A Survey of Cotton (*Gossypium hirsutum*) for Alleles $le_1$ and $le_2$

William Lloyd Rooney

University Undergraduate Fellow, 1986-1987

Texas A&M University

Department of Soil & Crop Sciences

APPROVED

Fellows Advisor: [signature]

Honors Advisor: [signature]

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# ABSTRACT

The tetraploid cultivated cotton Gossypium hirsutum (2n=4x=52) normally will not form viable $F_1$ hybrids with G. davidsonii or any tetraploid homozygous for $Le_2^{dav}$. Many or most G. hirsutum plants are homozygous for alleles $Le_1$ and $Le_2$; hybrids bearing an $Le_2^{dav}$ allele, and either or both $Le_1$ or $Le_2$, undergo a physiological reaction leading to seedling inviability. In contrast, $Le_2^{dav}$ is fully compatible with alleles $le_1$ and $le_2$; i.e. $le_1le_1le_2Le_2^{dav}$ plants are fully viable. Prior to this study, two facts suggested some G. hirsutum types may harbor $le_1$ and/or $le_2$ alleles. First, only a few lines had been tested. Second, homozygosity for $le_1$ or $le_2$ could be masked by homozygosity for $Le_2$ or $Le_1$, respectively, since the inviability reaction is epistatic over the compatability response. Successful deployment of new breeding procedures being developed for mass extraction of doubled haploids from G. hirsutum is contingent on the absence of the alleles $le_1$ and $le_2$. The purpose of the study was to determine the frequency of the alleles $le_1$ and $le_2$ in a representative sample of U.S. G. hirsutum germplasm, consisting of sixty-three varieties.

The varieties were tested (i) by crossing variety x $le_1le_1Le_2^{dav}Le_2^{dav}$ and observing progeny viability, and (ii) by crossing (variety x $le_1le_1le_2le_2$) x $le_1le_1Le_2^{dav}Le_2^{dav}$ and observing the frequencies of

progeny viability in seedling viability screenings. The first test identified any parental cultivars carrying the genotype $\underline{le}_1\underline{le}_1\underline{le}_2\underline{le}_2$, and the second test determined which varieties were homozygous dominant at one locus, but homozygous recessive at the other, e.g., $\underline{le}_1\underline{le}_1\underline{Le}_2\underline{Le}_2$ or $\underline{le}_1\underline{le}_1\underline{le}_2\underline{Le}_2$. The second test also served to identify any cultivars that were homozygous dominant, $\underline{Le}_1\underline{Le}_1\underline{Le}_2\underline{Le}_2$.

Of the sixty-three cultivars, the genotype at the $\underline{Le}_1$ and $\underline{Le}_2$ loci was determined for 42 cultivars. The genotype in all 42 cultivars was $\underline{Le}_1\underline{Le}_1\underline{Le}_2\underline{Le}_2$. For ten cultivars, enough testcross was not availible to reject one of the two segregation ratio hypotheses. However, with the incomplete data availible, a genotype of $\underline{Le}_1\underline{Le}_1\underline{Le}_2\underline{Le}_2$ is proposed. Therefore, the relative frequency of the alleles $\underline{le}_1$ and $\underline{le}_2$ appears to be zero or close to it. The rare frequency of the recessive alleles remains to be explained. Eight cultivars were eliminated from the study due to an outcross in one pollinator plants which caused segregation ratios to be erroneous. For the other three cultivars, the inability to produce $F_1$ seed caused the failure to successfully determine genotype.

After determining the genotypes, data gathered pertaining to rates of necrosis were compared to what should or could have been expected according to the hypothesis. It appears that the number of dominant alleles interacting with the $\underline{Le}_2^{dav}$ allele does affect the rate of death, but that this is not the sole factor affecting the rate of death. Results indicated that

environmental factors during seed development and during germination and seedling growth play a major role in the rate of expression of the lethality reaction. Additionally, data indicated the possibility that individual factors for each cultivar, possibly gene modifiers, affected each cultivar's rate of lethality expression. To positively identify the exact role of all variables involved, additional studies will be required. Nevertheless, results indicate that the HEHP doubled-haploid breeding system will be generally applicable to adapted U.S. G. hirsutum germplasm, in that $le_1$ and $le_2$ alleles seem to be very rare. However, in applying the HEHP system, some special considerations might be given to effects of environment on seed development, fruit retention, and thus, seed production and doubled haploid recovery

# INTRODUCTION

Throughout the years, plant breeders have worked intensively to develop "perfect" genotypes of economically important species. By utilizing a multitude of techniques and knowledge from numerous disciplines, but especially genetics, tremendous progress has been made in achieving the desired crop improvements. Plant breeding for genetic improvement often is a process that takes many generations of plant growth and calendar years before usable improved products are seen. The protractedness of plant breeding is well-exemplified by the development of inbred lines from $F_1$ plants. Obtaining an inbred, i.e., completely homozygous, line through traditional selfing normally requires at least five to six generations and as many as eight to ten, depending on criteria employed. Because inbreds serve a central role in the breeding process of many crops, any type of breeding that can decrease the number of generations required to obtain complete homozygousity would certainly be a welcome development to plant breeders.

New breeding techniques to facilitate mass extraction of doubled haploids from the cultivated tetraploid cotton, Gossypium hirsutum (2n=4X=52) have been proposed. (Stelly and Lee, submitted) Successful development of this procedure would offer several benefits, such as; (1) a single generation to obtain inbreds, (2) the process is simple and inexpensive, (3) it is compatible for large scale usage, (4) it

doubles the additive genetic variance and correspondingly increases epistatic interactions, and (5) eliminates dominance variance and corresponding epistatic interactions. The potential for this process to be useful in cotton breeding and research is immense.

Efficiency of the HEHP doubled-haploid procedure hinges on the use of the complementary lethality system that was discovered when the wild diploid cotton taxa Gossypium davidsonii (2n=2X=26) was crossed with the cultivated tetraploid G. hirsutum (Lee, 1981) Resulting seeds germinated normally but seedlings became necrotic very early in development, and died. This indicated a hybrid lethality system was operating as opposed to a much more common problem for interploidy crosses, i.e., endosperm failure. The lethal interaction observed in crosses between G. davidsonii and G. hirsutum results from complementary allelic interactions between G. hirsutum alleles $Le_1$ and/or $Le_2$ with the G. davidsonii allele $Le_2^{dav}$. The incompatability of most G. hirsutum cultivars with G. davidsonii had been is attributed to the prevalence of $Le_1$ or $Le_2$ alleles in the cultivated types (Stelly and Lee, submitted). Using hexaploid bridging, Lee transferred the complementary lethal gene $Le_2^{dav}$ from G. davidsonii to a G. hirsutum cross-compatible stock, designated 15-4 (Lee, 1981). The tester stock derived by Lee can be used for testing genotypes of G. hirsutum at these loci ($Le_1$, $Le_2$). If HEHP techniques are to be successfully deployed for research and

breeding, alleles $\underline{le}_1$ and $\underline{le}_2$ must be absent from the germplasm from which doubled haploids are to be extracted.

The complementary lethality system is based on the fact that the identified incompatibility alleles, $\underline{Le}_1$ and $\underline{Le}_2$ are each incompatible with the $\underline{Le}_2^{dav}$ allele (Lee, 1981). When either or both of the former alleles is combined with $\underline{Le}_2^{dav}$ in the same genotype, necrosis and death eventually result. Zygotes can form embryos and seeds may begin growing, but within two weeks, the seedlings will die. The time of death seems to be dosage-dependent, therefore, genotypes of $\underline{Le}_1\underline{le}_1\underline{Le}_2\underline{Le}_2^{dav}$ will become necrotic first, whereas genotypes $\underline{Le}_1\underline{le}_1\underline{le}_2\underline{Le}_2^{dav}$ and $\underline{le}_1\underline{le}_1\underline{Le}_2\underline{Le}_2^{dav}$ will react later. It is also possible that the differences between the latter two gentoypes could cause a change in the rate of lethality expression (Lee, 1981). According to this hypothesis, the days between germination and death should reflect the genotype of the parental cultivar.

It has been presumed that most cultivars of the tetraploid cotton G. hirsutum carry the alleles $\underline{Le}_1$ and $\underline{Le}_2$, but there have been no studies to determine the actual allelic frequencies at the loci $\underline{Le}_1$ and $\underline{Le}_2$. The purpose of this research was to determine the frequency of the alleles $\underline{le}_1$ and $\underline{le}_2$ in a representative sample of adapted U.S. germplasm G. hirsutum, based on the expectation that the results obtained will provide information critical for the successful testing and application of the HEHP doubled-haploid breeding procedures. Furthermore, results were

expected to corroborate and perhaps add to previous biological interpretations of the lethality system.

# OBJECTIVES

The objectives of this study are:

1. To determine the frequency of the alleles $\underline{le}_1$ and $\underline{le}_2$ in a representative sample of Gossypium hirsutum.
2. To determine genotype of each sample at the loci $\underline{Le}_1$ and $\underline{Le}_2$.
3. To determine if time of death is dosage dependent by recording days to death from emergence and germination and then comparing this data to the genotypic designations determined from objective 2.

# LITERATURE REVIEW

## Background Information

Genetic hybrid lethality is the failure of seed or embryos -- usually derived from an interspecific or an intergeneric cross -- to complete a life cycle and produce progeny. While genetic hybrid inviability reactions have been known to exist for over sixty years, the mechanisms and reasoning for the incompatible reactions are still not well understood. For example in 1921, when Sax first described a lethal reaction occurring in the $F_1$ progeny of a cross between two common wheat varieties of the time. In this earliest documentation of hybrid lethality, he suggested a genetic basis for the death of the plants. Since then, a number of other genetic hybrid inviability systems have been reported and in some cases, the genetic basis of the lethality system has been determined.

## Characteristics of Genetic Hybrid Lethality Systems

Hybrid inviabilty can be attributed to a wide range of causes, such as pollen, gametic growth failure. The inablity of two varieties to succesfully cross or once fertilization has occurred, endosperm failure can be result in the failure to produce hybrid seed. Ploidy differences between parent lines is a common cause of unsuccessful

hybrid production. There are general characteristics of a hybrid inviability system due to genetic factors that allow genetic hybrid inviabilty to be classified as a separate group. Even though each lethality reaction has its own idiosynchrasies, there are some general characteristics that apply to all lethality systems. Usually, the hybrid seed will germinate, but the plant subsequently becomes necrotic and dies at some specific stage of growth. The genetic mechanisms of lethality and the time of necrosis vary from crop to crop, but the very precise for the specific cross. Except in rare cases, the lethality reaction cannot be altered be changing environmental conditions. In addition to lethality, many forms of hybrid weakening, chlorosis and semi-lethals have been identified. Most hybrid incompatibilty reactions also appear to involve complementary alleles at two loci in the genotype.

Hybrid lethality has been identified and characterized in approximately twelve species of plants. While this may not be a large number, it must be remembered that this phenomenon is usually discovered only by accident in breeding programs when a desired cross fails to produce seed or viable progeny. Naturally, almost all lethality systems have involved agricultural crops. As the number of plant species used for genetic improvement of crops and plants continues to increase, so it is likely that hybrid lethality systems will be found in these species as well.

Wheat (Triticum spp.)

Hybrid necrosis was first described by Sax (1921) based on results from an intraspecific cross of common wheat varieties of Triticum aestivum. $F_1$ plants died before flowering although initial developmented had appeared the first case of interspecific hybrid necrosis in wheat when $F_1$'s from a cross of two common wheat varieties of the time died before flowering although initial development had appeared normal. The first report of hybrid lethality resulting from an intergeneric cross was by Sears (1944). In a cross of Triticum monococcum x Aegilops umbellulata he found early and late hybrid necroses. Genetic tests indicated single gene control of the lethal condition with variation attributed to gene modifiers. Heyne et al. (1943) reported interspecific lethality between the varieties 'Marquillo' (T. vulgare) and several other T. vulgare varieties. Soon after the second leaf appeared the primary leaf lost chlorophyll and died. The disappearance of chlorophyll gradually affected all of the leaves and stems and final lethality was expressed by the four to five leaf stage. A series of backcrosses and testcrosses, demonstrated that the reaction was controlled by a pair of complementary factors. Studies completed by Caldwell and Compton (1943) yielded very similar results.

In addition to lethality reactions in wheat, numerous instances of hybrid weakening, chlorosis, and semi-lethality in wheat have been

reported. Hermsen (1957) stated that while $F_1$ plants of a given cross are uniformly semi-lethal, the degree of semi-lethality is dependent on the nature of the cross. His results showed two dominant complementary genes controlled semi-lethality and modifiers accounted for the varying appearances of the $F_1$'s. In a separate case Kochumadhavan et al. (1984) crossed forty-four varieties of T. dicoccum by two testers of T. aestivum to determine their necrosis and chlorosis genes. These genes had been proven to exist in a series of studies done by Tsunewaki (1966, 1971). They determined the genotypes of these varieties at the critical gene loci and dicovered the different gene combinations that lead to lethality, semi-lethality and chlorosis.

Barley (Hordeum vulgare)

Wiebe (1934) reported lethal $F_1$ hybrids in barley where the lethal condition was determined by complementary factors. Each parent was responsible for contributing a single dominant factor. The lethality reaction in barley was very similar in symptoms as many of the hybrid lethality reactions in wheat. The genetic basis for the reaction was located at one locus.

Rice (Oryza spp.)

Oka, (1957) while investigating the sterility relations between a number of rice variety Oryza sativa hybrids, found hybrid inviability to be quite prevalent. Further research revealed two types of hybrid lethality. One type involved complementary dominant lethal factors where each parent contributed one lethal allele to the $F_1$ hybrid. This type of lethality occurred only in rare cases. The second type was due to complementary recessive factors where at least two of the four alleles involved had to be dominant in order for the plant to be viable. Oka proposed that this type of lethality was the reason many rice hybrids breakdown and become necrotic. Intergeneric hybrid weakness was also found to be prevalent in $F_1$ progeny from a cross of Oryza breviliguata and O. glaberrima. Chu and Oka (1972) determined that a set of two complementary dominant weakness genes, $W_1$ and $W_2$ controlled the observed $F_1$ weakness. Each parent contributed a dominant allele and modifier genes appearred to affect the intensity of the weakness expression.

