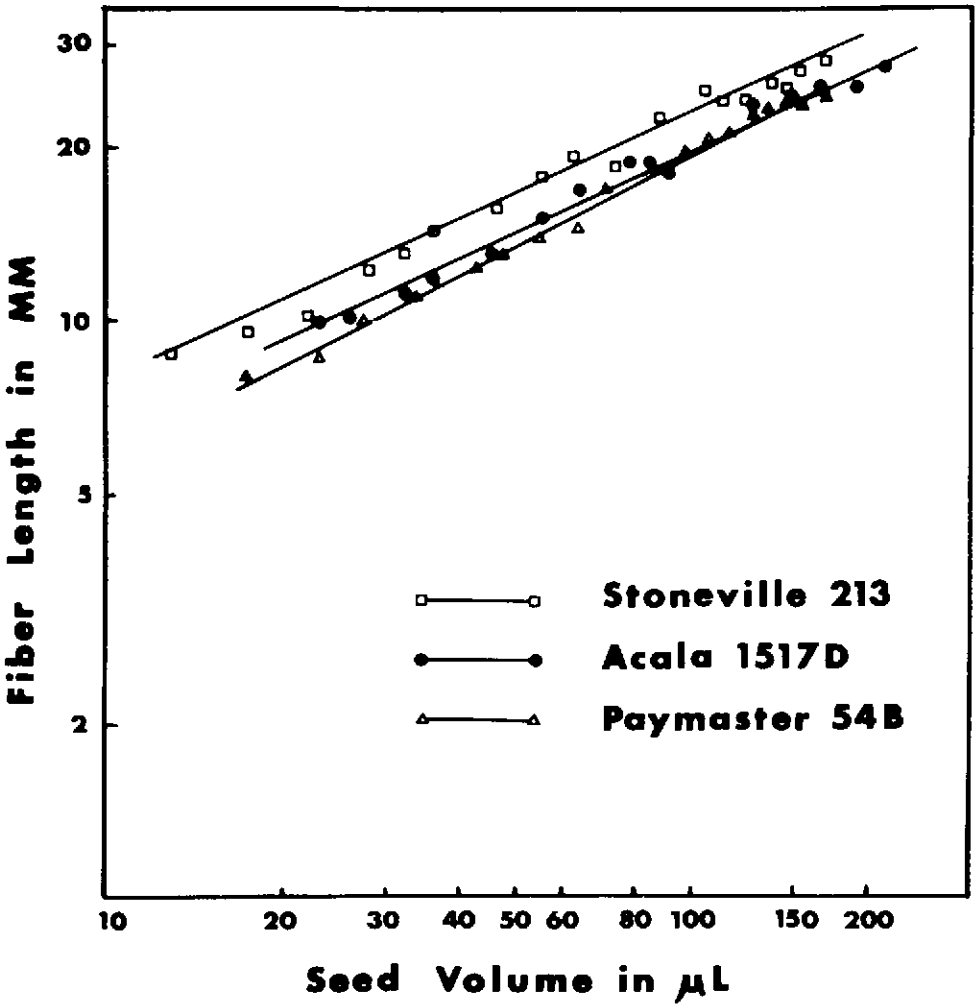

Figure 6. Relation of fiber length to seed volume during the enlargement phase (log transformation). $L = \beta V^{\alpha}$ or $\log L = \log \beta + \alpha \log V$. Length varies approximately as the square root of volume (Stewart and Kerr, 1974).

environments than among cultivars. The variation in β was about the same for cultivars and environment (Stewart, 1974). The β value represents parameters such as initial ovule size, time of fiber initiation and rate of enlargement of both ovule and fiber during the first week after anthesis.

Figure 7 shows the relative weight distribution between external and internal seed parts beginning the second week postanthesis up to 50 DPA. The results are expressed on a log-log scale for convenience. The cultivars Coker 310 and Dixie

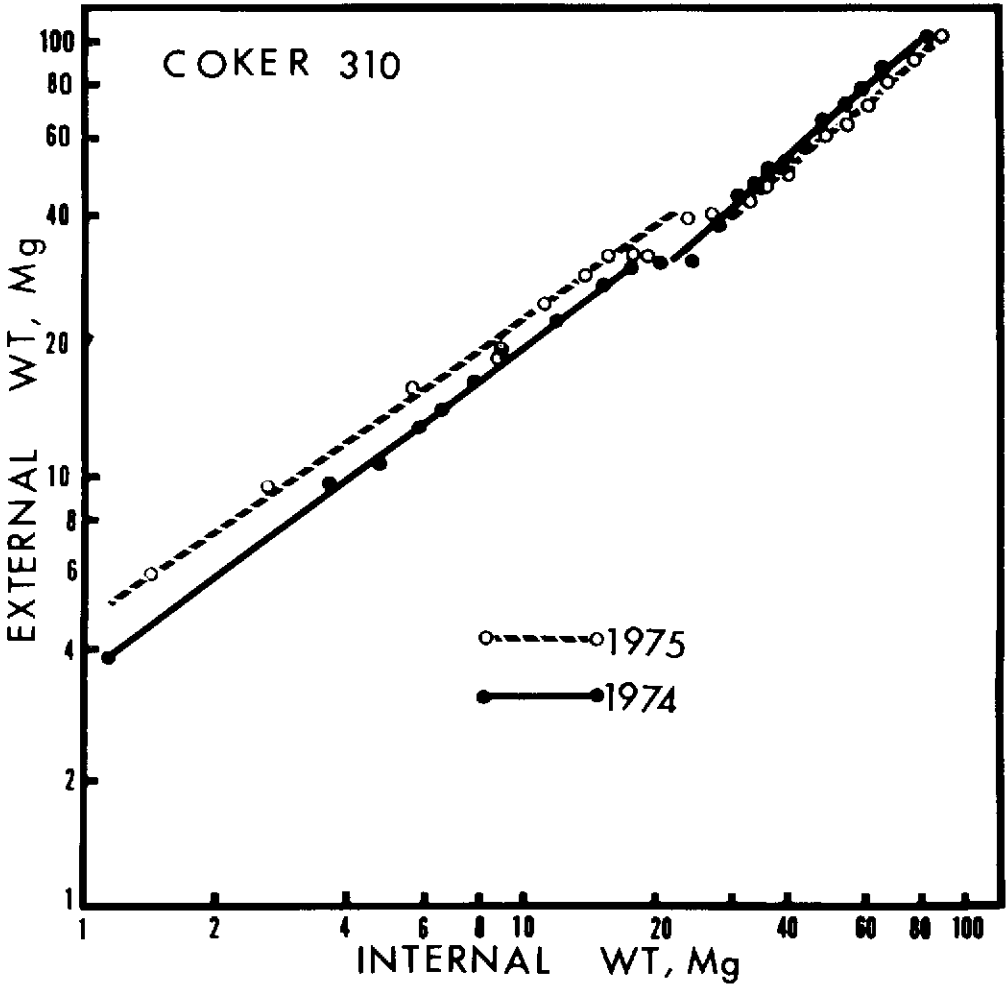

Figure 7. External weight versus internal weight of Coker 310 during the enlarging and accumulation stages. Two patterns of weight distribution are evident between the two stages.

