Diallel analysis of varying late season night temperatures on the development of a range of flue-cured tobacco (*Nicotiana tabacum* L.) genotypes.

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ABSTRACT

A study was conducted in the climate room facilities, at D.S.I.R. Plant Physiology Division, Palmerston North, on the effect of varying late season night temperatures on the development of a range of flue-cured tobacco genotypes.

The study involved imposing three night temperatures, 10°C, 15°C and 20°C, when the plants came into flower. Ten F1 genotypes of a five parent diallel cross (no parents, no reciprocals) were grown at each night temperature with three replications per temperature. Fourteen morphological, physical and chemical characters were measured.

The effect of late season night temperature was negligible but there was some evidence of genotype × environment interaction for some of the characters.

The experiment was conducted using single plants as plots and the statistical analysis showed acceptable coefficients of variation for biological studies.

The genetic analysis of the diallel showed that general combining ability variance is the most important type of genetic variance in the characters examined. This agrees with the majority of other tobacco diallel studies. As general combining ability variance is largely a measure of additive genetic variance, breeding homozygous lines from a heterozygous base population should be the best approach to follow.

Heritability values were of sufficient size for several of the commercially important characters to indicate that improvement through selection was possible.

General combining ability and phenotypic simple correlations between pairs of characters were generally in good agreement, demonstrating that phenotypic selection will result in altering the genotypes in the desired direction for the characters in question. The experiment showed a large negative correlation between the two economically important characters, yield and total nicotine alkaloids. This result is in agreement with similar studies carried out by other workers in this field.

The experiment revealed a number of improvements which could be useful in the conduct of future tobacco climate room studies.

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INTRODUCTION

Of the 65 presently recognised species in the Genus Nicotiana (Smith 1968; Ohashi 1976), only one, N. tabacum L. is of major commercial value. N. tabacum, or tobacco, is grown solely for its leaf, and is the most widely grown non-food plant in the world. It occupies a unique position in that it has considerable influence on the economics, finances and politics of many countries. Upon the discovery of the Americas by the Europeans, N. tabacum was quickly distributed round the world. The species has proved to be an extremely adaptable one and since its world-wide distribution in the 16th Century it has evolved into a number of distinct types, each with distinctive characteristics of commercial importance. In the evolution of N. tabacum to its highly specialized forms of today, artificial selection pressure, cultural and management practices, soils and climate have all influenced the formation of present types. The dominating feature of world tobacco production over the last three decades has been flue-cured tobacco. Flue-cured tobacco is the main constituent used in the cigarette industry. The distinguishing feature of flue-cured tobacco in comparison to other types is that it contains considerable amounts of soluble sugars as mono-and disaccharides. This is the result of artificial heat used during the flue-curing process, which prevents the enzymatic breakdown of sugars once they have been converted from starch.

N. tabacum is of sub-tropical origin (Goodspeed 1954) but has a wide range of temperature tolerance. The temperate climate of the Motueka district in the Nelson Province is one of the most extreme climatic areas of the world in which N. tabacum is grown commercially. The major type of tobacco grown in New Zealand is flue cured. The Motueka Coastal Plain and adjacent valleys produce, from 1750 hectares, approximately half of the country's requirements of leaf for cigarette production, thereby saving valuable overseas exchange. Each area of the world produces flue-cured tobacco with its own particular characteristics. The distinguishing features of New Zealand flue cured tobacco are its relatively high content of reducing sugars and large weight per unit area, in comparison with leaf produced in other areas of the world. Blick (1943) examined certain grades of USA and New Zealand leaf for various physical and chemical characters. He found that N.Z. leaf was heavier and had a greater specific leaf weight than corresponding USA grades. James (1973) reported a three year study on the chemical composition of New Zealand flue-cured tobacco. Sugar levels were relatively high by world standards. Another survey of New Zealand commercial tobacco has revealed similar trends (Rohrbach, pers. comm.).

The growing season in New Zealand is noted for its long hours of sunshine and short cool nights (Weybrew and Woltz 1975); a combination of its latitude and temperate climate. As the harvesting season progresses both day and night temperatures decline. It has been postulated that the long days (more photosynthesis) and cool nights (less respiration) may be part of the reason for the lemon coloured, high sugar, heavy bodied tobacco produced in New Zealand.