Cowpeas (Vigna sinesis)

Saunders (1952) discovered an intraspecific lethal hybrid reaction between two varieties of V. sinesis, 'Portuguese White' and 'Light Red'. $F_1$ seed germinated normally, but began to wilt and slow in growth rate at two weeks post emergence. By the sixth week all seedlings had died. A series of backcross and testcross genetic

tests, demonstrated that complementary lethal genes were responsible for the lethal reaction. The data showed that 'Portuguese White' and 'Light Red' carry the genes $L_1$ and $L_2$ respectively, and when these were combined in a genotype the seedlings become necrotic and moribund. A second lethal condition was disovered by Saunders (1952) in which the lethal reaction was controlled by two different complementary genes, designated $L_3$ and $L_4$. The former allele was found in the variety 'Portuguese White' while the latter was located in 'N.I. 31'. The second lethal reaction resulted in the lethal condition expressing itself much in a much earlier stage of growth.

Tomato (Lycopersicon esculentum)

Sawant (1956) described a semi-lethal condition that resulted from an interspecifie cross in the tomato genus. $F_1$ hybrids from the cross of L. esculentum x L. hirsutum showed a peculiar behavior of withering at the apex approximately ten weeks after sowing. The branches would turn pale and wilt as if suffering from drought and withering progressed towards the base of the plant. But before any given branch died, new shoots were issued forth from the base of the branch. For this reason, the hybrids never died, and never produced any seed of any kind. Sawant hypothesized that the semi-lethal condition was controlled by complementary gene action, although no studies to prove this were reported.

## Alfalfa (*Medicago sativa*)

Stringham and Elling (1966) studied a complementary lethality system in alfalfa discovered from crosses among several clones and their inbred progenies. They concluded that the lethality was determined by complementary dominant factors inherited tetrasomically. The results also indicated a general relationship between gene dosage and the rate of premature death.

## Tobacco (*Nicotiana* spp.)

In the genus *Nicotiana*, numerous interspecific and intraspecific crosses yield lethal combinations (East 1935; Kostoff, 1936). However, the genetics of these lethality reactions were not studied and no followup studies were found in the literature.

## Yellow Monkey Flower (*Mimulus guttatus*)

Vickery (1956) described a series of intraspecific lethality reactions occurring in the species *Mimulus guttatus*. Expression of lethality was recently found to be controlled by complementary factors in which each variety supplied a single factor (Christie and MacNair, 1984). Both inbred varieties were found to be polymorphic for the factors.

Hybrid Lethality in Cotton (Gossypium spp.)

Gerstel (1952) undertook the first intensive study to determine the causes of hybrid lethality that occurred in hybrids of Asiatic G. arboreum X Upland strains of G. hirsutum. Through a series of intensive crosses and backcrosses, data indicated that a genetic lethal combination was responsible for the lethality. It was discovered that G. arboreum carried a factor $Rl_a$. Further studies revealed that the Upland strains of G. hirsutum carried the $Rl_b$ factor. Any combination of these factors in a genome resulted in lethality being expressed. However, it was not determined whether $Rl_a$ and $Rl_b$ were alleles or complementary genes.

By the early 1930's, it was known that crosses of Gossypium davidsonii and G. klotzchianum with G. hirsutum, G. barbadense, G. stocksii, and G. thurberi all yielded hybrids that died early in the cotyledonary stage. Silow (1941) attributed the invability to general genotypic disharmony instead of to specific lethal genes. Even earlier, however, Skovsted (1935) proposed that the lethality reaction resulted from a complementary interaction of alleles harbored by both contributing parents. This inviability was also attributed to zygote endosperm disharmony Stephens (1945) and to an interaction involving the entire $D_3$ genome (Brown, 1951). While all four hypotheses had valid points, research in recent years has

discovered more inviable combinations and most of these lethal reactions are caused by lethal interactions of specific genes, in fact, specific alleles of these genes.

What were previously recognized as two varieties of G. klotzschianum, var. klotzschianum or var. davidsonii are now designated as species G. klotzschianum (2n=2x=26) and G. davidsonii (2n=2x=26) respectively. When crossed with several hundred accessions of new world cultivated tetraploids G. hirsutum and G. barbadense, embryo lethality reactions resulted. A few accessions of G. barbadense with G. klotzschianum combined to form seedling lethals and several more crosses yielded viable hybrids. The lethality reaction was caused by a complementary gene interaction (Phillips, 1963), in which the cultivated cottons contribute the factors $Le_1$ and $Le_2$ and G. klotzschianum contributes the factor $Le_2^{dav}$ (Figure 1 & 2).

Phillips and Reid (1975) described the morphological characteristics of the reaction between G. klotzschianum and the new world cultivated tetraploids. During embryo maturation in the severe reactions, tissue necrosis and death occurs causing the embryo to be aborted. When seed from a cross of these two types was produced and germinated, the seedling underwent tissue necrosis similar to what had occurred at the embryo stage for other crosses.

Phillips (1977) then discovered that this hybrid lethality system was temperature conditional. When normally lethal genotypes are grown in growth chambers at 40 C, hybrid ontogeny is essentially normal.

Figure 1 - Availible alleles at the $\underline{Le}_1$ and $\underline{Le}_2$ loci as described by Lee (1981).

Figure 2 - Viable and inviable hybrid combinations for the complementary lethality system. (Lee, 1981)

| LOCUS | AVAILIBLE ALLELES |
|---|---|
| $Le_1$<br>$Le_2$ | $Le_1$ $le_1$<br>$Le_2$ $le_2$ $Le_2^{dav}$ |

| VIABLE | INVIABLE |
|---|---|
| ----$\underline{le_2 le_2}$<br>----$\underline{le_2 Le_2}$<br>----$\underline{Le_2 Le_2}$<br>$\underline{le_1 le_1 le_2 Le_2}^{dav}$<br>$\underline{le_1 le_1 Le_2}^{dav}\underline{Le_2}^{dav}$ | $\underline{Le_1}$----$\underline{Le_2}^{dav}$<br>----$\underline{Le_2 Le_2}^{dav}$ |

When the lethal genotype is grown in a 32-39 C range, the lethal reaction was delayed for up to four weeks. Finally, if the lethal genotype is grown in the 26-32 C range, which is the optimum temperature range for cotton, the lethality reaction proceeds in its normal time frame. If the lethal genome is grown at 40 C and then moved to the normal temperatue range, the lethal reaction initiates immeadiately after temperature adjustment. Two other hybrid lethal combinations, G. hirsutum X G. arboreum (Gerstel, 1954) and G. hirsutum X G. gossypioides (Phillips and Merritt, 1972), were tested and found to be temperature insensitive.

Utilizing the same hybrid lethality system described by Phillips (1963) and Skovsted (1935), Lee (1981) isolated a rare line of G. barbadense (with a genotype of $le_1 le_1 le_2 le_2$) that produced viable $F_1$ hybrids when crossed with G. klotzschianum var. davidsonii. Then, using hexaploid bridging, Lee transferred $Le_2^{dav}$ from G. davidsonii to the cross compatible stock of G. barbadense. The resulting cotton has provided a valuable tester stock in transfers of cross-compatibility factors. Presently, the $Le_2^{dav}$ allele and other factors have been incorporated into several G. barbadense and G. hirsutum. Lee's preliminary data on crosses of $Le_2^{dav}$ lines with common cultivars have also seemed to indicate a dosage effect in terms of the stage of growth at which the lethal reaction will occur. Typically, in this reaction, seed carrying the lethal genotype will germinate and begin growth, but the seedling will become necrotic somewhere between the first and

fourth week of growth. Lee has proposed a dosage effect, specifically that if the cultivar contributes two dominant factors ($\underline{Le}_1$ and $\underline{Le}_2$) to the $F_1$ seedling instead of contributing just one ($\underline{Le}_1$ or $\underline{Le}_2$), the reaction with the $\underline{Le}_2^{dav}$ factor will be stronger and thus, the seedling will die sooner.

# MATERIALS AND METHODS

## Background Information

Two experiments were used to determine the relative frequencies of $\underline{le}_1$ and $\underline{le}_2$ (Figure 3A & 3B). A plan to determine whether the number of interacting dominant alleles ($\underline{Le}_1$ and $\underline{Le}_2$) interacting with $\underline{Le}_2^{dav}$ affected the time of seedling lethality was incorporated into each experiment. Each experiment consisted of (i) a series of crosses followed by (ii) a seedling viability screening to test for the presence of the lethality reaction. A set of 63 U.S. cultivars was used to represent the overall elite U.S. adapted G. hirsutum germplasm. Many of these 63 cultivars were, or still are, used for lint and cotton seed production by producers across the country, so both obsolete and current cultivars are included. It is assumed that these lines are all highly inbred, that all gene loci are homozygous, and the lines are homozygous.

The set of 63 varieties was obtained by Dr. David Stelly from the varietal component of the Cotton Germplasm Collection and given to me in the spring of 1986. Seven seed of each cultivar were planted in pre-soaked Jiffy-7 peat pots in April 1986. Mites and other insects were controlled in the greehouse by spraying with Omite, Orthene, Cygon, and Dipel. Three weeks after emergence, the five healthiest seedlings of each cultivar were mechanically transplanted into the

Figure 3 - Diagrammatic summary of basic experimental schemes.

A. Experiment I
B. Experiment II

A. EXPERIMENT 1

PURPOSE: TO IDENTIFY CULTIVARS HOMOZYGOUS FOR $le_1$ AND $le_2$

CULTIVAR x $le_1\ le_1\ Le_2^{dav}\ Le_2^{dav}$

↓

$F_1$ - SCREEN FOR SEEDLING VIABILITY

ONLY CULTIVARS WITH THE GENOTYPE $le_1\ le_1\ le_2\ le_2$
WILL YIELD VIABLE PROGENY.

B. EXPERIMENT 2

IDENTIFY CULTIVARS HOMOZYGOUS FOR EITHER $le_1$ OR $le_2$

CULTIVAR x $le_1\ le_1\ le_2\ le_2$

↓

$F_1$ x $le_1\ le_1\ Le_2^{dav}\ Le_2^{dav}$

↓

$F_2$ - SCREEN FOR VIABILITY

Calculate Segregation Ratio
Inviable : Viable To
Determine Genotype

cotton cytogenetic research field in College Station, Texas. Simultaneously, forty G. hirsutum seedlings with the genotype $\underline{le}_1\underline{le}_1\underline{le}_2\underline{le}_2$ and forty G. hirsutum tester stock seedlings of the genotype $\underline{le}_1\underline{le}_1\underline{Le}_2^{dav}\underline{Le}_2^{dav}$, descedent from seed kindly provided by Dr. Joshua Lee of USDA-ARS at North Carolina State University, were transplanted in the same field. The former group of seedlings was designated the $\underline{le}_1\underline{le}_2$ donor and the latter was designated as the Tester.

## Experiment I - $F_1$ Viability Screening

Experiment I was designed and especially well-suited to determine if any of the cultivars were $\underline{le}_1\underline{le}_1\underline{le}_2\underline{le}_2$, i.e., homozygous for both $\underline{le}_1$ and $\underline{le}_2$. During the summer of 1986, each of the 63 cultivars were crossed as females to the tester, $\underline{le}_1\underline{le}_1\underline{Le}_2^{dav}\underline{Le}_2^{dav}$. Enough crosses were made on a plant-by-plant basis to insure that adequate seed would be availible to obtain valid results in the seedling viability screening. In August 1986 the cottonseed was harvested, ginned, packaged and categorized according to each cultivar. Once the number of seed of each cultivar was counted the seed was placed in storage.

In February 1987, up to 30 $F_1$ seed from each of the 63 varietal testcrosses were planted in Jiffy-7 peat pots. The identity of seeds from different bolls (pollinations) was maintained throughout the

experiment, to facilitate detection of possible errors at any stage of experimentation (emasculation, pollination, tag-writing, harvesting, ginning, counting, packaging, planting or labelling). Throughout the trial seedlings were watered every evening and night. Twice a week, during watering, the seedlings were treated with the fungicide Captan, for control of cotton root rot caused by Pythium and Phytopthora. The total experiment was designed to last 21 days or until all the seedlings had died. If the lethality reaction had not occurred by the twenty first day after planting, the seedling was considered viable. This was a valid assumption based on studies published by Lee (1981).

Procedures for recording data, while relatively simple, were time-consuming. The two goals to be obtained were; (1) to determine the number of living and dead after the trial and (2) to determine the number of days to seedling necrosis after emergence. Emergence dates for seedlings were marked with colored toothpicks that were inserted into the peat pot for the day of emergence. Each day was signified by a different colored toothpick. Emergence data were taken daily for seven days after the first seedlings had emerged. Ten days after planting, all non-emerged seeds were exhumed and examined for possible causes of non-emergence. Daily ratings for the lethality reaction were made for each seedling according to both the maternal line and emergence date. Because the seedlings reacted so quickly, the total length of the experiment was only ten days.

Experiment II - Testcross Viability Screening

The second experiment was specially designed to determine which varieties, if any, were homozygous dominant at one locus, but homozygous recessive at the other, e.g., $\underline{\underline{Le}}_1\underline{\underline{Le}}_1\underline{\underline{le}}_2\underline{\underline{le}}_2$ or $\underline{\underline{le}}_1\underline{\underline{le}}_1\underline{\underline{Le}}_2\underline{\underline{Le}}_2$. This experiment also served to detect if the cultivar was homozygous dominant at both loci, ($\underline{\underline{Le}}_1\underline{\underline{Le}}_1\underline{\underline{Le}}_2\underline{\underline{Le}}_2$), or homozygous recessive at both loci, ($\underline{\underline{le}}_1\underline{\underline{le}}_1\underline{\underline{le}}_2\underline{\underline{le}}_2$), there also corraborating or rejecting results from Experiment I. In the summer of 1986, all 63 parental cultivars were crossed as female with a $\underline{\underline{le}}_1\underline{\underline{le}}_2$ homozygote. The plants used the same plants in Experiment I. Seed from these crosses was harvested, ginned and stored for approximately one month. In September 1986, five $F_1$ seeds from each cultivar were planted and grown in the greenhouse. During the winter of 1986-1987, these $F_1$ hybrid plants were pollinated with the tester, ($\underline{\underline{le}}_1\underline{\underline{le}}_1\underline{\underline{Le}}_2^{dav}\underline{\underline{Le}}_2^{dav}$). Seed from this second series of testcrosses were also used for the seedling viability tests.

Based on observations made in Experiment I and a preliminary screening with a small sub-sample of seed for Experiment II, some modifications were made in the screening techniques used in Experiment II. Testcross seed were chemically delinted with concentrated sulfuric acid and then physically scarified. Once the seed coat had softened sufficiently or the radicle had begun to emerge, the seed coat was peeled off. Germination was scored daily and each seed was rated at germination (defined as when the seed coat was removed) for

symptoms of the lethal reaction. This early evaluation provided a means to measure germination and an opportunity to determine the prevalence of innate lethal symptoms. Removal of the seed coat eliminated most causes for failure to emerge except those due to the lethal reaction. Subjective definitions and ratings for emergence, death, and germination were the same as in Experiment I. It had been determined from Experiment I that this information would valuable in providing answers to the objectives of the study.