King III (not shown) gave almost identical patterns for the 2 years. At about 45 mg total ovule weight (approx. 16-17 DPA), there was a hiatus in weight accumulation in the external part of the seed but not in the internal part. Reference to Figure 4 shows that the endosperm and palisade were accumulating weight at this time. After the pause, the fibers began to accumulate mass again but with a new distribution relationship compared to the previous distribution of mass.

The lower part of the curve in Figure 7 represents weight distribution during

the second and third week of growth. The slope of this line is approximately 1.7, indicating that the mass distribution is about 63 percent and 37 percent to the external and internal parts, respectively. This represents a considerable increase for the internal part of the ovule which was receiving only about 10 percent during the first 7 DPA.

The major structural additions to the external part are the initiation and development of the fuzz fibers, which begin at the start of the phase (Lang, 1938), and the length increase of the lint fibers. Also, it is likely that secondary cellulose deposition begins in the fibers during the latter part of this period (Schubert *et al.*, 1973). The non-fiber epidermal cells expand laterally and begin secondary deposition in conjunction with the fibers (Berlin, 1977). The outer integument does not increase in depth, but apparently the cells expand laterally and retain, and perhaps increase, their starch content (Baranov and Maltzev, 1937; Joshi *et al.*, 1967).

The development of the embryo with its associated endosperm has been examined by a number of individuals and groups (Baranov and Maltzev, 1937; Irvine, 1957; Joshi *et al.*, 1967; Pundir, 1972; Reeves and Beasley, 1935). There is little disagreement in the general sequence of events among these reports and only minor differences in the timing of events. The following descriptions on the embryo and endosperm are generalized concepts from the above works.

The internal part of the seed has the greatest structural changes during this period. At first the inner integument greatly expands and accumulates starch. The free nuclear endosperm also increases in size, and the embryo develops to the heart stage. The "liquid" of the endosperm has a high osmolarity. This is due mainly to malate salts (Mauney *et al.*, 1967) and probably simple sugars including fructose (Conner *et al.*, 1972). Unlike the fibers (Beasley *et al.*, 1974), the embryo apparently can utilize fructose (Mauney, 1961).

The endosperm-embryo complex is the only tissue undergoing mitosis during the second week of development. Around 10 DPA, the endosperm starts forming cell walls around the nuclei, especially in the micropylar area around the embryo. The sequence of this phenomenon suggests that the stimulus originates with the embryo. By the end of the third week, the endosperm is cellular and no additional divisions occur.

Shortly after the embryo is encased by cellular endosperm, it begins to elongate and form the cotyledons. Generally, by the end of the third week the cotyledons begin to enfold and the embryo begins to accumulate storage products. Also, the endosperm begins to be absorbed by the embryo. The maximum length of the embryo is obtained within a few days (ca. 25 DPA).

Coincident with, and perhaps related to, the changes occurring in the endosperm, the outer epidermis of the inner integument elongates to form the palisade layer which becomes the main protective layer of the seed coat. The stimulus that promotes this unique development is unknown. Secondary deposition in the palisade layer may precede or, at the very least, coincide with the onset of cellulose

deposition in the fibers and outer epidermal layer. The inner epidermis of the inner integument also acquires secondary deposition and eventually becomes the fringe layer.

During the enlarging phase of growth the hormonal interactions of the ovule do not seem quite as complex as the period immediately following anthesis. Auxin needed for the continued expansion of the fibers and ovule is probably produced by the endosperm-embryo complex. Excessive proliferation of integument cells has been related to exogenous GA treatments in several instances (Beasley, 1977b; Joshi and Johri, 1972; Stewart and Hsu, 1977b). Some motes may be related to excessive integument growth *in vivo* (Cognee, 1975; Joshi *et al.*, 1967). Most likely control of differentiation is maintained by a combination of IAA and ABA. These hormones are known to inhibit GA-mediated callus growth of the integuments (Beasley, 1977b; Stewart and Hsu, 1977b). Davis and Addicott (1972) showed that ABA in the young boll increased rapidly from 5 DPA to a maximum at 10 DPA. The amount in the lint and seed declined to near 0 by 15 DPA. Recall that fiber production was not affected by ABA after 4 DPA (Dhindsa *et al.*, 1976). Thus, the ABA may regulate integument development during this period. The influence of ABA on early embryo development *in vivo* is unknown, but there is evidence with somatic embryogenesis that it maintains the development of the embryoids in a more normal pattern of development compared to the absence of ABA (Ammirato, 1974). The absence of ABA at 15 DPA may permit elongation of the embryo. Onset of secondary deposition may also be related to the absence of ABA at this time (see Chapter 23).

C.A. Beasley and his coworkers have done extensive work on the responses of cultured cotton ovules to variations of minerals, carbohydrates and vitamins as well as the major hormones. The interested reader should refer to two reviews (Beasley, 1977b; Beasley *et al.*, 1974) for indepth discussion of their culture methodology and for more detailed discussions concerning hormonal interactions within the cotton ovule (see Chapter 39).

Accumulation Phase—During the latter part of the enlargement period accumulation begins, but apparently the two phases are not exclusive. Benedict and coworkers (Benedict *et al.*, 1972; Schubert *et al.*, 1976) showed that elongation of the fibers continues until about 25-26 DPA. Reeves and Beasley (1935) and Pundir (1972) indicate that the full length of the embryo is not achieved until about that same time, even though accumulation of reserve material begins several days earlier. The information at this point suggests that the two processes are independently regulated, but serious investigation of this area is lacking.