The aim of the thesis study was to examine the effect of one environmental factor, late season night temperatures, on the development of a range of flue-cured tobacco genotypes. The controlled environment facilities of Plant Physiology Division, DSIR, Palmerston North were used for the study which included a study of inheritance, heritability, and genotype x temperature interaction of the various characters measured. Recommendations on conducting future climate room tobacco studies as a result of findings from this first experimental work, was also an aim.

CHAPTER 1

LITERATURE REVIEW

1:1 CONTROLLED ENVIRONMENT STUDIES IN TOBACCO

Early studies on temperature effects on tobacco growth and development were carried out by Camus and Went (1952) and Coolhaas (1955). Camus and Went concluded from their study of 3 Cigar and Turkish varieties, that night temperature is the most critical factor influencing developmental processes of tobacco.

Pearse (1960a, 1960b, 1961a, 1961b, 1962) conducted a series of glasshouse experiments on flue-cured tobacco. He (1961a) found that low night temperature increased the weight per unit area of leaves and also increased their sugar levels. Pearse suggested that low night temperatures could be expected to result in a saving of carbohydrate due to a decrease in respiration at night.

Geisler (1966), in a phytotron study, examined the effects of day and night temperatures on 3 flue-cured tobacco cultivars. Geisler concluded that the optimum combination of temperature for the study was 28°C day and 22°C night. Night temperature had a marked effect on dry matter production. Lowering night temperatures from the optimum resulted in lower yields. However the drop in night temperature did not result in a significant reduction in leaf area. Lower night temperatures resulted in a substantial thickening of leaves.

Hopkinson (1967) in a phytotron study,also examined the effect of night temperature on flue-cured tobacco growth. He was investigating why Australian-produced tobacco crops did not yield very highly in comparison to many other countries. He found that one of the critical determinants of yield in tobacco is the number of leaves developed before flowering initiation. The number of leaves per plant increased with increasing night temperature.

Matsuyama _et.al._ (1972a, 1972b) in a phytotron study concluded that flue-cured tobacco exposed to high temperatures at the early stage of vegetative growth experienced an increase in leaf number and an increase in dry matter at the flowering stage. They found that starch content of leaves exposed to low temperature was higher than high temperature treatment at any stage of development.

An extensive series of experiments have been conducted at the North Carolina State University Phytotron, to establish the conditions necessary to obtain a field phenotype within artificial environments, for flue-cured tobacco (Raper Johnson & Downs 1971; Raper & Johnson 1971;

Raper 1971; Raper & Downs 1973; Raper, Weeks, Downs & Johnson 1973; Raper Smith & Downs 1975; Raper & Thomas 1972; Raper & Smith 1975).

The conditions which appear to be critical for approaching field phenotype in phytotrons for flue-cured tobacco are:

1. Seasonal simulation of temperatures
2. Decreasing availability of nitrogen and potassium
3. Maintenance of CO_2 at ambient levels
4. Humidity Control

Characters of flue-cured tobacco used to investigate field phenotype in phytotrons were leaf area, specific leaf weight, leaf shape (Leaf length:leaf width ratio), distribution and levels of soluble carbohydrates, alkaloids and nitrogen content of leaves at different nodal positions (Raper & Downs 1975).

1:1:1 TEMPERATURE

Constant temperatures in controlled environments fail to reproduce the physical characteristics of field grown plants. Normal leaf size ordering is obtained by the seasonal simulation of temperature (Raper 1971). Early post transplant temperatures are particularly important in determining the final shape of leaves from the upper half of the plant (Raper & Thomas 1972). Low temperatures early after transplanting cause an elongation of the upper leaves characteristic of field grown tobacco. Thomas,Anderson, Raper & Downs (1975) found that fewer leaves are produced at low temperatures than at high temperatures.

Raper (1971) found that seasonal progression of temperature along with progressive reductions of nitrogen and potassium supply are essential for obtaining field phenotypes within phytotrons.

1:1:2 NUTRITION

Normal climate room work involves the periodic application of a complete balanced nutrient solution to the plants. Commercial production of flue-cured tobacco is based on high levels of available nitrogen and potassium during early post transplant growth and then rapid diminuition of nitrogen and potassium as the plant reaches maturity.

Combined with seasonal progression of temperatures, a sequential nutrient application schedule results in the normal leaf size ordering characteristic of field grown tobacco (Raper 1971). Sequential nutrient application enables the harvesting of mature leaf with characteristic levels of soluble carbohydrates, alkaloids and nitrogen (Raper 1971; Raper & Johnson 1971).