Seed then were planted in Jiffy-7 peat pots, the dates of germination being marked by by a color-coded label. The seedling trial subsequently followed the same format as in Experiment I. Final segregation ratios were obtained by counting the number of viable plants showing no signs of the reaction after 21 days.

## Photography

All black-and-white photographs were exposed on Kodak Panatomic-X film. All magnified close up photographs were taken using a Zeiss Stereo Microscope with a Zeiss MC-63 camera system. Non-magnified close-up pictures were taken using a Nikon FE2 camera with 55mm Micro Nikkor lens with a Tiffen +4 close up filter. Prints were developed on half-weight Kodak polycontrast paper. All color pictures were exposed using Kodak Ektachrome slide film taken with the same photographic equipment.

## Statistics

Databases were entered on DBASE II and transferred via ASCII files to SYSTAT for graphic and uni-variate statistical analyses. Chi-square analyses were calculated without Yates correction factor.

# RESULTS

## Experiment One

Three types of phenotypic responses were observed: seeds either (1) emerged but then died, (2) failed to emerge, but had germinated and died prior to emergence, or (3) did not germinate, thus rotting. No emerging viable progeny were observed. The results indicated that none of the cultivars tested had the $\underline{le}_1\underline{le}_1\underline{le}_2\underline{le}_2$ genotype being sought. The cumulative results are presented in figure 4A. Of the 1797 seed that were planted, only 1015 (56%) of these emerged, all of which became necrotic within a period of seven days after emergence. The average interval from emergence to death was 3.72 days.

To establish a method of measuring the days to necrosis after emergence, it was necessary to establish a consistent definition of emergence and full necrosis. Subjective ratings were established for each condition. A seedling was considered emerged once the hypocotyl had cracked the surface and the cotyledons were beginning to emerge. The process os seedling death was divided into five stages ( Appendix, figures 10-14) based on the following parameters;

Stage One: Symptomless, normal seedling. (Figure 10)

Stage Two: Tiny necrotic areas formed on the cotyledons (initial observable reaction), (Figure 11).

Stage Three: Progressive necrosis and wilting, (Figure 12).

Stage Four: Cotyledons totally necrotic, stem becoming necrotic, (Figure 13).

Stage Five: Cotyledons and stem totally necrotic and moribund (Figure 14).

At stage five the seedling was recorded as dead and number of days from emergence to death were determined from this date.

The percentage of emergence was very low (56.4% as compared to a normal 90% to 100%), so non-emergent seeds were exhumed and observed to determine the causes of non-emergence. Nearly half (327 seeds) of the non-emergent seeds were found to have germinated and died before emergence. Closer inspection under a dissecting microscope revealed symptoms characteristic of the lethal reaction. Darkened necrotic tissue was scattered throughout the young cotyledons and contrasted sharply with the light yellow healthy tissue. Interestingly, the radicle and any other developing roots were unaffected by the reaction.

Approximately one-half of the non-emergent seed had rotted or simply failed to initiate any form of germination. It must be noted that in all likelihood, a large number of these seed had simply become necrotic immediately following the initiation of germination or perhaps during embryo growth and differentiation. This interpretation is compatible with data gathered in Experiment II.

Cumulative results for the 63 cultivars on the rate of death, relative to the day of emergence, are depicted in the frequency

histogram given in Figure 5. Although a significant number of germinated seeds failed to emerge, due to necrosis, a more-or-less normal distribution was observed among emergent seedlings in their rates of necroses and death between one and seven days post-emergence. No viable progeny were recovered from crosses with any of the cultivars, but the average number of post-emergent days-to-death by line were quite varied, ranging from 0.0 days (all seed was non-emergent) to 5.8 days. The cumulative average for sixty three lines, excluding non-emergent seed, was 3.72 ± 1.32 days. If non-emergent seed was included the cumulative average dropped to 2.81 days.

Testcross seed from individual cultivars varied in rate of seedling necrosis, percent emergence and germination, but overall the results were consistent (Tables 1 & 2). Of the 1342 germinating seeds, all carried genotypes that caused a lethal reaction within the first week after germination. That all testcross seedlings died indicated none of the 63 parental cultivars had the genotype $\underline{le}_1\underline{le}_1\underline{le}_2\underline{le}_2$. The quickness with which the lethal reaction onset and subsequently killed the seedlings was suprising. Some reactions occurred even before the seedling emerged. These results indicate that the number of dominant alleles interacting with the $\underline{Le}_2^{dav}$ allele could possibly affect on the time of death.

Experiment Two

Two basic types of progenies were detected among testcross progenies of $F_1$ plants - those that segregated viable and inviable progenies (table 3), and those that included only inviable progenies (table 4). Given the orgin of the $F_1$ plants, all were expected to produce at least some $\underline{le}_1\underline{le}_2$ gametes and thus some viable testcross progeny. All non-segregating families originated from $F_1$ plants obtained involving D35.17-85, putatively a $\underline{le}_1\underline{le}_1\underline{le}_2\underline{le}_2$ plant. In all, no viable progeny were observed in testcross families from seven of fourteen $F_1$ plants produced by testcrosses of respective cultivars with D35.17-85. The results clearly indicate D35.17-85 was heterozygous for $\underline{Le}_1$ and/or $\underline{Le}_2$. Insufficient numbers of testcross seed were obtained from four other $F_1$ plants, so analysis of Experiment II were not completed for the respective cultivars. Due to these complications, Experiment II analyses were completed on only 52 of the original 63 cultivars.

Segregation ratios among 52 of the 63 cultivars were used to determine genotypes of individual cultivars at the $Le_1$ and $Le_2$ loci. Testcross progenies descendent from each cultivar were expected to segregate in either a 3:1 or a 1:1 inviable : viable seedling ratio. Chi-square tests indicated that 42 of the 52 cultivars had the genotype $\underline{Le}_1\underline{Le}_1\underline{Le}_2\underline{Le}_2$ (table 3). Chi-square tests for the other ten testcross families were inconclusive, but not discordant

with the possibility that they too share the same genotype. Chi-square tests for pooled data and for sample homogeniety were made for all 63 cultivars, including the eight lines that did not segregate. Tests of pooled data indicated lack of fit between observed ratios (1375:338) and those expected $P(3:1) < .01$ ($X^2_{1d.f.} = 30.54$) and $P(1:1) < .01$ ($X^2_{1d.f.} = 627.00$). However, test of the 52 cultivars that did segregate revealed overall segregation (1122:338) fit results expected for digenic segregation $P(3:1) > .20$ ($X^2_{1d.f.} = 2.67$), but not that for monogenic segregatio $P(1:1) < .01$ ($X^2_{1d.f.} = 421.00$). Moreover, whereas homogeniety lacking among the group of all 63 cultivars ($P < .05$; $X^2 = 3.84$), there was no detectable heterogeniety among the group of 52 cultivars ($P > .995$; $X^2_{51\ d.f.} = 27.085$) (table 3).

The cumulative results of Experiment II are presented in Figure 4B. When the seed coat had softened or the radicle had begun to emerge (signs of seed germination), the seed was examined for signs of the lethal reaction (Figure 15). Germination was approximately the same as for normal seed (95 %). The frequency of emergence (89%), was higher than in Experiment I, and more than sixty percent of all non-emerging seedlings exhibited of the lethal reaction at germination, indicating the lethal reaction was the causal agent for lack of emergence in those seedlings. Seed affected at germination was characteristically smaller and somewhat shriveled and the cotyledons had areas of tissue decay. This inference was substantiated by the fact that of the 412 seeds that showed lethal

signs at germination, 310 (75%) never emerged and that all of these seedlings were killed by the reaction. In contrast, of 1057 seed with a normal appearance at germination, only 10 (1%) died due to a lethal reaction prior to emergence. Therefore, it can be assumed that the reaction was definitely occurring at a very early stage and symptoms at germination foreshadowed eventual necrosis of the seedling.

Results in post-emergence and post-germination days-to-death for individual cultivars were somewhat variable with emergence averages from 5.00 to 10.17 days (figure 6) and germination averages from 9.81 to 14.64 days (figure 7). Taken relative to emergence there was an average of 4.5 extra days-to-death from germination. However, the two measures of days-to-death, relative to emergence versus germination were highly correlated ($r = .965$, 33 data) (Figure 8 & 9).

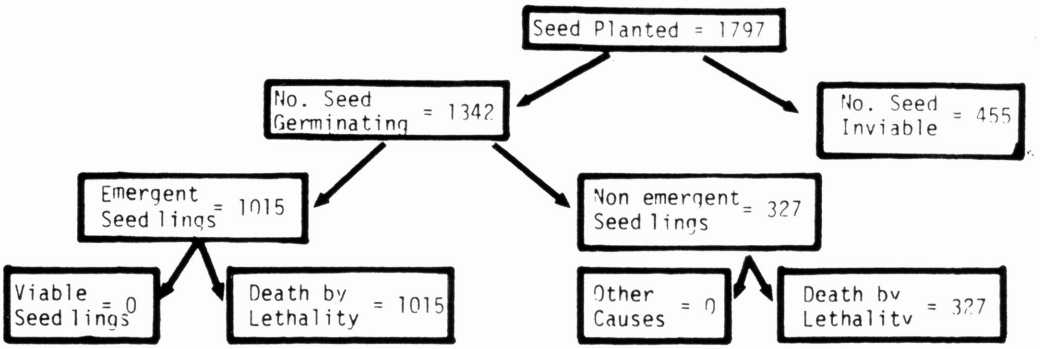
Figure 4 - Diagrammatic breakdown of results

A. Experiment I
B. Experiment II

A.

TRIAL 1 - RESULTS

Cumulative Totals - 63 Cultivars




B.