The relative distribution of mass during the time that enlargement and accumulation coincide reflects the latter phase. The break between the curves in Figure 7 occurs at about the developmental stage that accumulation of cellulose and embryo reserves starts (16-17 DPA). The slope of the line for the new distribution relationship is approximately 0.9. This means that 47 percent of the

mass is accumulating in the external part of the seed and 53 percent goes to the embryo. Reference to Figure 4 shows that the palisade is the main recipient of mass internally until about 30 DPA. During this time the gain in embryo weight is approximately matched by loss in endosperm weight. After 30 DPA, the embryo weight surpasses that of the endosperm and all additional weight changes internally are explained by embryo weight increases. Externally, essentially all mass accumulation is in the fibers (including the fuzz fibers). From this relationship, it is easy to see why immature seeds generally have a higher lint percentage than mature seeds (Meredith *et al.*, 1967; Walhood and Counts, 1955). The longer a seed accumulates mass, the greater will be the absolute amount in the embryo. Also, data presented by Walhood and Counts (1955) indicate that mass continues to accumulate in the seed (seed index) after the fibers reach maximum weight (fiber index).

During the development of the embryo, the 30 DPA to 32 DPA period is one that has attracted considerable interest, speculation and controversy. In order to explain certain results of their experiments with precocious germination and mRNA synthesis in developing embryos (see Chapter 28), Ihle and Dure (1972) proposed that the vascular connection between the ovule and parent plant atrophies at 32 DPA. Subsequent weight gain of the embryo supposedly would come from the rest of the ovule. In subsequent communications (Dure, 1975) the tenuousness of the proposal was overlooked, and it reached a wide audience and acceptance outside the cotton discipline. However, Benedict *et al.* (1976) adequately demonstrated that ^{14}C was transported into the seed up to 45 DPA (see Chapter 22). The results of Figure 4 support those of Benedict and coworkers and show that the remainder of the ovule, with the exception of the endosperm, does not lose weight to the embryo. An interesting feature can be seen in the weight changes of the embryo and endosperm. At about 30-32 DPA the embryo weight exceeds that of its supporting environment. Perhaps the restraints Ihle and Dure (1972) attributed to the parent plant reside in the endosperm and as this tissue is lost, the restraints are removed. That germination after 30 DPA is inhibited by ABA is well established (Davis and Addicott, 1972; Dure, 1975; Halloin, 1974). Davis and Addicott (1972) showed that ABA in the developing seed and boll is near zero at 30 DPA but increases rapidly to 40 DPA. The level of ABA in the seeds declines with age thereafter, but continues to increase in the carpel wall until boll dehiscence. The ABA within the seed is probably in the integuments since embryos removed from the seed will germinate (Dure, 1975).

While the external part of the seed accumulates only cellulose, the embryo accumulates both lipids and proteins. The proteins are generally accumulated first in association with the elongation of the embryo and then at a more or less continuous rate associated with the weight increase of the embryo (Elmore and Leffler, 1976; El-Nockrashy *et al.*, 1976; Grindley, 1950; King and Leffler, 1979). There is a low but progressive accumulation of polar lipids during embryo development. The neutral lipids, which constitute the bulk of the storage reserve,

increase rapidly in the seed from about 26-30 DPA to about 45 DPA and then increase only slowly (Brown and Kurtz, 1949; El-Nockrashy *et al.*, 1976; Grindley, 1950; Touma-Touchan, 1977).

Maturation and Boll Opening—The final period in boll development has not received the attention other periods such as the enlargement phase have received, and often it is not recognized as a time during which significant changes occur. Overall mass accumulation declines, probably with oil being the only carbon reserve that continues to increase (Brown and Kurtz, 1949; El-Nockrashy *et al.*, 1976; Grindley, 1950; Tharp, 1948; Touma-Touchan, 1977). The accumulation of cellulose in fibers apparently stops before reserve accumulation ceases in the embryo (Walhood and Counts, 1955).

Other changes occur during the maturation period. Active accumulation of minerals, in contrast to photosynthate, continues in the embryo; however, minerals in the carpel walls and in the fibers generally decline (Leffler and Tubertini, 1976; see also Chapter 21). Water-soluble nitrogen, including protein and small peptides, increases in the embryo, whereas accumulation of storage protein ceases (King and Leffler, 1979). Also, dramatic changes in the level of certain enzymes occur during this period (see Chapter 29).

The hormones in the fruit may play a significant role in maturation. The ABA concentration is high in the carpel wall and continues to increase during the maturation period; however, the amount of ABA in the seeds declines during this period (Davis and Addicott, 1972). It is not known if the decline in ABA in the seed is related to other observed changes. In the carpel walls the ABA increase may stimulate ethylene production and dehiscence of the boll. Ethylene production by the fruit increases abruptly during the maturation period (Lipe and Morgan, 1972a).

The final maturation of the seed coat occurs immediately before and during capsule opening. The various layers of the seed coat are not cemented together well and remain permeable to water until oxidative processes occur (Halloin, 1976b). Coloration of seed coats is apparently due to enzymic oxidation of catechin, and the development of impermeability to water requires O_2. In "hard" seed the oxidation process extends across the chalazal end, whereas normal seed take up water easily in this region (Christiansen and Moore, 1959). A "hard" seed condition may also result from a high level of ABA in the seed.

The stimulus that triggers the sequence of events leading to boll dehiscence is not known. One would expect that the progression to maturity in the seed would control events in the carpel wall. Ray (1963) found that the boll period of parthenocarpic bolls was 4 days less than fertile bolls. He suggested that dehiscence of capsules was independent of seed maturity. However, that the seeds delay dehiscence cannot be ruled out. In either circumstance, the results suggest that dehiscence can be controlled independently of seed maturation. This may have significance in developing techniques for green boll harvesting.

Once the sutures of the carpels separate, the walls begin to dry and reflex open. The vascular system is tangential to the carpel in the inner part but radial in the middle and outer parts of the carpel wall. Upon drying, the outer part can contract more, hence the walls reflex outward (Baranov and Maltzev, 1937; Simpson and Marsh, 1977).

ENVIRONMENTAL INFLUENCES

Boll development is controlled by the genetic-environment interaction. While recognizing that there is considerable genetic diversity among cultivars, in the following discussion I will attempt to give general physiological trends that occur in response to changes in the environment. Obviously all plant responses are to the total environment, but for simplicity the environmental parameters are divided into water, mineral and temperature effects.

Water—As discussed earlier, during the first 14 DPA the primary response of a young boll to stress is abscission. If water stress occurs after that time, the boll generally does not abscise (McMichael *et al.*, 1973). Since bolls are extremely resistant to water loss and can be considered non-transpiring (McMichael and Elmore, 1976; Radin and Sell, 1975), they are less susceptible to dehydration than leaves. The bolls exhibit 3 to 5 bars higher potential than the leaves, but their diameters will fluctuate with water status (McMichael and Elmore, 1976).