1:1:3 CARBON DIOXIDE

Raper and Downs (1973) and Raper, Weeks, Downs & Johnson (1973) conclude that self induced CO_2 depletion by growing plants within climate rooms results in several abnormalities of tobacco. These include a low concentration of reducing sugars in cured leaves, a shortening of internodes, and epinasty of leaves during maturation. Raper & Downs (1975) conclude that the maintenance of CO_2 supply at ambient levels in climate rooms is essential in preventing the effects of self induced CO_2 depletion by the growing plants.

1:1:4 HUMIDITY

Raper and Smith (1975) found that the effects of humidity on leaf shape and size were dependent on the age of the plants. They found that humidity and nitrogen nutrition have a close relationship during the growth of the tobacco plant. Low relative humidities in early post-transplant growth ensure adequate tissue levels of N during leaf formation. High humidity levels during later stages of plant growth promote a greater efficiency of dry matter production and greater production of starch and alkaloids (Raper & Smith 1975).

Raper & Downs (1975) conclude from the series of experiments at North Carolina that field phenotype of flue-cured tobacco can be approached in climate rooms.

1:2 QUANTITATIVE GENETICS AND PLANT BREEDING

Quantitative genetics deals with the inheritance of those differences among individuals that are expressed in terms of degree rather than kind (Moll and Stuber 1974). In the study of quantitative inheritance, plant breeders conduct genetic variability experiments to obtain estimates of the magnitude and type of genetic variation, to determine the magnitude of the genotype x environment interaction variance, and to evaluate the relationship among various characters. These estimates are obtained from measurements on the phenotype.

1:2:1 ANALYSIS OF VARIANCE

The analysis of variance is defined by Fisher (1948) as "the separation of the variance ascribable to one group of causes, from the variance ascribable to other groups".

The variability due to components of a model is measured in terms of sums of squares of deviations about the general mean. The residual variability, after the removal of all defined sources of variation, is termed the residual error.

The application of the analysis of variance to experimental data is appropriate only when the data conform to the basic assumptions pertinent to the development of the statistical technique. Failure to fulfill the assumptions will affect the significance levels as well as the sensitivity tests - F-test and the t-test. Four assumptions are considered essential for the analysis of variance (Cochran, 1947; Eisenhart, 1947).

1. Independence of Experimental Errors

The observations that comprise the treatment from which the error variance is estimated, should be independently distributed. If they are not independent the estimates of variance components may be biased and consequently impair tests of significance. Independence of experimental errors assumption is reasonably fulfilled by the assignment of treatments at random to the experimental units.

2. Normal Distribution of Experimental Errors

It is necessary that the samples from which the error variance is derived are from normally distributed populations. Non-normality affects the efficiency of the F-test because it leads to the acceptance of too many results as significant. Eden & Yates (1933) found that the F-test as used in the analysis of variance is affected very little by departures from normality encountered in experimental work. Transformations such as logarithm, square root, arcsin, can be used to rectify non-normal distributions (Bartlett, 1947).

3. Homogeneity of Experimental Errors

The third assumption requires that the treatments have the same variances, although the treatment means may differ. That is, the components of error contributed by several treatments must all be estimates of a common population variance. To fulfill this assumption it is necessary to examine the error variances for homogeneity. This can be done by Bartlett's (1937) chi square test.

4. Additivity of Variances

It is necessary in the analysis of variance that the treatment and environmental effects are additive. That is in a randomised complete block experiment treatment and replication effects are independent and do not interact, or in other words treatment effects bear the same relation to one another from replication to replication.

Tukey (1949) has developed a test to determine non-additivity of data and he also presents information on the use of transformations to restore additivity to data. Tukeys test for non-additivity involves the isolation of a single degree of freedom associated with non-additivity.

The method serves to:

(a) determine whether the transformation of data would make them amenable to the analysis of variance.

(b) indicate whether one or more observations are discrepant.

(c) indicate whether the effects of environment and treatments are additive.

(d) when not additive, to indicate a suitable transformation to restore additivity to the data.

Non-additivity will result in the inflation of the error term with a consequent reduction on the level of significance of the treatments.