TRIAL 2 - RESULTS

Cumulative Totals - 52 Cultivars

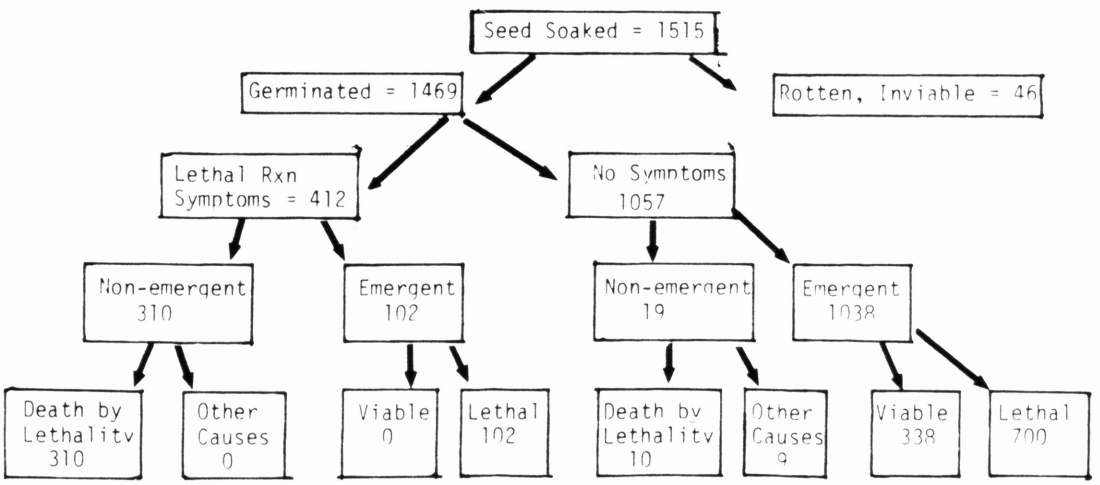


Figure 5 - Experiment I - Post-emergent Days-to-Death

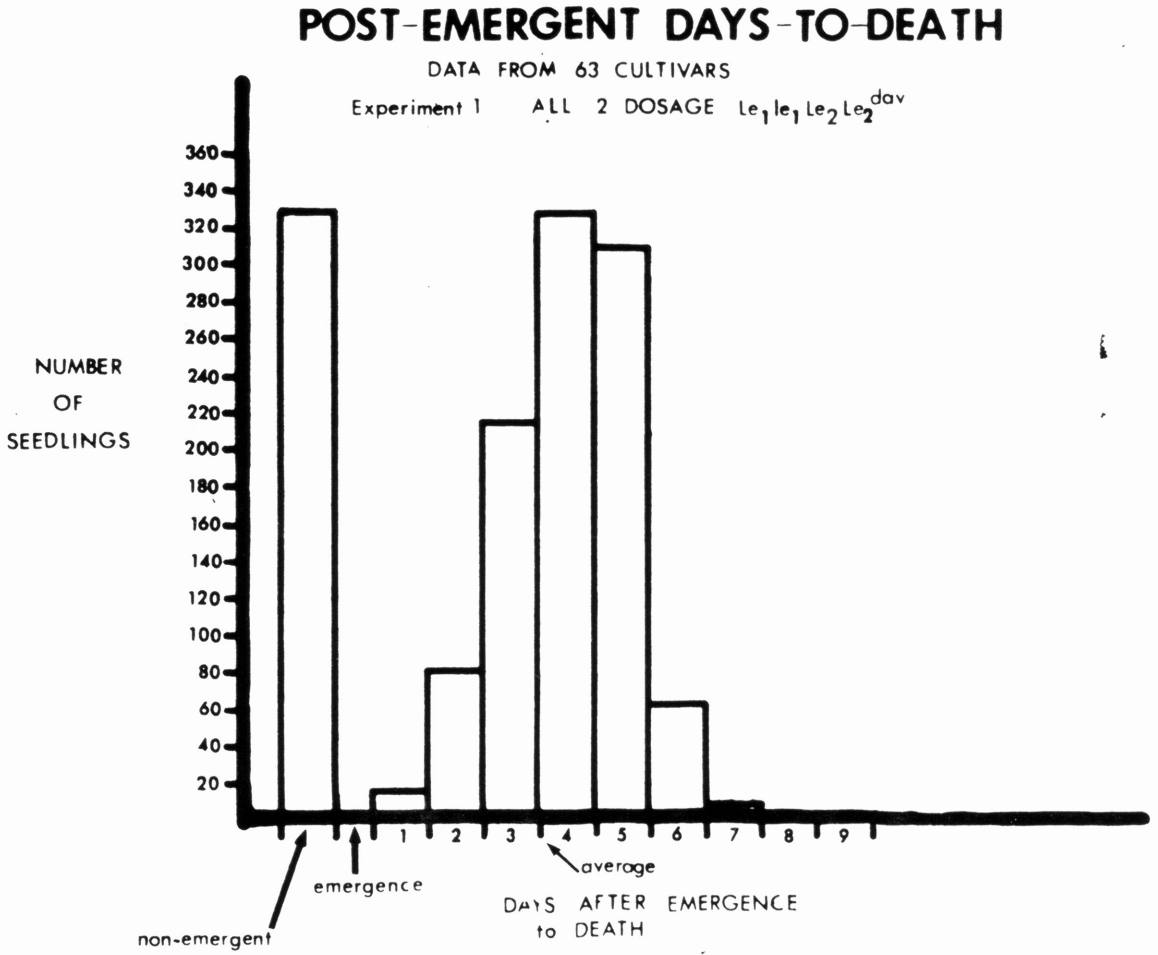
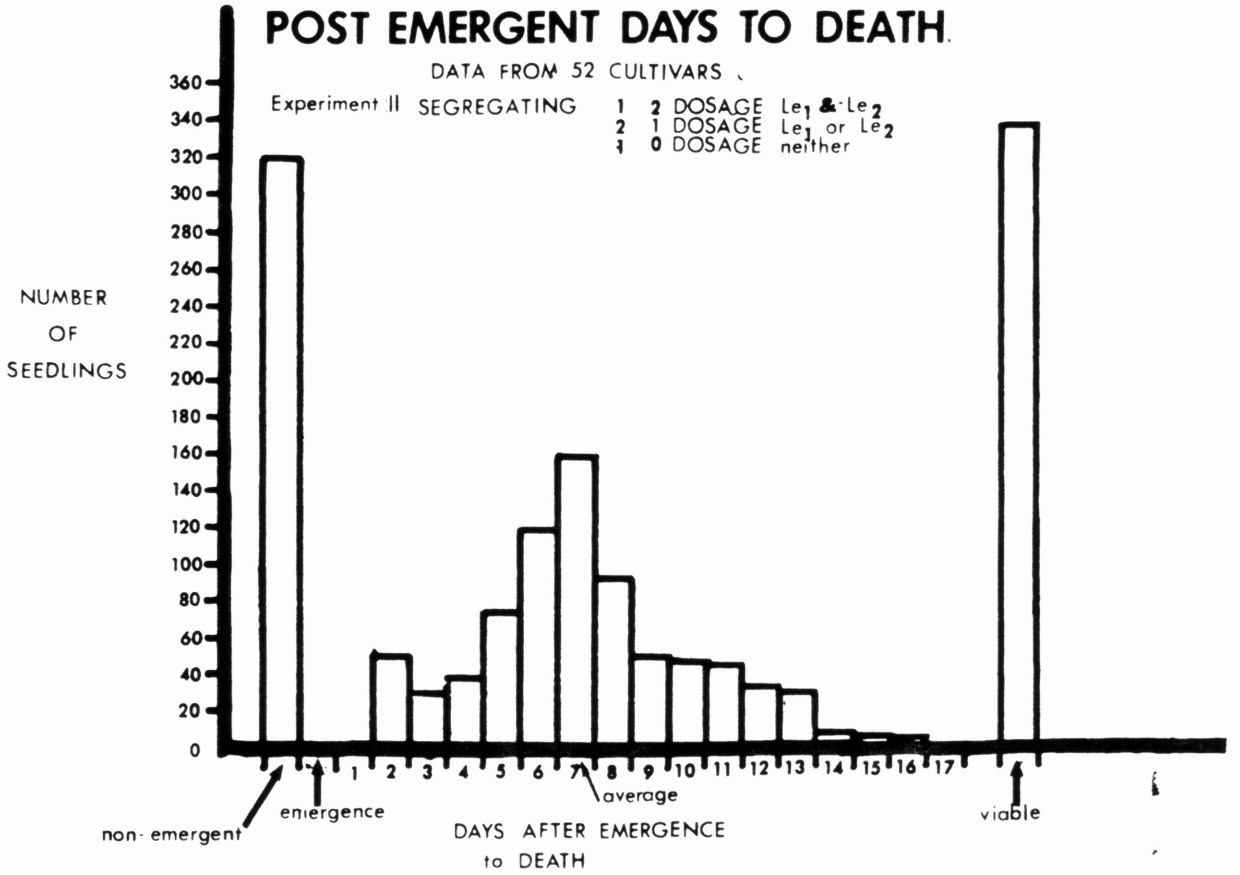

POST-EMERGENT DAYS-TO-DEATH
DATA FROM 63 CULTIVARS
Experiment 1 ALL 2 DOSAGE $le_1 le_1 Le_2 Le_2^{dav}$
NUMBER OF SEEDLINGS
360
340
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
1
2
3
4
5
6
7
8
9
average
emergence
non-emergent
DAYS AFTER EMERGENCE to DEATH

Figure 6 - Experiment II - Post-emergence Days-to-Death

Figure 7 - Experiment II - Post-germination Days-to-Death


POST EMERGENT DAYS TO DEATH
DATA FROM 52 CULTIVARS
Experiment II SEGREGATING
2 DOSAGE Le1 & Le2
1 DOSAGE Le1 or Le2
0 DOSAGE neither
NUMBER OF SEEDLINGS
0 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360
-1 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
non-emergent
emergence
average
viable
DAYS AFTER EMERGENCE to DEATH

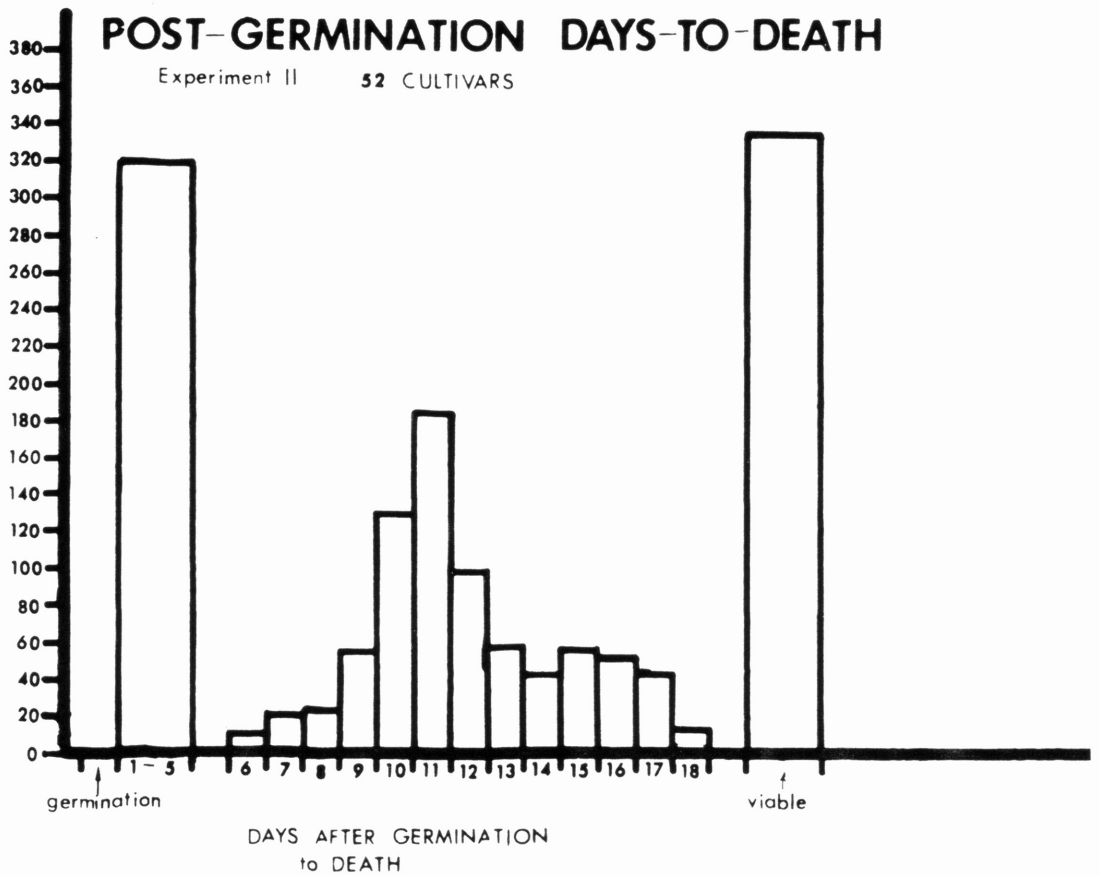
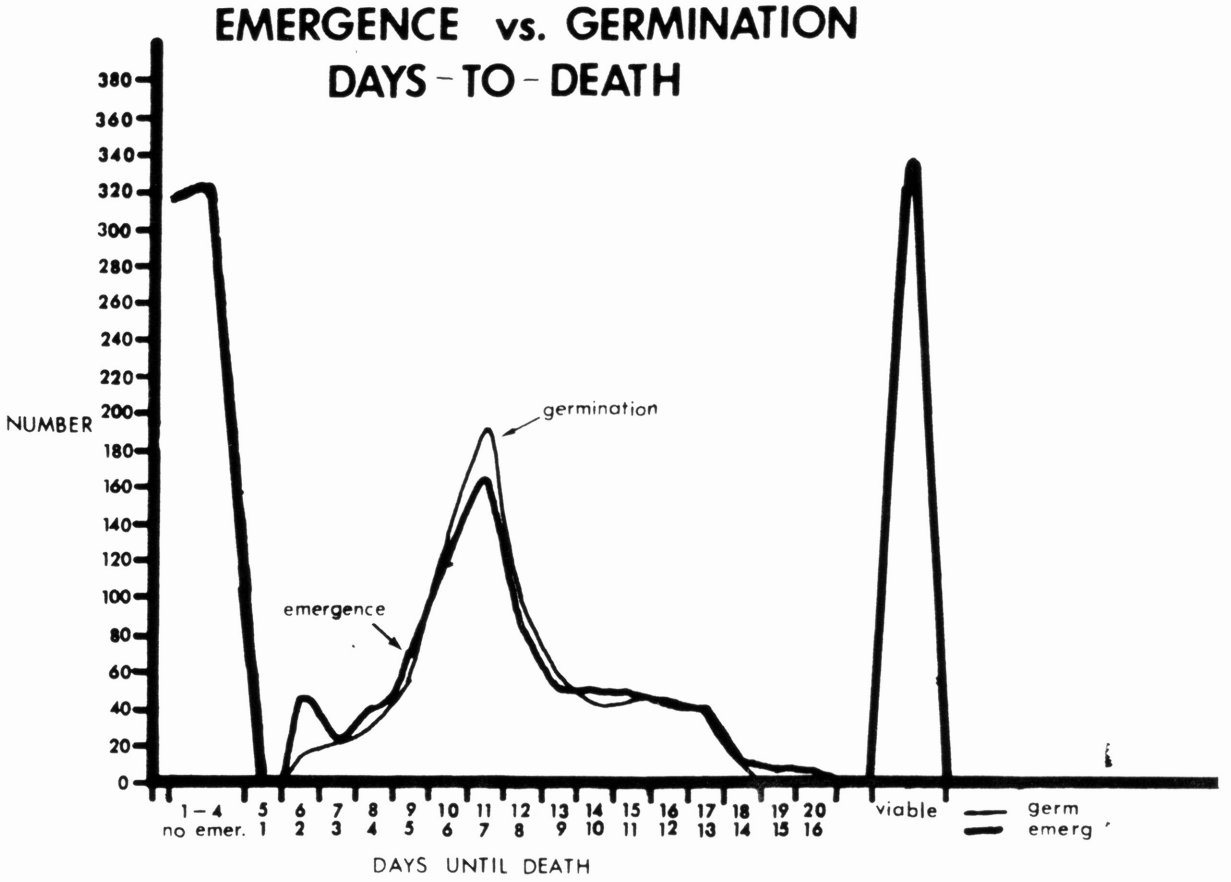

POST-GERMINATION DAYS-TO-DEATH
Experiment II 52 CULTIVARS
0 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380
1-5 6 7 8 9 10 11 12 13 14 15 16 17 18
germination
viable
DAYS AFTER GERMINATION to DEATH

Figure 8 - Graph overlay of Experiment II - Comparison of Post-emergent Days-to-Death vs. Post-germination Days-to-Death.

Figure 9 - Experiment II - Correlation of Post-emergent Days-to-Death vs. Post-germination Days-to-Death.


EMERGENCE vs. GERMINATION
DAYS - TO - DEATH
NUMBER
380
360
340
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
germination
emergence
1 – 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
no emer. 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
viable
germ
emerg
DAYS UNTIL DEATH

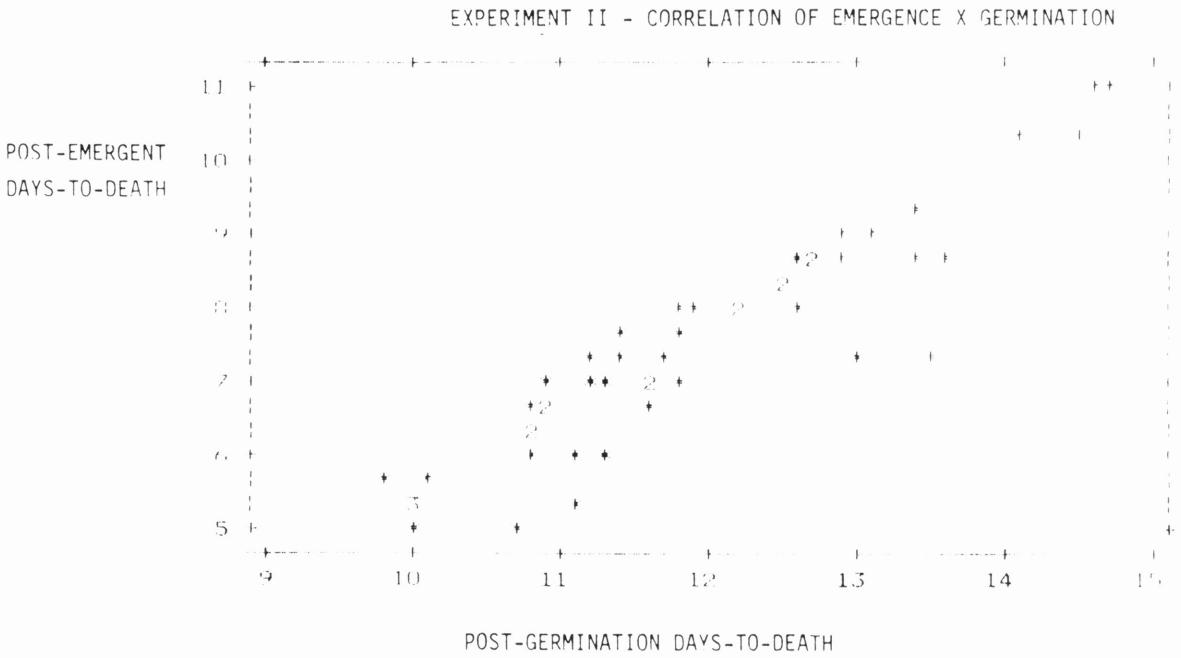

EXPERIMENT II - CORRELATION OF EMERGENCE X GERMINATION
POST-EMERGENT
DAYS-TO-DEATH
11
10
9
8
7
6
5
9
10
11
12
13
14
POST-GERMINATION DAYS-TO-DEATH
r = .929
52 entries