Moisture level during the fruiting cycle of the cotton plant does not affect all parts of the seed equally. During the enlarging stage a stress will reduce fiber length; conversely, irrigation will increase length slightly (Antony and Kutty, 1975; Bennett *et al.*, 1967; Hearn, 1976; Newman, 1967; Spooner *et al.*, 1958). The effects of water availability on the seed at this stage have not been determined directly. Also, these effects cannot be deduced from published reports since the customary measure is seed index, which is determined by both the enlargment and accumulation phase. However, one would expect seed volume to vary with available moisture much the same as fiber length.

Overall, seed index is probably more sensitive to moisture stress than the commonly measured parameters of fiber quality such as length and micronaire (Longenecker and Erie, 1968). This is indicated by the observation that cotton produced under dryland conditions generally has a higher lint percentage than irrigated cotton. Newman (1967) found higher micronaire values under non-irrigated culture and Bilbro (1962) found no differences compared to irrigated. However, under severe water stress, low micronaire values and immaturity of fibers can result (Antony and Kutty, 1975).

The relationship between water availability and percent oil in the seed has long been recognized (Anonymous, 1918). Stansbury *et al.* (1954) found a highly significant correlation of 0.59 for oil content and rainfall during the accumulation period. Correlations between oil percent and rainfall during the enlarging period were much less and were non-significant.

As with most environmental factors, the percent protein in the cottonseed seems to be less affected by water stress than by other seed parameters. However, environmentally controlled experiments are lacking in this area.

Mineral Environment—The influence of the mineral environment on cotton fruit development has been examined primarily as the response to fertilization under production conditions. Specific physiological studies of the essential elements usually are limited to yield, but some information on quality parameters in relation to fertilization is available. These studies were reviewed previously by a

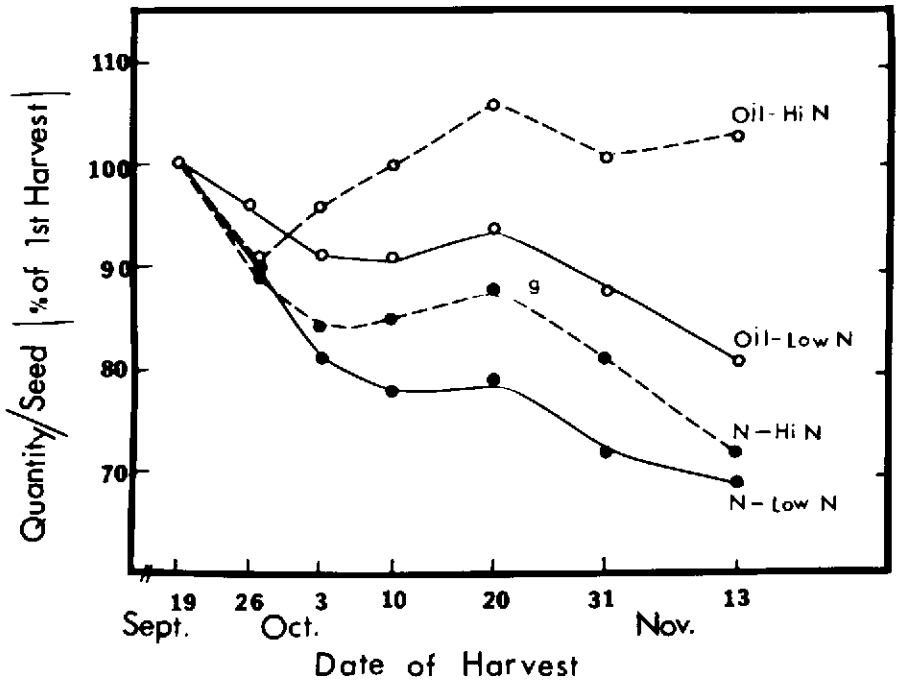

Figure 8. Percent change of seed oil and protein from first harvest to last harvest at high and low nitrogen fertilization. High N reversed the decline in oil but not protein. Results calculated from data given by Leffler *et al.* (1977).

group of authors in another symposium (Elliott *et al.*, 1968). Tharp (1960) presented a general treatment of the response of cotton to essential nutrients. In Chapter 9, Joham discusses the effects of minerals on the relative fruitfulness of cotton; in Chapter 21, Leffler discusses the accumulation and distribution of minerals in the fruit. In view of the availability of information elsewhere, my

discussion will be limited to generalizations and recent observations.

Nitrogen relationships in the developing boll are not yet completely understood. When comparing responses to adequate versus deficient nitrogen supply, the following are usually observed: increases in seed per boll, fiber length, lint weight, seed weight and seed nitrogen, but decreases in percent oil and in the ratio of lint to seed (Elliott *et al.*, 1968; Tharp, 1960). The increases are probably related to the general increase in vegetative vigor of the plant in response to N fertilization. The increase in seed nitrogen is due almost entirely to increase in storage protein (Leffler *et al.*, 1977).

The decrease in percent oil does not necessarily indicate a decrease in oil per seed. In fact, the absolute amount of oil probably increases. Tharp *et al.* (1949) and Leffler *et al.* (1977) noted that conditions that result in high protein may result in high oil content per seed. (The former authors pointed out the need for biologists to express oil and protein concentrations of the cotton seed as amount per kernel or amount per seed rather than the percent of seed used by industry.) Leffler *et al.* (1977) compared percent protein and percent oil of cottonseed from plants grown under high and low nitrogen fertility for different harvest dates. Also, from their data I calculated quantity per seed of oil and protein. As expected, percent N and N per seed increased with high-N fertilization, but they both declined from bottom harvest (early) to top harvest (late). Percent oil was decreased by high-N compared to low-N, but the percent increased from bottom to top harvest regardless of N. Oil per seed was reduced by high-N in the bottom harvest, but the relationship was reversed in the middle and late harvests. As a result of the treatment-environment-boll position interaction, total reserve per seed decreased in the low-N but not in the high-N as the season progressed. The effect of high-N was to maintain a high level of oil accumulation throughout the season. The percent changes of oil and protein with date-of-harvest at high and low-N are shown in Figure 8.

Although lint per seed increased with N fertilization, the observed decrease in lint percentage indicates that seed weight is increased more than lint. Fiber maturity is not significantly changed by nitrogen supply unless it is excessive, in which case micronaire is decreased (Hearn, 1976; Koli and Morrill, 1976). The influence of nitrogen on fibers per seed is not known; however, MacKenzie and van Schaik (1963) reported that N increased lint index (LI) but did not change length (M) or micronaire (Mic) under their conditions. Their observations indicate that the number of fibers per seed increased since $LI = M \times Mic \times F/S$.