In summary then, the analysis of variance depends on the assumptions that (i) the treatment and environmental effects are additive, and (ii) that the experimental errors are random, independently and normally distributed about zero mean and with a common variance. In practice, one can never be certain that all of the above assumptions are fulfilled with a particular set of data. The consequences of failures in the assumptions and the remedial procedures in cases of non-conformance to these assumptions have been summarized by Eisenhart (1947), Cochran (1947), and Bartlett (1947). It is generally agreed that for most types of biological data the disturbances resulting from the failure of the data to fulfill the above requirements are not sufficiently great to invalidate the technique (Cochran & Cox 1957; Steel and Torrie 1960). The procedures for testing significance levels and confidence limits must be considered approximate rather than exact (Cochran 1947).

1:2:2 PARTITIONING GENETIC VARIANCE

Measurements made on the phenotype can be partitioned into genetic and non genetic components. The genotypic variance component can be further partitioned into additive dominance and epistatic components, as demonstrated by Fisher (1918). It was not until much later that experimental mating designs were worked out to obtain estimates of these genetic components. (Comstock & Robinson 1948, 1952; Mather 1949; Yates 1947; Sprague & Tatum 1942; Rojas & Sprague 1952; Hayman 1954,1958; Jinks 1954; Matzinger & Kempthorne 1956; Matzinger & Cockerham 1963; Cockerham 1963; and Griffing 1956). The several mating designs result in different types of relatives being developed from which the genotype partitioning is effected. The estimation of genetic parameters is obtained by the analysis of variance or regression techniques applied to data collected on various combinations of parents and their progenies. The components of variance and covariance and parent-offspring regression coefficients have been estimated and interpreted in view of their genetic expectations based on the particular genetic model assumed (Cockerham 1954, 1963; Anderson & Kempthorne 1954; Kempthorne 1957; Falconer 1960; Hayman & Mather 1955). The estimation of genetic parameters in relation to the present work is presented later on in the review.

The very widely reported experimental design to obtain estimates of genetic parameters is the diallel. The diallel was first described by Yates (1947) and was developed further by Hayman (1954) and Jinks (1954) and Griffing (1956). Of particular relevance to this study is the combining ability diallel of Griffing (1956).

The analysis of data from the various mating designs has a set of genetic assumptions:

1. homozygous parents,
2. diploid segregation,
3. no reciprocal differences,
4. no epistasis,
5. no multiple alleles,
6. uncorrelated gene distributions.

Assumptions 1 and 3 can be either tested for or justifiably assumed. Assumption 6 can rarely be satisfied in a practical plant breeding programme since the parents selected do not represent a random population sample. However, if the population of inference is redefined to be a "random sample of all selected genotypes" then assumption 6 is satisfied. Assumptions 4 and 5 are difficult to ascertain in a given set of parents (Williams 1964). Crumpacker & Allard (1962) conclude that the partial failure of one or more of the assumptions was unlikely to lead to serious bias in the analysis of their experiment. Matzinger, Mann & Robinson (1960) using a design estimating additive x additive epistatic component of genetic variance in a tobacco population conclude that this type of epistasis did not alter the relative importance of additive and dominance variance estimates to any great extent. Other results on tobacco (Matzinger Mann & Cockerham 1966; Matzinger 1968; Legg & Collins 1971 a, 1971 b, 1975; Matzinger, Wernsman & Cockerham 1972) have shown a similar trend, with the additive x additive epistatic variance component of minor importance only. Povilaitis (1964) using Hayman's generation means analysis concluded that epistatic effects are of minor importance in the tobacco population he studied.

A diallel crossing system can be defined as a set of p parent genotypes crossed amongst themselves in all possible combinations (p^2). The diallel design then is a special case of the factorial mating design of Comstock & Robinson (1948) (also known as the North Carolina design II). The p^2 crosses can be divided into three portions:

1. p parents.

2. $\frac{p\ (p - 1)}{2}$ F1's and

3. $\frac{p\ (p - 1)}{2}$ reciprocal F1's

Griffing (1956) has presented models for the four possible diallel combinations:

1. All possible combinations (p^2 genotypes),

2. Parents and one set of F1's ($\frac{p\ (p + 1)}{2}$ genotypes) ,

3. Both sets of F1's ($p\ (p - 1)$ genotypes),

4. One set of F1's only ($\frac{p\ (p - 1)}{2}$ genotypes).

Hayman (1954) and Jinks (1954) proposed an analysis of diallel cross data based on estimates of variances and covariances of a sample of parents and their F1's. The analysis can also be used to construct graphs that can be interpreted in terms of the dominance and epistasis of the character under consideration (Hayman 1960). The graph is called the "VrWr" graph which is obtained by plotting the regression of the array covariances (Wr) on the array variances (Vr). The "VrWr" graph shows:
(i) the type of dominance present
(11) the proportion of dominant and recessive genes in each parent
(iii) the presence of epistasis.