Table 1. Experiment I: Seedling Classification of $F_1$ Seed Obtained from the crosses of Cultivar X $\underline{le}_1\underline{le}_1\underline{Le}_2$ $^{dav}\underline{Le}_2{}^{dav}$.

| Cultivar | Emergent Seedlings | | Non-Emerged Seedlings | | Total |
|---|---|---|---|---|---|
| | Lethal | Viable | Rotten | Germ. & Lethal | |
| Acala Nunn 5-37 | 7 | 0 | 17 | 6 | 30 |
| Acala 1064 NM | 8 | 0 | 13 | 9 | 29 |
| Coker's Cleveland | 19 | 0 | 7 | 4 | 30 |
| Coker's WildS2 | 0 | 0 | 20 | 10 | 7 |
| Lone Star | 29 | 0 | 0 | 1 | 30 |
| Wannamakers Early Wilt | 12 | 0 | 0 | 2 | 14 |
| Stoneville 28 Original | 28 | 0 | 2 | 0 | 30 |
| Acala 5675 | 13 | 0 | 10 | 1 | 24 |
| Pandora | 23 | 0 | 0 | 2 | 25 |
| Deltatype Webber | 0 | 0 | 11 | 2 | 13 |
| Deltapine 12 | 30 | 0 | 0 | 0 | 30 |
| Delta Smooth Leaf | 23 | 0 | 2 | 5 | 30 |
| Acala No. 101 Rogers | 27 | 0 | 2 | 1 | 30 |
| Half & Half | 23 | 0 | 0 | 7 | 30 |
| Stoneville 213 | 14 | 0 | 8 | 1 | 23 |
| Carolina Queen | 19 | 0 | 6 | 5 | 30 |
| Bayou B-10 | 28 | 0 | 1 | 1 | 30 |
| Acala SJ-1 | 27 | 0 | 1 | 2 | 30 |
| Coker 201 | 24 | 0 | 3 | 3 | 30 |
| Deltapine 45A | 26 | 0 | 2 | 1 | 29 |
| Dixie King | 9 | 0 | 5 | 16 | 30 |

Table 1. Experiment I: Seedling Classification of $F_1$ Seed Obtained from the crosses of Cultivar X $\underline{le}_1\underline{le}_1\underline{Le}_2^{dav}\ \underline{Le}_2^{dav}$.

| Cultivar | Emergent Seedlings | | Non-Emerged Seedlings | | Total |
|---|---|---|---|---|---|
| | Lethal | Viable | Rotten | Germ. & Lethal | |
| Kasch | 17 | 0 | 2 | 11 | 30 |
| MissDel | 26 | 0 | 1 | 3 | 30 |
| Coker 124 | 16 | 0 | 3 | 11 | 30 |
| Hi-Bred | 13 | 0 | 5 | 12 | 30 |
| Plains | 7 | 0 | 10 | 7 | 24 |
| StarDel | 13 | 0 | 9 | 8 | 30 |
| Wacona | 2 | 0 | 27 | 1 | 30 |
| Blightmaster | 3 | 0 | 22 | 5 | 30 |
| Paymaster 101 | 0 | 0 | 17 | 13 | 30 |
| Coker 100A | 22 | 0 | 3 | 5 | 30 |
| Greg 35 | 8 | 0 | 17 | 5 | 30 |
| Lankart Sel 611 | 4 | 0 | 18 | 8 | 30 |
| DelFos 9169 | 6 | 0 | 10 | 14 | 30 |
| Deltapine 15 | 10 | 0 | 9 | 11 | 30 |
| Empire WR-61 | 29 | 0 | 1 | 0 | 30 |
| Fox 4 | 4 | 0 | 19 | 7 | 30 |
| DelCot 277 | 22 | 0 | 4 | 4 | 30 |
| Lockert 4789A | 2 | 0 | 18 | 10 | 30 |
| Coker 5110 | 20 | 0 | 4 | 6 | 30 |
| Acala 1517-70 | 22 | 0 | 3 | 4 | 29 |
| Stoneville 731N(98731) | 26 | 0 | 1 | 3 | 30 |

Table 1. Experiment I: Seedling Classification of $F_1$ Seed Obtained from the crosses of Cultivar X $\frac{le_1}{le_1}\frac{Le_2}{Le_2}$ dav dav.

| Cultivar | Emergent Seedlings | | Non-Emerged Seedlings | | Total |
|---|---|---|---|---|---|
| | Lethal | Viable | Rotten | Germ. & Lethal | |
| Deltapine 16 | 23 | 0 | 4 | 3 | 30 |
| Deltapine 14 | 24 | 0 | 5 | 1 | 30 |
| Cleveland 54 | 0 | 0 | 3 | 4 | 7 |
| Coker 100 | 15 | 0 | 2 | 7 | 24 |
| Lockert 140-46 | 6 | 0 | 16 | 8 | 24 |
| Nebane Watson | 27 | 0 | 0 | 2 | 29 |
| Bobshaw 1A | 6 | 0 | 18 | 6 | 30 |
| Lankart Sel 57 | 29 | 0 | 1 | 0 | 30 |
| Pope | 22 | 0 | 7 | 1 | 30 |
| Wilds 15 | 8 | 0 | 11 | 11 | 22 |
| Acala 8 | 27 | 0 | 0 | 3 | 30 |
| Stoneville 7 | 23 | 0 | 1 | 6 | 30 |
| RilCot | 2 | 0 | 14 | 9 | 25 |
| Acala 1517C | 8 | 0 | 14 | 8 | 22 |
| Acala 44 | 28 | 0 | 0 | 2 | 30 |
| Acala 4-42 | 24 | 0 | 3 | 3 | 30 |
| Coker 310 | 25 | 0 | 2 | 3 | 30 |
| Paymaster 54 | 13 | 0 | 10 | 8 | 31 |
| Northern Star 5 | 14 | 0 | 12 | 4 | 30 |
| Rowden 41B Bryant | 6 | 0 | 15 | 9 | 24 |
| Parrott | 24 | 0 | 4 | 2 | 30 |
| TOTALS | 1015 | 0 | 455 | 327 | 1797 |

Table 2. Rates of seedling mortality due to lethal interaction.

| Cultivar | Experiment I | Experiment II | |
|---|---|---|---|
| | Days Post emerg. to death | Days Post emerg. to death | Days Post germ. to death |
| Acala Nunn 5-37 | 4.1 | 7.73 | 11.82 |
| Acala 1064 NM | 3.6 | 7.77 | 11.89 |
| Coker's Cleveland | 4.7 | 8.39 | 12.71 |
| Coker's Wild | 0.0 | 8.69 | 12.92 |
| Lone Star | 5.3 | 7.14 | 11.43 |
| Wannamakers Early Wilt | 3.7 | 8.50 | 13.40 |
| Stoneville 28 Original | 4.8 | 8.20 | 12.50 |
| Acala 5675 | 4.7 | 7.78 | 12.56 |
| Pandora | 3.9 | 6.70 | 11.60 |
| Deltatype Webber | 0.0 | 8.30 | 12.50 |
| Deltapine 12 | 4.5 | 9.26 | 13.42 |
| Delta Smooth Leaf | 4.2 | 8.36 | 12.68 |
| Acala No. 111 Rogers | 3.4 | 6.30 | 10.83 |
| Half & Half | 2.6 | 8.50 | 12.88 |
| Stoneville 213 | 4.6 | 6.93 | 11.64 |
| Carolina Queen | 4.9 | 5.27 | 11.06 |
| Bayou B-10 | 5.0 | 7.17 | 13.00 |
| Acala SJ-1 | 4.7 | 7.04 | 13.48 |
| Coker 201 | 4.9 | 5.72 | 10.83 |
| Deltapine 45A | 4.6 | 7.39 | 11.83 |
| Dixie King | 4.3 | 6.88 | 10.88 |

Table 2. Rates of seedling mortality due to lethal interaction.

| Cultivar | Experiment I | Experiment II | |
|---|---|---|---|
| | Days Post emerg. to death | Days Post emerg. to death | Days Post germ. to death |
| Kasch | 3.9 | 7.73 | 12.18 |
| MissDel | 4.8 | 8.39 | 12.61 |
| Coker 124 | 4.3 | 10.33 | 14.06 |
| Hi-Bred | 4.0 | 6.53 | 10.77 |
| Plains | 4.0 | 7.29 | 11.71 |
| StarDel | 3.8 | 6.91 | 11.18 |
| Wacona | 3.0 | 6.70 | 11.80 |
| Blightmaster | 4.0 | 6.60 | 10.93 |
| Paymaster 101 | 0.0 | 6.40 | 10.95 |
| Coker 100A | 4.6 | ---- | ----- |
| Greg 35 | 3.8 | 5.17 | 10.00 |
| Lankart Sel 611 | 3.8 | 8.20 | 12.85 |
| DelFos 9169 | 4.3 | 8.53 | 13.59 |
| Deltapine 15 | 3.6 | 5.27 | 10.00 |
| Empire WR-61 | 5.7 | 5.83 | 11.29 |
| Fox 4 | 2.8 | 6.56 | 11.63 |
| DelCot 277 | 4.8 | 6.30 | 10.82 |
| Lockert 4789A | 3.0 | 5.33 | 9.81 |
| Coker 5110 | 3.8 | 7.50 | 11.42 |
| Acala 1517-70 | 3.0 | 5.52 | 10.71 |
| Stoneville 731N(98731) | 3.6 | 7.10 | 11.20 |

Table 2. Rates of seedling mortality due to lethal interaction.

| Cultivar | Experiment I | Experiment II | |
|---|---|---|---|
| | Days Post emerg. to death | Days Post emerg. to death | Days Post germ. to death |
| Deltapine 16 | 4.9 | 5.78 | 11.11 |
| Deltapine 14 | 4.2 | 6.77 | 11.31 |
| Cleveland 54 | 0.0 | 5.00 | 10.00 |
| Coker 100 | 4.7 | 10.25 | 14.50 |
| Lockert 140-46 | 3.2 | 10.91 | 14.64 |
| Nebane Watson | 5.0 | 10.89 | 14.67 |
| Bobshaw 1A | 5.0 | ----- | ----- |
| Lankart Sel 57 | 5.2 | 5.00 | 10.67 |
| Pope | 5.6 | 9.00 | 13.06 |
| Wilds 15 | 1.9 | 8.00 | 12.25 |
| Acala 8 | 4.1 | ---- | ----- |
| Stoneville 7 | 4.2 | ---- | ----- |
| RilCot | 2.0 | ---- | ----- |
| Acala 1517C | 3.6 | ---- | ----- |
| Acala 44 | 5.8 | ---- | ----- |
| Acala 4-42 | 5.4 | ---- | ----- |
| Coker 310 | 5.2 | ---- | ----- |
| Paymaster 54 | 3.4 | ---- | ----- |
| Northern Star 5 | 4.4 | ---- | ----- |
| Rowden 41B Bryant | 4.8 | ---- | ----- |
| Parrott | 5.0 | ---- | ----- |
| CUMULATIVE AVERAGES | 3.72 | 7.37 | 11.95 |

Table 3. Segregation among testcross progeny (cultivars X $\underline{le}_1\underline{le}_1\underline{le}_2\underline{le}_2$) X $\underline{le}_1\underline{le}_1\underline{Le}_2{}^{dav}$ $\underline{Le}_2{}^{dav}$ for viability versus inviability.

| Cultivar | No. of seedlings | | | Chi-square values | | Genotype |
|---|---|---|---|---|---|---|
| | Lethal | Viable | Total | 3:1 | 1:1 | |
| Acala Nunn 5-37 | 16 | 5 | 21 | .016 | 5.762* | $\underline{Le}_1\underline{Le}_1\underline{Le}_2\underline{Le}_2$ |
| Acala 1064 NM | 24 | 9 | 33 | .091 | 6.818* | $\underline{Le}_1\underline{Le}_1\underline{Le}_2\underline{Le}_2$ |
| Coker's Cleveland | 23 | 7 | 30 | .044 | 8.533* | $\underline{Le}_1\underline{Le}_1\underline{Le}_2\underline{Le}_2$ |
| Coker's WildS2 | 18 | 8 | 26 | .462 | 3.846* | $\underline{Le}_1\underline{Le}_1\underline{Le}_2\underline{Le}_2$ |
| Lone Star | 10 | 2 | 12 | .444 | 5.333* | $\underline{Le}_1\underline{Le}_1\underline{Le}_2\underline{Le}_2$ |
| Wannamakers Early Wilt | 26 | 5 | 31 | 1.301 | 14.226* | $\underline{Le}_1\underline{Le}_1\underline{Le}_2\underline{Le}_2$ |
| Stoneville 28 Original | 26 | 7 | 33 | .253 | 10.939* | $\underline{Le}_1\underline{Le}_1\underline{Le}_2\underline{Le}_2$ |
| Acala 5675 | 36 | 6 | 42 | 2.571 | 21.429* | $\underline{Le}_1\underline{Le}_1\underline{Le}_2\underline{Le}_2$ |
| Pandora | 26 | 6 | 32 | .667 | 12.500* | $\underline{Le}_1\underline{Le}_1\underline{Le}_2\underline{Le}_2$ |
| Deltatype Webber | 20 | 7 | 27 | .012 | 6.259* | $\underline{Le}_1\underline{Le}_1\underline{Le}_2\underline{Le}_2$ |
| Deltapine 12 | 25 | 7 | 32 | .167 | 10.125* | $\underline{Le}_1\underline{Le}_1\underline{Le}_2\underline{Le}_2$ |
| Delta Smooth Leaf | 26 | 8 | 34 | .039 | 9.529* | $\underline{Le}_1\underline{Le}_1\underline{Le}_2\underline{Le}_2$ |
| Acala No. 111 Rogers | 30 | 6 | 36 | 1.333 | 16.000* | $\underline{Le}_1\underline{Le}_1\underline{Le}_2\underline{Le}_2$ |
| Half & Half | 24 | 9 | 33 | .091 | 6.818* | $\underline{Le}_1\underline{Le}_1\underline{Le}_2\underline{Le}_2$ |
| Stoneville 213 | 23 | 7 | 30 | .044 | 8.533* | $\underline{Le}_1\underline{Le}_1\underline{Le}_2\underline{Le}_2$ |
| Carolina Queen | 26 | 5 | 31 | 1.301 | 14.226* | $\underline{Le}_1\underline{Le}_1\underline{Le}_2\underline{Le}_2$ |
| Bayou B-10 | 26 | 8 | 34 | .039 | 9.529* | $\underline{Le}_1\underline{Le}_1\underline{Le}_2\underline{Le}_2$ |
| Acala SJ-1 | 25 | 6 | 31 | .527 | 11.645* | $\underline{Le}_1\underline{Le}_1\underline{Le}_2\underline{Le}_2$ |
| Coker 201 | 32 | 4 | 36 | 3.704 | 21.778* | $\underline{Le}_1\underline{Le}_1\underline{Le}_2\underline{Le}_2$ |
| Deltapine 45A | 26 | 8 | 34 | .039 | 9.529* | $\underline{Le}_1\underline{Le}_1\underline{Le}_2\underline{Le}_2$ |
| Dixie King | 24 | 6 | 30 | .400 | 10.800* | $\underline{Le}_1\underline{Le}_1\underline{Le}_2\underline{Le}_2$ |