All nutrient supply to the boll must be viewed in terms of active transport, since there is essentially no transpiration stream to carry minerals passively (McMichael and Elmore, 1976; Radin and Sell, 1975). This is particularly notable when the cotton plant receives nitrogen (N) as nitrate. No nitrate occurs in the ovules, and almost all N supplied to the developing seed is in reduced form (Radin and Sell, 1975). Evidence strongly indicates that the major form of N transported into the seed is asparagine (Elmore and Leffler, 1976).

There are a number of indications that the ammonium ion (NH_4^+) is critical to developmental events in the ovule. Beasley (1977a) found that the response to IAA of cultured ovules was qualitatively influenced by NH_4^+. I noted (unpublished data) a synergistic response between NH_4^+ and callus induction from cultured ovules by ethephon (Stewart and Hsu, 1976). Stewart and Hsu (1977a) found that the ammonium ion was critical for the *in ovulo* culture of embryos. All these reports demonstrate that seed development is influenced by the availability of the ammonium ion. How this ion functions is not known.

Potassium (K) availability is closely associated with seed development and strongly influences the quality parameters of the mature product. Where K is deficient, addition of the mineral will increase mean fiber length and length uniformity (Bennett *et al.*, 1967; Sabino, 1975; Tharp, 1960). These observations are understandable since K is the counter-ion for malate in the elongating fiber (Dhindsa *et al.*, 1975). Potassium malate probably acts as the major osmoticum for fiber expansion; thus, an adequate supply should assure that all fibers reach their maximum potential. Beasley *et al.* (1974) found that high levels of KNO_3 were beneficial to ovule and fiber growth *in vitro*. A high level of KNO_3 was also used for *in ovulo* embryo culture (Stewart and Hsu, 1977a).

During the accumulation stage of seed development, K may increase fiber micronaire (Sabino, 1975), but this response is not consistently reported. Perhaps the most consistent response to K fertilization is an increase in the percent oil and oil per seed (Tharp *et al.*, 1949). Seed weight is usually increased. Potassium is intimately involved in transport of photosynthate from the leaves to the bolls and decreases in the K content reduce both the quantity and distance carbohydrates move (Ashley & Goodson, 1972). With a deficiency of K, the symptoms of low carbohydrate status can be expected.

Phosphorus (P) generally does not have a major impact on seed development. If sufficient P is available for the fruiting form to remain on the plant, there is a sufficient amount for seed development. Sabino (1975) found that P fertilization increased fiber length slightly. The main role of P in fiber growth would be in sugar metabolism and in membrane synthesis. The major increase of P occurs in the seed during the accumulation phase (see Chapter 21). More than 80 percent of this accumulation at maturity occurs as phytin (Ergle and Guinn, 1959). The accumulation of P is not important in terms of seed processing/utilization quality but may be extremely important in planting seed quality. This apparently has not been investigated.

Information on the specific effects of other elements on the development of boll components is limited. Liming (Ca,Mg) was found to reduce micronaire without changing length or uniformity (Sabino, 1975). Anter *et al.* (1976a,b) sprayed micronutrients at various concentrations on cotton plants before flowering and/or before (what they called) boll-setting to determine the effects on fiber quality. Zinc (Zn) had no effect; length was decreased by manganese (Mn), iron (Fe), and boron (B); micronaire was decreased by copper (Cu) and Fe but was increased by

molybdenum (Mo) and B. EDTA also increased micronaire significantly and may have increased length slightly. The soil in their experiments was calcareous, so the EDTA may have had its beneficial effect by relieving calcium (Ca) inhibition of micronaire (Sabino, 1975).

Ovules grown in culture require B for normal growth and for development of fibers (Birnbaum *et al.*, 1974). A deficiency of B may disrupt the flow of metabolites through the pyrimidine synthesis pathway (Wainwright *et al.*, 1980) and cause reduced synthesis of UDPG (Birnbaum *et al.*, 1977). UDPG is probably the major intermediate in cellulose synthesis. Thus, sufficient-to-excess B should promote fiber maturity as observed by Anter *et al.* (1976a). The influence of B on embryo development is not known. Anderson and Worthington (1971) found no effect of B fertilization on oil or protein of the seed.

Temperature—The influence of temperature, especially low temperature, on cotton boll and seed development has been examined more than any other environmental factor. It is well established that boll period is inversely related to temperature (Gipson and Joham, 1968a; Hesketh and Low, 1968; Morris, 1963; Yfoulis and Fasoulas, 1978; see also Chapter 5). Whether mean day or night temperature is most important depends upon the range of the temperatures in relation to the maximum and minimum. Yfoulis and Fasoulas (1978) found that night temperature must be above a certain minimum rather than a certain mean before an economically acceptable boll-period results.

Other physiological factors not related to temperature influence boll period. Morris (1963) found that the physiological age of the plant when a boll sets has a strong influence on the boll-period. Also, there is great variation in the boll-period of fruit set the same day. Specific reasons for this variation have not been determined but are conveniently explained away as differences in microclimate.

Field experiments (Meredith *et al.*, 1967; Turner *et al.*, 1979; Verhalen *et al.*, 1975) indicate that most of the boll parameters other than seeds per boll (S/B) decline with the season. The declines are usually attributed to the cooler temperatures the cotton crop experiences in the latter part of the growing season. Quisenberry and Kohel (1975) quantified environments by daily and accumulated heat units (HU = mean temperature minus 18.3C). Correlation and regression analyses between HU and boll parameters showed that fiber weight per seed, micronaire and seed size were highly correlated to HU as expected. The maximum temperatures in the three environments they examined were not exceptionally high.