Table 3. Segregation among testcross progeny (cultivars X $le_1 le_1 le_2 le_2$) X $le_1 le_1 Le_2 Le_2$ dav dav for viability versus inviability.

| Cultivar | No. of Seedlings | | | Chi-Square Values | | Genotype |
|---|---|---|---|---|---|---|
| | Lethal | Viable | Total | 3:1 | 1:1 | |
| Kasch | 26 | 8 | 34 | .039 | 9.529* | $Le_1 Le_1 Le_2 Le_2$ |
| MissDel | 25 | 8 | 33 | .010 | 8.758* | $Le_1 Le_1 Le_2 Le_2$ |
| Coker 124 | 23 | 7 | 30 | .044 | 8.533* | $Le_1 Le_1 Le_2 Le_2$ |
| Hi-Bred | 26 | 6 | 32 | .667 | 12.500* | $Le_1 Le_1 Le_2 Le_2$ |
| Plains | 26 | 8 | 34 | .039 | 9.529* | $Le_1 Le_1 Le_2 Le_2$ |
| StarDel | 22 | 5 | 27 | .605 | 10.704* | $Le_1 Le_1 Le_2 Le_2$ |
| Wacona | 19 | 8 | 27 | .309 | 4.481* | $Le_1 Le_1 Le_2 Le_2$ |
| Blightmaster | 19 | 6 | 25 | .013 | 6.760* | $Le_1 Le_1 Le_2 Le_2$ |
| Paymaster 101 | 28 | 6 | 34 | .980 | 14.265* | $Le_1 Le_1 Le_2 Le_2$ |
| Coker 100A | 15 | 4 | 19 | .158 | 6.368* | $Le_1 Le_1 Le_2 Le_2$ |
| Greg 35 | 24 | 6 | 30 | .400 | 10.800* | $Le_1 Le_1 Le_2 Le_2$ |
| Lankart Sel 611 | 25 | 8 | 33 | .010 | 8.758* | $Le_1 Le_1 Le_2 Le_2$ |
| DelFos 9169 | 25 | 8 | 33 | .010 | 8.758* | $Le_1 Le_1 Le_2 Le_2$ |
| Deltapine 15 | 16 | 6 | 22 | .061 | 4.545* | $Le_1 Le_1 Le_2 Le_2$ |
| Empire WR-61 | 23 | 8 | 31 | .011 | 7.258* | $Le_1 Le_1 Le_2 Le_2$ |
| Fox 4 | 23 | 5 | 28 | .762 | 11.571* | $Le_1 Le_1 Le_2 Le_2$ |
| DelCot 277 | 31 | 8 | 39 | .419 | 13.564* | $Le_1 Le_1 Le_2 Le_2$ |
| Lockert 4789A | 29 | 5 | 34 | 1.922 | 16.941* | $Le_1 Le_1 Le_2 Le_2$ |
| Coker 5110 | 17 | 6 | 23 | .014 | 5.261* | $Le_1 Le_1 Le_2 Le_2$ |
| Acala 1517-70 | 26 | 8 | 34 | .039 | 9.529* | $Le_1 Le_1 Le_2 Le_2$ |
| Stoneville 731N(98731) | 17 | 6 | 23 | .014 | 5.261* | $Le_1 Le_1 Le_2 Le_2$ |

Table 3. Segregation among testcross progeny (cultivars X $\underline{le}_1\underline{le}_1\underline{le}_2\underline{le}_2$) X $\underline{le}_1\underline{le}_1\underline{Le}_2{}^{dav}$ $\underline{Le}_2{}^{dav}$ for viability versus inviability.

| Cultivar | No. of Seedlings | | | Chi-Square Values | | Genotype |
|---|---|---|---|---|---|---|
| | Lethal | Viable | Total | 3:1 | 1:1 | |
| Deltapine 16 | 22 | 12 | 34 | 1.922 | 2.941 | $\underline{Le}_1\underline{Le}_1\underline{Le}_2\underline{Le}_2$ |
| Deltapine 14 | 21 | 11 | 32 | 1.500 | 3.125 | $\underline{Le}_1\underline{Le}_1\underline{Le}_2\underline{Le}_2$ |
| Cleveland 54 | 5 | 1 | 6 | .222 | 2.667 | $\underline{Le}_1\underline{Le}_1\underline{Le}_2\underline{Le}_2$ |
| Coker 100 | 8 | 4 | 12 | .444 | 1.333 | $\underline{Le}_1\underline{Le}_1\underline{Le}_2\underline{Le}_2$ |
| Lockert 140-46 | 14 | 7 | 21 | .778 | 2.333 | $\underline{Le}_1\underline{Le}_1\underline{Le}_2\underline{Le}_2$ |
| Nebane Watson | 12 | 6 | 18 | .667 | 2.000 | $\underline{Le}_1\underline{Le}_1\underline{Le}_2\underline{Le}_2$ |
| Bobshaw 1A | 6 | 2 | 8 | .000 | 2.000 | $\underline{Le}_1\underline{Le}_1\underline{Le}_2\underline{Le}_2$ |
| Lankart Sel 57 | 6 | 1 | 7 | .429 | 3.571 | $\underline{Le}_1\underline{Le}_1\underline{Le}_2\underline{Le}_2$ |
| Pope | 22 | 12 | 34 | 1.922 | 2.941 | $\underline{Le}_1\underline{Le}_1\underline{Le}_2\underline{Le}_2$ |
| Wilds 15 | 9 | 6 | 15 | 1.800 | .600 | INSUFFICIENT DATA |
| TOTALS | 1122 | 338 | 1460 | 29.755 | 441.341 | |
| SUM OF $X^2_2$ (52 d.f.) | | | | 29.755 | 441.341 | |
| POOLED $X^2$ (1 d.f.) | | | | 2.670 | 420.900 | |
| HOMOGENIETY (51 d.f.) | | | | 27.085** | 20.441 | |

* $P(X^2_{1 d.f.} > 3.84) < .05$

** $P(X^2_{51 d.f.} > 67.5) < .05$

Table 4. Segregation among testcross progeny (cultivars X D35.17-85) X $\frac{le_1}{--_1}\frac{le_1}{--_1}\frac{Le_2^{dav}}{--_2}$ $\frac{Le_2^{dav}}{--_2}$ for viability versus inviability.

| Cultivar | No. of Seedlings | | Chi-Square Values | |
|---|---|---|---|---|
| | Lethal | Viable | 3:1 | 1:1 |
| Acala 8 | 13 | 0 | 4.33 | 13.0 |
| Stoneville 7 | 35 | 0 | 11.67 | 35.0 |
| RilCot | 39 | 0 | 13.00 | 39.0 |
| Acala 1517C | 33 | 0 | 11.00 | 33.0 |
| Acala 44 | 33 | 0 | 11.00 | 33.0 |
| Acala 4-42 | 38 | 0 | 12.54 | 38.0 |
| Coker 310 | 15 | 0 | 5.00 | 15.0 |
| Paymaster 54 | 0 | 0 | N/A | N/A |
| Northern Star 5 | 0 | 0 | N/A | N/A |
| Rowden 41B Bryant | 1 | 0 | N/A | N/A |
| Parrott | 0 | 0 | N/A | N/A |
| TOTALS (including Table 3) | 1329 | 338 | 98.295 | 647.341 |
| SUM OF $X^2_2$ (63 d.f.) | | | 98.295 | 647.341 |
| POOLED $X^2$ (1 d.f.) | | | 30.540 | 627.76 |
| HOMOGENIETY (62 d.f.) | | | 67.755 | 19.581 |

* $P(X^2_{1d.f.} > 3.84) < .05$

# DISCUSSION

## Determination of Cultivar Genotypes

Two main conclusions are derived from interpretation of the data obtained: (1) all 63 cultivars are homozygous for $\underline{Le}_1$ or $\underline{Le}_2$, if not both and (2) at least 42 cultivars are homozygous at both loci. The extensive prevalence of the dominant alleles will be beneficial for the implementation to the newly proposed scheme for mass extraction of doubled haploids in cotton. Of the 23 cultivars that have not been identified at both loci for genotype, four cultivars were eliminated for lack of testcross seed, and seven cultivars were eliminated due an outcross which caused segregation to occur in a plant used as a pollinator. The other ten cultivars did not have enough testcross progeny in order to reject either of the segregation ratio hypotheses.

Using procedures described by Mather (1941), it was determined that 50 testcross seed for each testcross family would be adequete to differentiate between the two expected ratios (3:1 or 1:1, inviable : viable). However, due the lack of sufficient greenhouse space and other factors, about 30 seed were obtained from each testcross family. For 42 of the 52 cultivars, approximately 30 seed yielded segregation ratios adequete to reject the 1:1 hypothesis, but for the other ten cultivars it will be necessary to produce additional testcross seed in

order to obtain additional data on segregation ratios to positively the genotypes of involved cultivars. The reason seven cultivars failed to segregate in Experiment II (all the progeny was necrotic), was traced to a pollinator that apparently was segregating for allele(s) $\underline{Le}_1$ or $\underline{Le}_2$, if not both. The pollinator was grown from open pollinated greenhouse seed harvested from a plant with a known genotype $\underline{le}_1\underline{le}_1\underline{le}_2\underline{le}_2$. Cotton rarely cross pollinates in greenhouse conditions because there is no wind or insect activity. Obviously though, outcrossing occasionally occurs. Successful identification of this problem was through accurate recording of parental plants involved in each cross.

The results of the genotype determination and apparent absence of alleles $\underline{le}_1$ and $\underline{le}_2$ in cultivated G. hirsutum varieties poses some interesting biological, genetic, and evolutionary questions. An interesting question is why dominant alleles $\underline{Le}_1$ and $\underline{Le}_2$ are so prevalent when no apparent detrimental effects have been associated with recessive alleles $\underline{le}_1$ or $\underline{le}_2$. Another problematic question is why does the allele $\underline{Le}_2^{dav}$ even exist. It seems obvious that some type of mutation would have occurred and remained because the recessive alleles have not been found to harbor any deleterious effects. The reasons as to why these phenomenom occur is beyond the scope of this study, but they are puzzling question.

Determination of Dosage Related Time of Death

A secondary objective of this study was to determine if there were a dosage effect, i.e., if the number of dominant $\underline{Le}_1$ or $\underline{Le}_2$ alleles interacting with the $\underline{Le}_2$ allele would effect the rate of the lethality reaction. In studies done by Lee, he proposed that hybrid seed carrying a genotype of $\underline{Le}_1\underline{le}_1\underline{Le}_2\underline{Le}_2^{dav}$ would become necrotic approximately six to thirteen days after planting while hybrids harboring a genotype of either $\underline{Le}_1\underline{le}_1\underline{le}_2\underline{Le}_2^{dav}$ or $\underline{le}_1\underline{le}_1\underline{Le}_2\underline{Le}_2^{dav}$ would become necrotic 13-21 days after planting. Since the genotypes of the parental cultivars were determined in this study, data gathered and recorded corresponding to days to death from various stages should have produced results similar to Lee's hypothesis.

In Experiment I, only 56.4 % of all seed planted emerged. Under greenhouse conditions, normal cotton seed less than one year old has approximately 90 - 100% emergence. Upon examination of the non-emergent seed, nearly half had germinated but had died prior to emergence. Close examinantion of the germinated seedlings, showed characteristic symptoms of the lethality reaction. The cotyledons had extensive dark necrotic regions, but the radicle seemed to be unaffected by the lethality. Evidently, these seedlings had carried a lethal genotype and the lethal reaction was responsible for the seedling death. This created a problem in measuring days to death

from emergence since it had been assumed that the lethal reaction would not appear until after the seedling had emerged. This was obviously an incorrect assumption.

Since we now knew that measuring days-to-death relative to day-of-emergence presented some problems and introduced more error, additional parameters were evaluated in Experiment II. While date of emergence was again recorded, the date of germination was recorded and each seedling was rated for symptoms of the lethal reaction at germination (Figure 15). At germination, almost one third of the seed had begun to show signs of an impending reaction. Since the reaction had already progressed extensively by germination, it can be hypothesized that it had begun during the late stages of seed maturation while still on the parent plant. As in previous experiments, the radicle was not affected by the reaction.

Each of the two parameters has advantages and disadvantages. An advantage to peeling the seed coat for measurement of days from germination to death was seedlings that never emerged but had germinated could be counted as deaths due to lethality. But measurement from germination required additional work and the exact time to death of those seedlings that had germinated but not emerged could still not be determined. For post emergence measurements, less work was required to gather data but many seedlings that died due to the lethality reaction prior to emergence could not be counted as such because the seed remains rotted before they were exhumed for evaluation. Results for both measurement parameters in Experiment II

followed very similar patterns (figure 6 & 7) and when plotted, a correlation value of r = .929 is obtained (figure 9). Therefore, it appears that either emergence or germination are equally effective in rating the number of days to death.

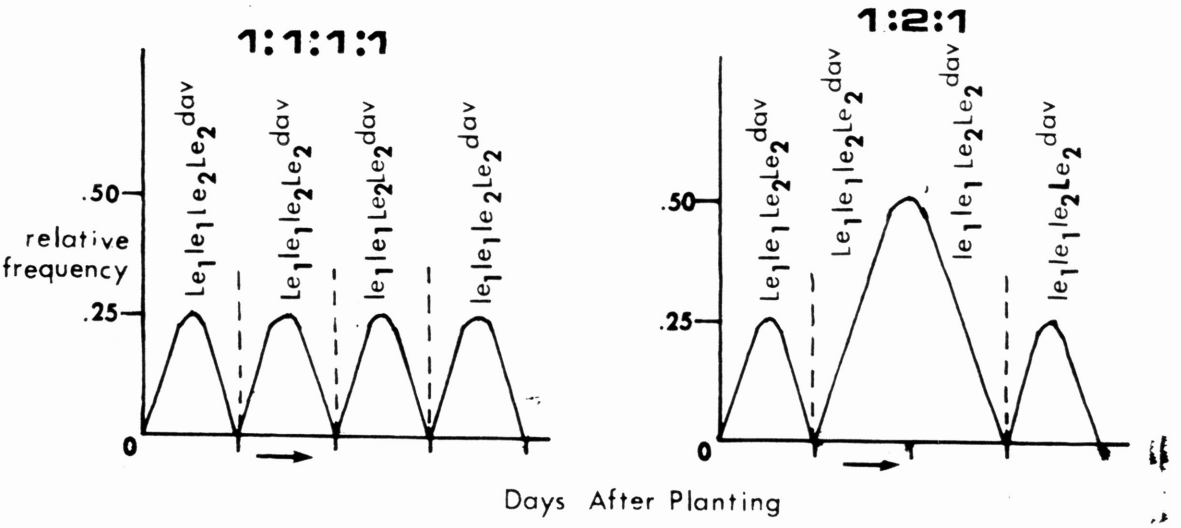
From the genotype determination, it was known that all seedlings in Experiment I screening were genotype $\underline{\underline{Le}}_1\underline{\underline{le}}_1\underline{\underline{Le}}_2\underline{\underline{Le}}_2^{dav}$. Therefore, according to Lee's proposal, these seedlings should have died approximately 4-11 days after emergence. The cumulative average for Experiment I was 2.81 days after emergence, which is a much faster than Lee hypothesized. The quickness of the reaction could be attributed to environmental effects such as temperature.

In Experiment II, the $F_2$ seed entered into the seedling screening should have segregated in the following manner:

| Seg Group | Ratio | Genotype | No. of Dom. Allele |
|---|---|---|---|
| A | 1 | $\underline{\underline{Le}}_1\underline{\underline{le}}_1\underline{\underline{Le}}_2\underline{\underline{Le}}_2^{dav}$ | 2 |
| B | 1 | $\underline{\underline{Le}}_1\underline{\underline{le}}_1\underline{\underline{le}}_2\underline{\underline{Le}}_2$dav | 1 |
| C | 1 | $\underline{\underline{le}}_1\underline{\underline{le}}_1\underline{\underline{Le}}_2\underline{\underline{Le}}_2$dav | 1 |
| D | 1 | $\underline{\underline{le}}_1\underline{\underline{le}}_1\underline{\underline{le}}_2\underline{\underline{Le}}_2$ | 0 |

Segregation group A would die first and groups B and C should react second while the D group progeny are viable. Another possibility that could occur is differing lethality rates between segregation groups B and C, i.e., genotype $\underline{\underline{Le}}_1\underline{\underline{le}}_1\underline{\underline{le}}_2\underline{\underline{Le}}_2^{dav}$ may express lethality before $\underline{\underline{le}}_1\underline{\underline{le}}_1\underline{\underline{Le}}_2\underline{\underline{Le}}_2^{dav}$. So when time to

death from either germination or emergence was plotted against number of seedling to die that day the graph should appear analogous to one of the following:




Comparing figures 6 & 7 with the above shows a strong similarity to the graph of a 1:2:1 ratio, with the middle genotypes combining to form a single phenotype. The following genotypic-phentypic relationship is proposed for Experiment II:

| Ratio | Genotype | Post-Emergent Days-to-Death | No. |
|---|---|---|---|
| 1 | $\underline{Le}_1\underline{le}_1\underline{Le}_2\underline{Le}_2^{dav}$ | 0-2 | 371 |
| 2 | $\underline{Le}_1\underline{le}_1\underline{le}_2\underline{Le}_2^{dav}$<br>$\underline{le}_1\underline{le}_1\underline{Le}_2\underline{Le}_2$ | 3 + | 739 |
| 1 | $\underline{le}_1\underline{le}_1\underline{le}_2\underline{Le}_2^{dav}$ | Viable | 338 |

While the breaking point between the second and third categories was obvious (viable plants contained the non-lethal genotype), the line between the first and second categories was not so clear. In the

graph on figure 6, the low number occurred at three days after emergence. This was assumed to be the breaking point between the first categories. One may wonder why no seedlings died at one day after emergence. This was due to the fact that the day of emergence was itself rated as day "one", therefore for a seedling to be rated lethal on day one would have required it to emerge, grow, and then die the same day. If growth were to cease a day before death, then "day 1" deaths would not be expected to have been observed. When the chi-square test is applied a goodness of fit to the 1:2:1 ratio $P(1:2:1) > .50$, ($X^2_{2d.f.} = 2.12$) is obtained. These results fits the hypothesis that the number of dominant alleles interacting with $\underline{Le}_2^{dav}$ affects the rate of death quite well. The only factors different from Lee's hypothesis is the time frame required for the complete reactions.

Upon returning to Experiment I, however, and applying the same criteria as used in Experiment II, all of the seedlings in the first experiment should have died before emergence or at least by the second day after emergence. Clearly by observing figure 5, this did not happen, with the lethal reaction occurring in trial one up to seven days after emergence. These results can summarized to mean one of two options; either the interaction of dominant alleles with the $\underline{Le}_2^{dav}$ allele is not the only factor in rate of lethal expression or a large amount of variability was present due to some unknown reason. While both of these statements are possibilities, the

observed results of these studies can be best explain by the combination of the two reasons.

While the results indicate that the number of dominant alleles interacting with the $\underline{Le}_2$ allele is not the sole factor that determines the rate of the lethality expression, it still must be considered the primary factor. Several other factors could be involved in the rate of lethality reaction as well. First, there was a moderate amount of variability and range of the days to death between lines in both trials that followed no set pattern or that could be attributed to any other cause. Therefore, variability between cultivars may be due to modifiers within each cultivar that affect the expression of the lethality reaction within that cultivar. Second, the environmental conditions under which the seed was produced may have a major effect on how fast the lethal condition is expressed. Seed produced for Experiment I was grown in field conditions in the summer of 1986. Seed used in Experiment II was produced in the greenhouse in the winter of 1986-87. Reports by Phillips (1975) indicated that the expression of the lethal reaction was suppressed at temperatures of greater than 37 C. Seed grown in the field in 1986 could easily reached this temperature during the day while it was developing. Thus, the reaction had not been able to begin to express itself at that early stage. This could then have caused the seedlings in Experiment I to live longer than expected when compared to genotypic-phenotypic relationships of Experiment II. The seed for Experiment II was produced in the greenhouse during the winter, in

which, temperatures never reached as high as 37 C and often were very cool. Since these seedlings underwent temperature conditioning, the lethal condition would continue at the normal rate without any suppression, as was seen.

To positively identify the specific control of the rate of lethality expression, it will be necessary to produce isolines and completely homozygous lines of G. hirsutum with the genotypes of $\underline{Le}_1\underline{Le}_1\underline{Le}_2\underline{Le}_2$, $\underline{Le}_1\underline{Le}_1\underline{le}_2\underline{le}_2$, $\underline{le}_1\underline{le}_1\underline{Le}_2\underline{Le}_2$, and $\underline{le}_1\underline{le}_1\underline{le}_2\underline{le}_2$. Utiliztion of these materials will eliminate genotypic variation and these lines would be crossed with an $\underline{Le}_2^{dav}$ tester stock, genotype $\underline{le}_1\underline{le}_1\underline{Le}_2^{dav}\underline{Le}_2^{dav}$. All crosses should be made in one growing season in order to eliminate environmental variances. Seedling screenings of these crosses would yield exact results on the effects of dominant allele interaction with the $\underline{Le}_2^{dav}$ allele.

# CONCLUSIONS

The main objectives of this study were; (i) to determine the relative frequency of the alleles $\underline{le}_1$ and $\underline{le}_2$ in a representative sample of G. hirsutum, (ii) to determine the genotype of each cultivar of G. hirsutum in the sample, and (iii) to determine whether the number of dominant alleles interacting with the $\underline{Le}_2^{dav}$ alleles affected the rate of necrosis.

Of the sixty-three cultivars entered into the representative sample, the genotype at the $\underline{Le}_1$ and $\underline{Le}_2$ loci was determined for 42 cultivars. The genotype in all 42 cultivars was $\underline{Le}_1\underline{Le}_1\underline{Le}_2\underline{Le}_2$. For ten cultivars, enough testcross was not availible to reject one of the two segregation ratio hypotheses. However, with the incomplete data availible, a genotype of $\underline{Le}_1\underline{Le}_1\underline{Le}_2\underline{Le}_2$ is proposed. Therefore, the relative frequency of the alleles $\underline{le}_1$ and $\underline{le}_2$ appears to be zero or close to it. The rare frequency of the recessive alleles remains to be explained. Eight cultivars were eliminated from the study due to an outcross in one pollinator plants which caused segregation ratios to be erroneous. For the other three cultivars, the inability to produce $F_1$ seed caused the failure to successfully determine genotype.

After determining the genotypes, data gathered pertaining to rates of necrosis were compared to what should or could have been

expected according to the hypothesis. It appears that the number of dominant alleles interacting with the $\underline{Le}_2^{dav}$ allele does affect the rate of death, but that this is not the sole factor affecting the rate of death. Results indicated that environmental factors during seed development and during germination and seedling growth play a major role in the rate of expression of the lethality reaction. Additionally, data indicated the possibility that individual factors for each cultivar, possibly gene modifiers, affected each cultivar's rate of lethality expression. To positively identify the exact role of all variables involved, additional studies will be required. Nevertheless, results indicate that the HEHP doubled-haploid breeding system will be generally applicable to adapted U.S. G. hirsutum germplasm, in that $\underline{le}_1$ and $\underline{le}_2$ alleles seem to be very rare. However, in applying the HEHP system, some special considerations might be given to effects of environment on seed development, fruit retention, and thus, seed production and doubled haploid recovery.

# BIBLIOGRAPHY

Brown, M.S. and Menzel, M. 1952. Additional evidence on the crossing behavior of Gossypium gossypioides. Bull. Torr. Bot. Club. 71: 285-292.

Brown, M.S. 1951. The spontaneous occurance of amphidiploidy in species of Gossypium. Genetics, 27: 25-54.

Caldwell, R.M. and L.E. Compton. 1943. Complementary lethal genes in wheat. J. of Hered. 34: 66-70.

Christie, P. and M.R. MacNair. 1984. Complementary lethal factors in two North American populations of the yellow monkey flower. J. of Hered. 75: 510-511.

Chu, Y.E. and H.I. Oka. 1972. The distribution and effects of genes causing $F_1$ weakness in Oryza breviligulata and O. glaberrima. Genetics, 70: 163-173.

East, E.M. 1935. Genetic reactions in Nicotiana. I. Compatibility. Genetics 20: 403-413.

Gerstel, D.U. 1954. A new lethal combination in interspecific cotton hybrids. Genetics, 39: 628-639.

Hanna, W.W. and K.F. Schertz. 1970. Inheritance and trisome linkage of seedling characters in Sorghum bicolor. Crop Sci. 10: 441-443.

Hebert, T.T. and Middleton, G.K. 1955. Lethality in a wheat cross. Agro. J. 47: 196.

Hermsen, J.G. 1957. Semilethality in hybrid offspring of wheat. Euphytica 6: 18-25.

Heyne, E.G., G.A. Weibe and R.H. Painter. 1943. Complementary genes in wheat causing death of $F_1$ plants. J. of Hered. 34: 243-245.

Hollingshead, L. 1930. A lethal factor in Crepis effective only in an interspecific hybrid. Genetics. 15: 114-140.

Knowles, P.F. 1962. Complementary genes cause a lethal seedling character in flax. Crop Sci. 2: 270.

Kostoff, D. 1930. Ontogeny, genetics, and cytology of Nicotiana hybrids. Genetica 12: 33-139.

Kostoff, D. 1936. Polyploid hybrids Nicotiana rustica var. texana x N. glauca. Grah. Bull. Appl. Bot., Genet., Plant Breed., Ser. II, no. 9: 153-162.

Kochumadhavan, M., et al. 1984. Hybrid necrosis and hybrid chlorosis in Indian varieties of Triticum dicoccum. Euphytica, 33: 853-858.

Lee, J.A. 1981. Genetics of $D_3$ complementary lethality in Gossypium hirsutum and G. barbadense. J. of Hered. 72: 299-300.

-----, 1981. A Genetical scheme for isolating cotton cultivars. Crop Sci. 21: 339-341.

Mather, K. 1951. Measurement of Linkage in Heredity, 2nd edition., John Wiley & Sons, Birmingham, NY.

Morrison, J.W. 1957. Dwarfs, semi-lethals, and lethals in wheat. Euphytica, 6: 213-223.

Oka, H.I. 1957. Phylogenetic differentiation of the cultivated rice. XV. Complementary lethal genes in rice. Jpn J. of Genet., 32: 83-87.

Phillips, L.L. 1963. The cytogenetics of Gossypium and the orgin of New World cottons. Evol. 17: 460-469.

-----, and Merritt, J.F. 1972. Interspecific compatability in Gossypium. I. Stem histogenesis of G. hirsutum x G. gossypioides. Amer. J. of Bot., 59: 203-208.

-----, and Reid, R.K. 1975. Interspecific incompatibility in Gossypium. II. Light and electron microscope slides of cell necrosis and tumorogenesis in hybrids of G. klotzschianum. Amer. J. of Bot., 62: 790-796.

-----. 1977. Interspecific incompatability in Gossypium. IV. Temperature-conditional lethality in hybrids of G. klotzschianum. Amer. J. of Bot., 64: 914-915.

Saunders, A.R. 1952. Complementary lethal genes in the cowpea. S. Afr. J. of Sci., 48: 195-197.

Sawant, A.C. 1956. Semilethal complementary factore in a tomato species hybrid. Evol., 10: 93-96.

Sax, K. 1921. Sterility in wheat hybrids. I. Sterility relations and endosperm development. Genetics, 6:399-416.

Sears, E.R. 1944. Inviability of intergeneric hybrids involving Triticum monococcum and T. aegilopoides. Genetics 29: 113-127.

Silow, R.A. 1941. The comparative genetics of Gossypium anomalum and the cultivated Asiatics cotton. J. of Genet., 42: 259-358.