Gipson and coworkers (Gipson and Joham, 1969; Gipson and Ray, 1968, 1969a; Chapter 5) examined the influence of night temperature on fiber elongation. They concluded that the initial stages of fiber elongation were highly temperature-dependent, whereas the later stages appeared to be less sensitive to temperature. Their conclusions are justified when fiber initiation is included as a part of fiber elongation. However, close examination of their data revealed that no

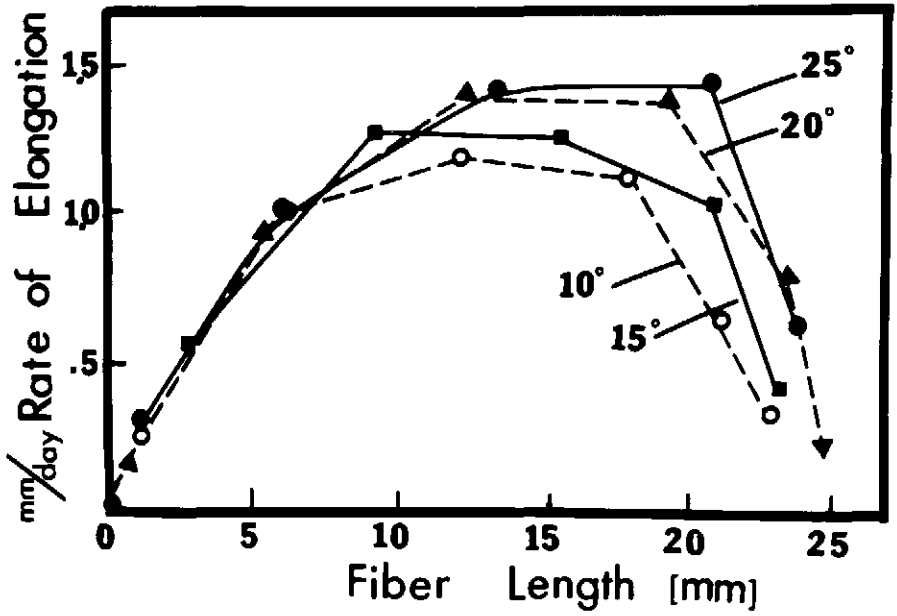

Figure 9. Rate of fiber elongation as a function of length at various night temperatures. Elongation of short fibers is relatively independent of night temperature. Results calculated from data given by Gipson and Ray (1968).

fiber growth was detected for a number of DPA at the lower night temperatures. Therefore, elongation-rate-per-day of fibers at the higher temperatures was being compared to a zero rate at the lower temperatures since there were no fibers. When their data (Gipson and Ray, 1968) for elongation rate were plotted against similar physiological stages of fiber development (length), the early stages of elongation seemed to be independent of night temperature while the later stages became more affected (Figure 9). The novel feature of their data is that fiber initiation (as opposed to elongation) seems to be very sensitive to night temperature.

High temperatures can have a detrimental effect on boll development. Turner (unpublished data) compared boll characteristics of three cultivars grown for 2 years in the Imperial Valley (hot) and the northern San Joaquin Valley (mild) of California. The cotton grown in the Imperial Valley had reduced boll size, seed index, weight per seed, seeds per boll, lint percent and mean length. Micronaire was higher in the hot environment of the Imperial Valley. Stockton and Walhood (1960) also found that boll size and fiber length decreased with increasing boll temperature but maturity increased. Since both low night temperature (Gipson

and Joham, 1968b; Gipson and Ray, 1969a) and high temperature reduce lint length, the response is hyperbolic. Boll weight also is related to temperature in a hyperbolic fashion, with the maximum occurring around a 20-22C night temperature and a day temperature less than 30C (Gipson and Ray, 1970; Hesketh and Low, 1968). Gipson and Ray (1976) found that lint index, lint percentage and lint per boll were decreased by high (37C) or low (13C) night temperature. Contrary to other reports, they found that seed index was increased by high night temperature (however, their day temperatures were probably less than 30C). All reports agree that micronaire is linearly related to temperature.

Information concerning the influence of temperature on embryo development is meager. Gipson and coworkers (Gipson and Ray, 1970; Gipson *et al.*, 1969) found that seed N content was linearly related to night temperature. Percent oil tended to respond hyperbolically, with the optimum being near 20C. Stansbury *et al.* (1954) examined eight cultivars at 13 locations for 3 years and found the correlation of percent oil to maximum temperature to be $r = 0.57^{**}$. Low temperature changes the ratio of simple sugars and the time of maximum accumulation in the seed (Conner *et al.*, 1972). The rates of accumulation of minerals and reserves are also altered by low temperature (Kreig *et al.*, 1973). Other than time required to develop, qualitative changes in the seed are difficult to recognize. However, certain interrelationships discussed below suggest that temperature (and other environmental factors) cause qualitative as well as quantitative changes.

COMPETITIVE INTERACTIONS

Many events and structures are involved in the development of the mature cotton fruit. Those events or structures which develop simultaneously compete for a share of the available mineral and photosynthate within the constraints imposed by the genetic complement and any differential response to environment that may exist. In addition, there is the possibility that the competitive results of one developmental sequence may influence the results in a subsequent sequence. For example, the number of fibers per seed which develop during initiation might influence the mean fiber length during enlargement, and these two in turn influence the amount of cellulose deposited in each fiber during accumulation.

The influence of some of these interactions can be detected in the mature harvested product. However, caution must be exercised in the interpretation of correlation analyses involving events that are separated temporally (environmentally) or involve multiple interactions. Almost no studies are available in which environment is controlled, but in some situations partial regression analysis has been used to separate the relative contribution of the various factors that influenced an observation. When two unrelated factors are influenced in parallel, they will give a positive correlation. That is, two parameters may be correlated but not related. Relatedness must be determined on the basis of their developmental history.

The obvious overall area of competition is between fruits. When the plant acquires a "boll load," the competition is such that no new fruit can set (see Chapters 2 and 12). Schubert *et al.* (Chapter 22) showed that sink demand will determine the distribution of carbohydrate. Since older bolls have the highest demand, new fruit is discouraged, and a boll in the accumulation stage will be at an advantage to one in the enlarging stage. Sink demand may explain the observations of Kittock *et al.* (1979). They estimated the effects of bolls at branch node 1 on bolls at other branch nodes. When branches had 2 or more bolls, the boll at node 3 averaged 8 percent fewer seed, 9 percent lower weight/seed, 13 percent less lint/seed and 22 percent less lint/boll than bolls at branch node 1. About 50 percent of the reduction in seed weight and 75 percent of the reduction in S/B and lint/seed (L/S) was the result of onbranch competition.

The second level of competition occurs between the seeds within a boll. Baranov and Maltzev (1937) demonstrated that the rate of development of embryos in different positions of the lock varied greatly. The slowest-developing position was the basal one. The degree of sunlight (amount of photosynthate) strongly influenced the rate of development, also. Porter (1936) examined fiber length as a function of lock position and found that the position nearest the apex, especially in the many seeded bolls, had the shortest fibers. Seeds near the base of the boll had shorter fibers than seeds at the middle positions (Figure 10).