Skovsted, A. 1935. Cytological studies in cotton: A hybrid between Gossypium davidsonii Kell. and G. sturtii Muell. J. of Genet., 32: 397-405.

Snedecor, G.W. and Cochran, W.G. 1980. Statistical Methods. 7th edn. Iowa State Uni. Press, Ames Iowa.

Stebbins, G.L. 1950. Variation and evolution in plants. New York.

Stelly, D.M. and Lee, J.A. 1987. Proposed Schemes for mass-extraction of doubled haploids in Cotton. Submitted for publication in Crop Sci.

Stephens, S.G. 1945. Colchicine produced polyploids in Gossypium II. Old world triploid hybrids.

Stringham, G.R. and L.J. Elling. 1966. Tetrasomic inheritance of a lethal character in Medicago sativa. Crop Sci., 6: 587-589.

Tsunewaki, K. 1966. Gene Analysis on chlorosis of the hybrid Triticum aestivum var. Chinese Spring X T. macha var. subletchumicum and its bearing on the genetic basis of necrois and chlorosis. Jpn. J. of Genet., 41: 413-426.

-----, 1971. Distribution of necrosis genes in wheat, Triticum macha, T. spellata, and T. vavilov. Jpn. J. of Genet., 46: 93-101.

Vickery, R.K. 1956. Data on Interracial hybridizations on Mimulus guttatus. Proc. Utah Acad. 33: 37-43.

Wiebe, G.A. 1934. Complementary factors in barley giving a lethal progeny. J. of Hered., 25: 273-274.

# APPENDIX

Figure 10 - Normal Cotton Seedling

A normal cotton seedling at approximately ten days after emergence. Note that both cotyledons are fully extended and the first leaves are beginning to expand.

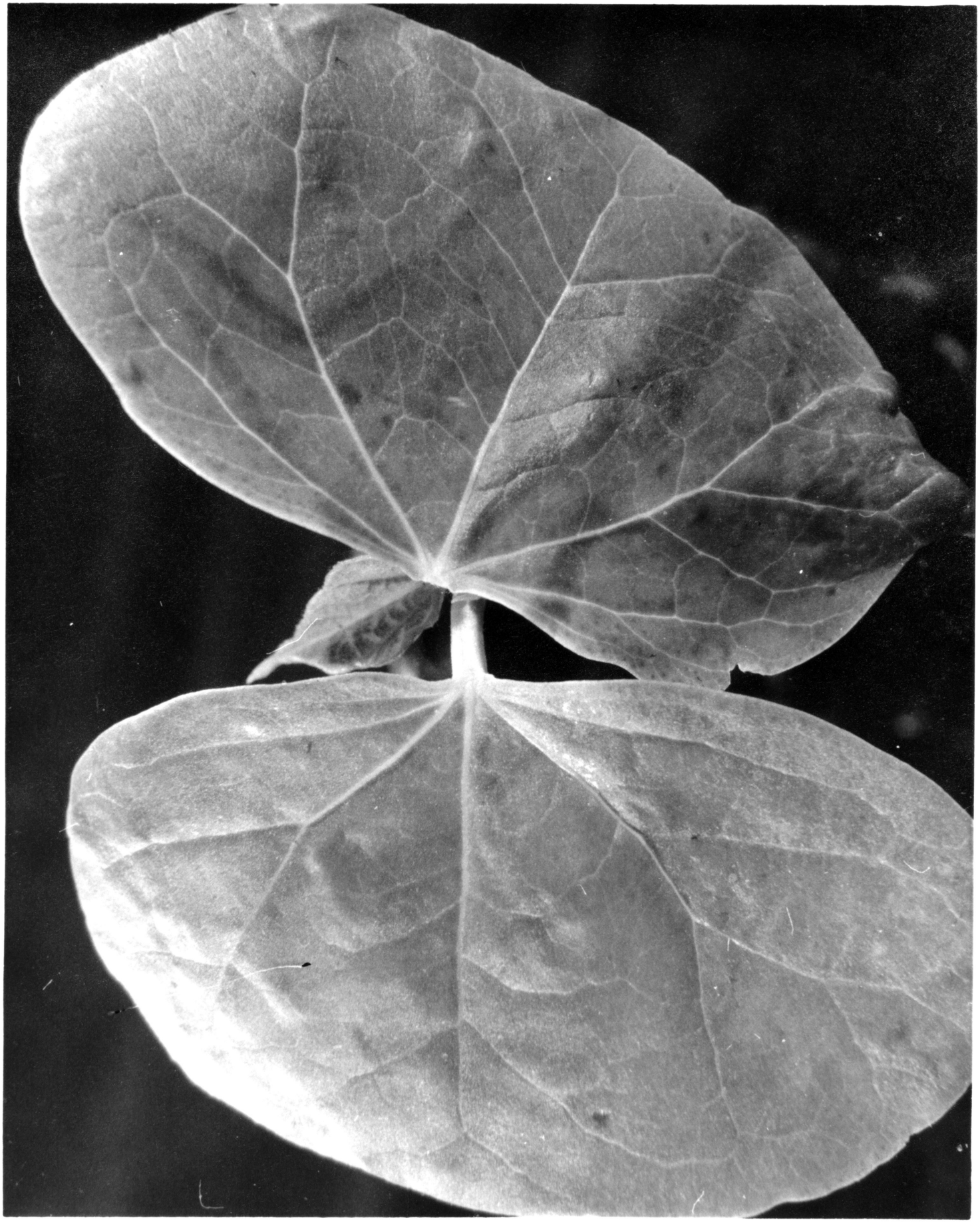


Figure 11 - Initial Stages of Necrosis

The first evidence of impending necrosis for this seedling occurred seven days after emergence. The dark spots on the cotyledons are intial areas of necrosis. Wilting along the edges of the leaves has also initiated. While the time of initial onset of the reaction varied from pre-germination up to ten days post emergence, these symptoms were universal. Growth rate has slowed dramatically.

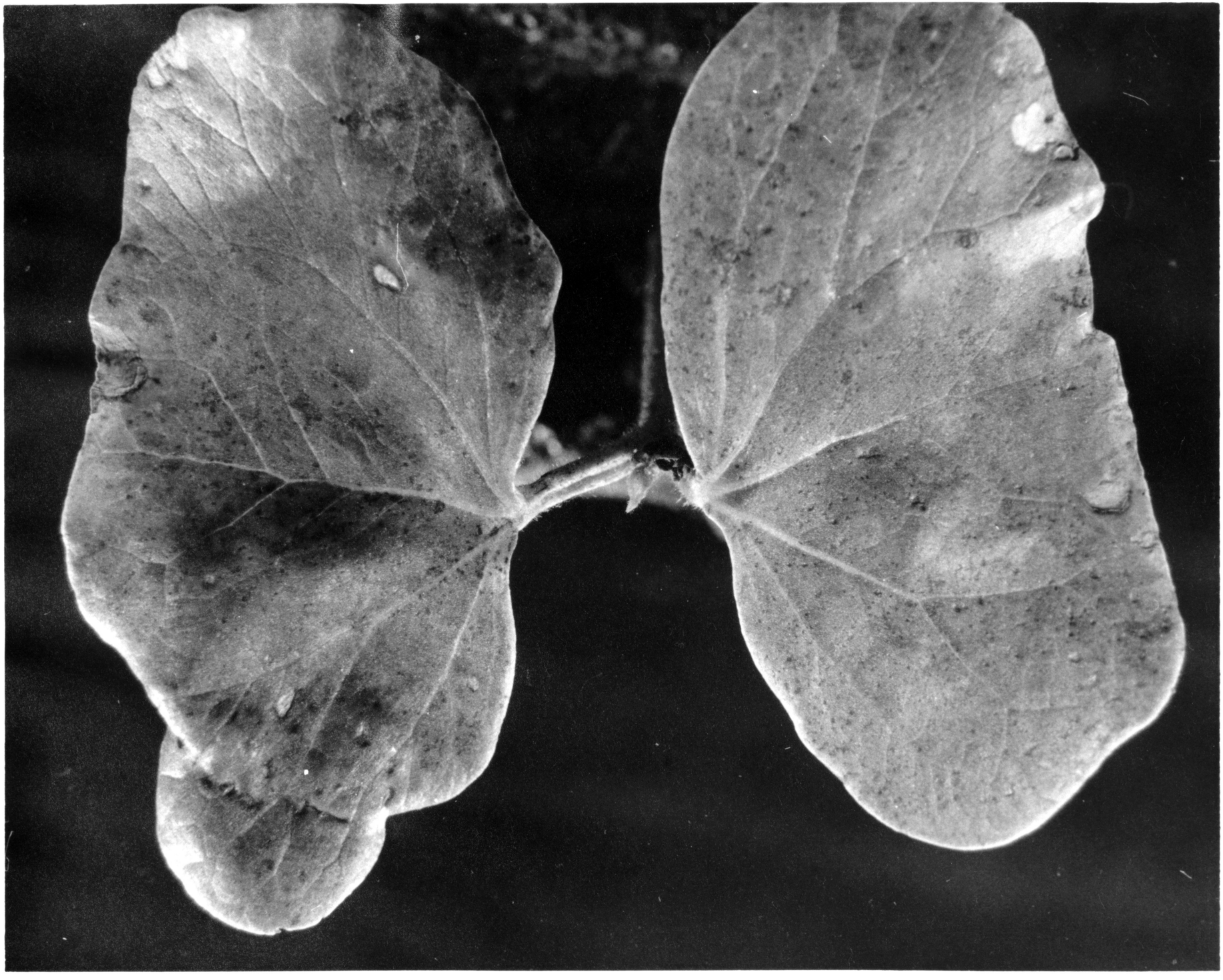


Figure 12 - Progressive Stages of Necrosis

Additional tissue surrounding the initial area of tissue dieback has become necrotic. Necrosis affects almost every area of the leave. Wilting has progressed so that the cotyledons, once smooth and glabrous are now wrinkled, and very irregular. The tissue along the edges of the cotyledons have begun to shred and tear. Growth has stopped completely.

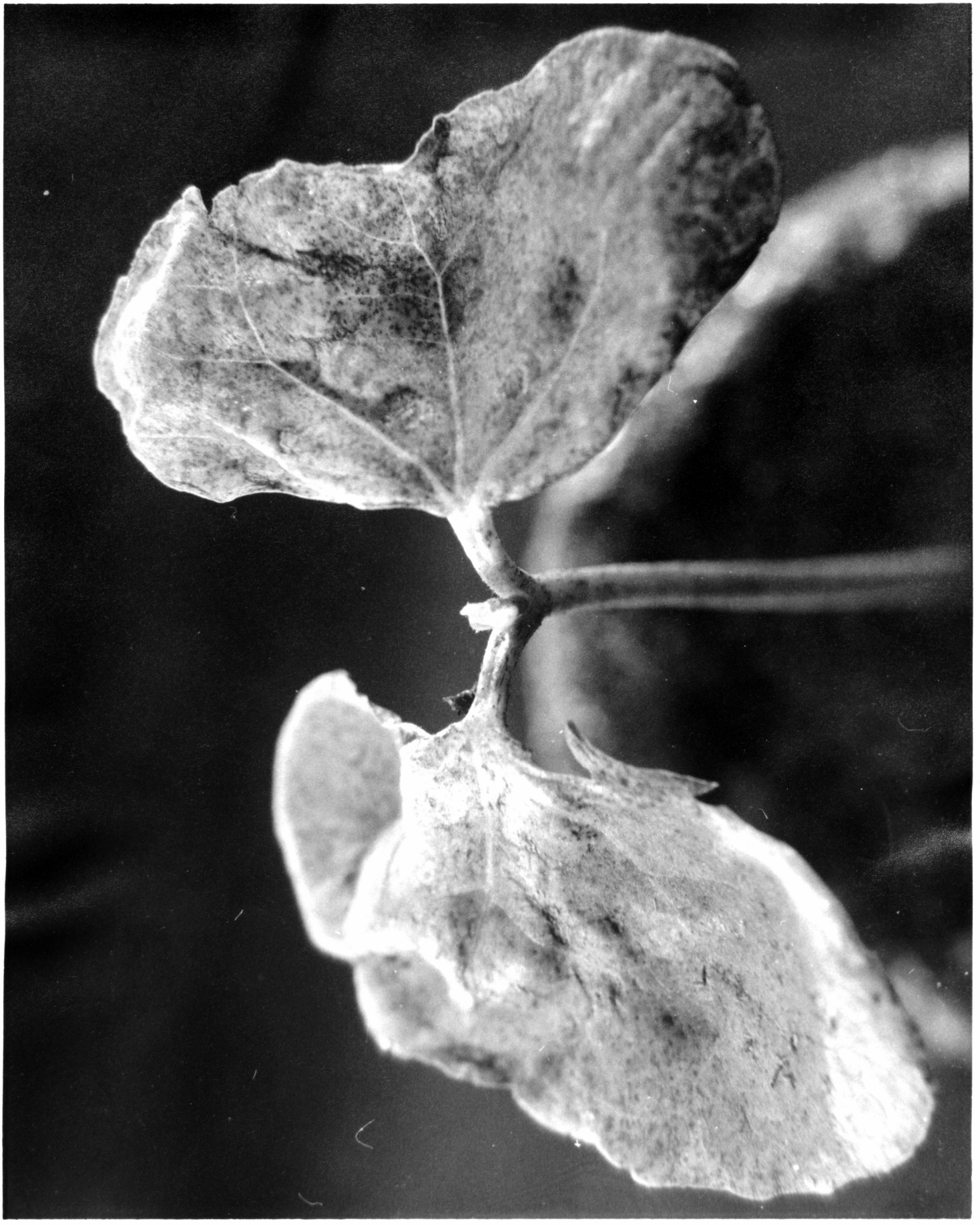


Figure 13 - Final Stages of Necrosis

Most all cotyledonary tissue has become necrotic and moribound and wilting had become severe enough to cause the cotyledons to droop down onto the stem, unable to support themselves. The stem has begun to show signs of lethality and necrotic tissue.

Figure 14 - Death of Seedling

The cotyledons have completely dried out and all cotyledonary tissue is necrotic and moribund. When pressure is applied, it shatters into pieces. The stem shows necrotic regions and wilting as well. The root was not directly affected by the lethality reaction until the seedling had died.

Figure 15 - Symptoms of Hybrid Incompatibility at Germination

A comparison of a normal cotton seed (bottom) and cotton seed showing symptoms of the lethal reaction (top). The dark necrotic regions on the cotyledons are typical evidence of the onset of lethality. The seed coat was removed eight hours after initial soaking.