Although an increase in S/B should increase the sink strength of the fruit, the relationship is not linear. Kittock and Pinkas (1971) measured individual bolls of Pima and found that seed weight decreased as S/B increased. Others (Kearney, 1926; Scholl and Miller, 1976; Turner, unpublished) found a similar correlation in Pima and Upland cotton. In a study involving male-sterile lines, bolls which varied widely in their numbers of seed allowed examination of S/B effects over 5-seed increments from 5 to 40 (L. L. Ray, personal communication). Seed size and fiber-per-seed were strongly inversely related to S/B; however, there seemed to be a definite breakpoint at about 15 S/B. Micronaire, which is related to weight per unit length of fiber, was also inversely related to S/B except that the lowest seed class had a low value. Length was not related to S/B except that the lower category had the shortest length. It appeared that below a certain number of S/B, the strength of the boll as a sink was insufficient to provide for optimum growth.

Turner (unpublished data) determined correlation values for 8 cultivars at 67 or more environments. When all cultivars were examined for trends, the results in Table 1 were observed. These are generalizations concerning the direction of change of seed parameters as influenced by environment. Geneticists often use correlation analyses of diverse genetic lines to predict changes that may occur when selection pressure is placed on a given trait. A survey of a number of these (Innes, 1974; Kearney, 1926; Quisenberry *et al.*, 1975; Scholl and Miller., 1976; Woodward and Malm, 1976) revealed that genetic or intrinsic correlations do not differ greatly from the trends shown in Table 1. (Not all results were in agreement in every case, but generalizations can be made).

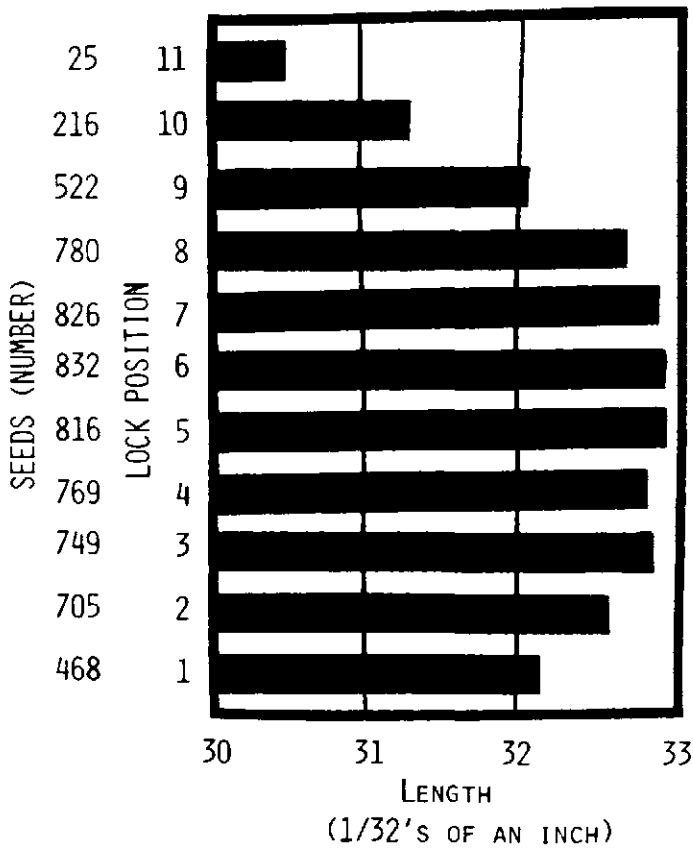

Figure 10. Influence of seed position in the lock on fiber length. From Porter (1936).

The number of S/B is negatively related to most other seed parameters. This is understandable in terms of competition among seeds. The relationship of mean length and S/B is uncertain. Turner found a high positive correlation but Ray found no correlation. The available genetic correlations were also in disagreement. All correlations show that SI or weight per seed is positively associated with fiber quality. Conditions which support good seed growth and development also support fiber development. This may be an example of the parallel influence of environment. Lint per seed (L/S) showed a negative correlation with micronaire across environments, but Quisenberry *et al.* (1975) found a positive correlation in his genetic material. Developmentally, a negative correlation would be expected.

Perhaps one of the more detailed studies of fiber interactions is that of Moore

Table 1. Trends in correlation values for 8 cultivars grown under 67 or more environments (location x years).

	Mic.	M.L.	L/S	SI
S/B	0	+	−	−
SI	+	+	+	
L/S	−	+		
M.L.	0			

(1941). He found that an increasing fiber population on the seed was associated with fiber weight decreases, with the percentage of thin-walled fibers, and with a decreasing fiber length. There was also a negative association of average fiber length and the average fiber weight per inch.

Many of the interactions involved in fiber and seed yield per seed and boll are not clear. Information of the parameter, fiber density (f/mm^2), is lacking even though it is a basic yield component: fiber yield/boll = wt/f x f/mm^2 x mm^2/S x S/B (Turner *et al.*, 1979). Also, from this relationship, it is obvious that surface area of the seed is important.

Surface area is related to seed volume, another parameter that may be important, but whose contribution is unknown. What is the relationship of seed volume developed during the enlarging stage upon kernel (embryo) weight that develops during the accumulation stage? What is the relationship of the enlarging stage to seed density?

A correlation between oil and protein in the cottonseed has been recognized for many years (Pope and Ware, 1945). Hanny *et al.* (1978) examined seed oil and seed protein across 39 genotypes and found a negative correlation. Turner *et al.* (1976a) examined four cultivars grown at 17 locations across the Cotton Belt. The correlation of percent protein with percent oil in the seed was -0.71 and highly significant. When their data were expressed as grams (g) protein vs. grams (g) oil, the correlation was 0.27 and non-significant. When oil and protein were expressed as g/g seed, the correlations were +0.79** and +0.72**, respectively. However, the regression coefficient for oil was 0.29 but only 0.19 for protein. This indicates that conditions which favor high seed weight will increase oil more than protein.

Turner *et al.* (1976b) found that oil percentage was positively correlated to micronaire. Developmentally, these two parameters occur simultaneously; therefore, the correlation is due to environment. The competitive interaction is not known. However, Quisenberry (personal communication) reanalyzed data of Gipson and Ray (1959b) and Gipson *et al.* (1969) and obtained estimates of the differences between seed and fiber mass accumulation at different night temperatures. The result is shown in Figure 11. At low night temperature, more seed mass accumulated than fiber mass. At high night temperature the reverse was true. In

this instance, it is clearly shown that environment can differentially change the competitive balance between two coincidental processes.

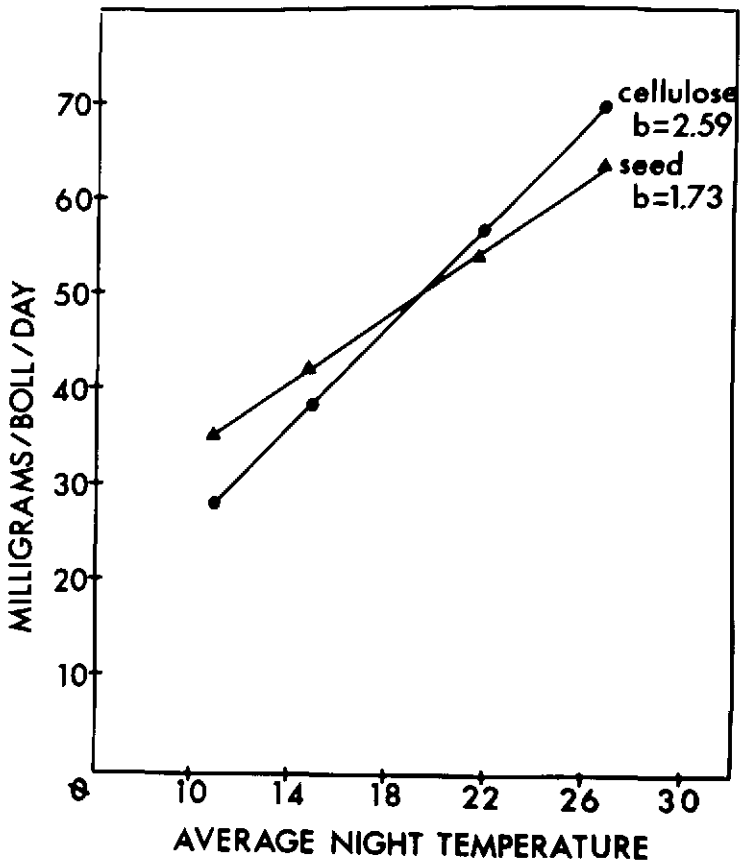

Figure 11. Influence of night temperature on rate of mass accumulation by fibers and by seeds. Graph courtesy of J. E. Quisenberry, data from Gipson *et al.* (1969) and Gipson and Ray (1969a).

SUMMARY

The development of the cotton boll from flower induction to boll opening involves a complex series of events that are interrelated either sequentially or competitively. Each stage in the developmental sequence must reach a minimum level of completion before the next stage begins. When two or more structures develop simultaneously, they compete for the available photosynthate based on

Table 2. Developmental events in relation to days before and after anthesis.

Age	Events
-40	Floral stimulus
-32	Carpel and anther number established
-23	Ovule number established
-22	Pollen mother cell meiosis; "Pin-head square"
-14	Megaspore mother cell meiosis
-7	Begin exponential expansion of corolla
-3	Begin fiber differentiation
0	Flower open; pollen shed, germinates; fiber initiation; K accumulation in fiber
+1	Fertilization of egg and polar nuclei; division of primary endosperm nucleus; zygote shrinks
+2	Liquid endosperm developing; fibers begin elongating; most dry mass goes to fibers
+3-4	Zygote divides
+5-6	Ovule integument division stops; fuzz fibers initiated; globular embryo dividing but not increasing in size; ovule enlargement stage proceeding rapidly; dry mass to internal parts increase
+12-13	Endosperm becomes cellular around embryo; palisade cells elongate; embryo differentiation begins
+14-16	Secondary deposition in fibers, outer integument and palisade begins; embryo elongating, accumulates Ca and Mg; fibers begin slow accumulation of Ca, outer integument begins rapid weight increase
+20	Endosperm completely cellular and at maximum weight; fiber elongation slows rapidly; P translocated from fiber; embryo begins accumulating protein; weight distribution about equal between fiber and embryo
+25	Fiber elongation complete; bur weight maximum; cotyledons complete; embryo maximum length; endosperm declining; oil accumulation starts
+30-32	Embryo enters period of grand weight gain; endosperm nearly depleted; maximum rate of cellulose deposition in fiber, oil and protein in embryo; rapid P and K accumulation in embryo; fibers begin losing K
+42	Dry weight of boll nearly maximum; some oil accumulation; fibers lose Mg; cellulose deposition stops
+45-50	Internal changes in seed hormones and enzymes; seed coat hardens; boll sutures dehisce in response to ethylene

the genetic control but influenced by their previous developmental history and the current environment.

In this paper I attempt to illustrate how the various parts of the seed are related during their development and how environmental factors influence the development of the cotton fruit. An abbreviated summary of the sequence of events from flower induction to boll opening in relation to days before and after anthesis is given in Table 2. Of course, the days are approximations and can change drastically with environment. Table 3 summarizes some of the critical periods during

Table 3. Developmental periods and events particularly sensitive to competition or environmental factors.

Period (Days)	Event	Factor
-40 to -35	Initiation of floral buds	Temp., N, HOH
-35 to -30	Carpel number, maybe	
	Anther number	CHO
-25 to -22	Ovules/ovary	CHO
	Anther number	R.H.
-19 to -15	Pollen viability	High temp.
		R.H.
-2 to 12	Fiber density (f/mm^2)	Temp, CHO
0	Anther dehiscence	Temp, R.H.
0 to 3	Rate of fiber initiation	Temp., K
	Pollen tube growth,	
	fertilization	Temp., R.H.
1 to 14	Boll abscission	CHO, HOH
3 to 25	Fiber length, seed volume	Temp., K
15 to 45	Fiber cellulose	Temp.
25 to 50	Protein and oil accumulation;	
	oil/protein ratio	Temp., HOH, N, K
49 to 50	Boll opening	Temp., R.H.

which an environmental stress can cause a measurable response in the development of the flower or seed.

While much is known about the overall development of the cotton boll, less is known of the specific effects of the environment on photosynthate partitioning. Also, information is limited on the relationship between sink (boll) strength and competition between elements (seeds, fibers, etc.) of the sink. For example, demand increases sink strength so, theoretically, increasing seeds/boll should increase sink strength of bolls. However, more seeds/boll is related to smaller seed so competition is increased. Many such opposing effects probably occur during development of the total crop.

The cotton fruit and seed is a unique biological system that can be used as a model for many of the questions common to all of biology. For example, the development of fibers could be used (or is used) as a model for differentiation, for K transport, for cell extension and for cellulose synthesis; the seed could be used (or is used) for seed development studies, carbohydrate utilization and partitioning and biochemical pathway analysis. Hopefully, the material presented in this discussion will stimulate interest in the potential of cotton, both as a model plant for basic research, and as a plant that still can be improved for economic gain.