Sprague and Tatum (1942) originally defined the terms general and specific combining ability. General combining ability is the "average performance of a line in hybrid combination". Specific combining ability is defined as the "deviations from expectation of a hybrid on the basis of the average performance of its parents". Griffing (1956) used the combining ability concept to provide an alternative method of analysis of diallels to that of Hayman-Jinks.

Estimates of general combining ability and specific combining ability variances can be related to covariances among relatives (Griffing 1956).

$$\sigma^2_g = \text{cov (HS)}$$

$$\sigma^2_s = \text{cov (FS)} - 2 \text{ cov (HS)},$$

where, from Kempthorne (1955):

$$\text{cov FS} = \frac{1 + F}{2} \sigma^2_A + \frac{(1 + F)^2}{2} \sigma^2_D + \frac{(1 + F)^2}{2} \sigma^2_{AA} + \frac{(1 + F)^3}{2} \sigma^2_{AD} + \frac{(1 + F)^4}{2} \sigma^2_{DD} + \ldots\ldots\ldots\ldots$$

and

$$\text{cov HS} = \frac{(1 + F)}{4} \sigma^2_A + \frac{(1 + F)^2}{4} \sigma^2_{AA} + \ldots\ldots\ldots\ldots$$

where σ^2_g is general combining ability variance estimate, σ^2_s is specific combining ability variance estimate, cov (FS) is full sib covariance, cov (HS) is half sib covariance, F is inbreeding coefficient, σ^2_A is additive genetic variance, σ^2_D dominance genetic variance σ^2_{AA} σ^2_{AD} and σ^2_{DD} are apistatic variances. Assuming no epistasis and an inbreeding coefficient of 1, $\sigma^2_A = 2 \sigma^2_g$ and $\sigma^2_D = \sigma^2_s$.

When $F = 0$, $\sigma^2_A = 4 \sigma^2_g$ and $\sigma^2_D = 4 \sigma^2_s$

In the literature, σ^2_A and σ^2_D are not usually computed as the F coefficient of the parents is not usually known. The genetic variances obtained from diallels can be used to find various heritability ratios. Diallels are often conducted over more than one environment, to obtain information on the importance of genotype x environment variance, and to enable estimates of genetic variance free from confounding to be calculated.

Diallels have been widely used in tobacco breeding studies (Matzinger, Mann & Cockerham 1962, Legg, Collins & Litten 1970; Vandenberg & Matzinger 1970; Chaplin 1966, 1967; Gwynn 1966; Povilaitis 1966, 1970a,1971; Lamprecht 1964, 1967, 1969, 1973; Lamprecht and Nuss 1973; Lamprecht and Van Wyk 1969, 1971; Wright 1968; Jones, Gupton & Terrill 1972; Matzinger, Wernsman & Ross 1971; Aycock 1972; Dean 1974; Gopinath, Ramanarao, Subrahmanyam & Narayama 1966; Gritton, Jones, Powell, & Matzinger 1965; Luthra 1964; Murty 1965; Fan & Aycock 1974; Marani & Sachs 1966).

Almost without exception, the diallel studies on tobacco have shown a predominance of additive genetic variance. Dominance variance has been of minor importance. Other mating designs used in tobacco studies - hierarchical mating design (North Carolina design I) (Robinson, Mann & Comstock 1954), Hayman's (1958) generation means analysis (Povilaitis 1964) and the combination design of Matzinger & Cockerham (1963) (Matzinger, Mann & Robinson 1960; Legg & Collins 1971 a, 1971 b, 1974, 1975; Matzinger 1968; Matzinger, Mann & Cockerham 1966; Matzinger, Wernsmann & Cockerham 1972) - have also shown a predominance of additive genetic variance over other forms of genetic variance.

In tobacco then, the best breeding procedure to use is one in which the gene frequencies of those genes acting additively for the character in question, are increased. This is achieved by inbreeding the lines from a heterozygous population for the character/s desired.

1:2:3 GENOTYPE X ENVIRONMENT INTERACTION

When the magnitude and possibly the nature of genotypic estimates obtained from phenotypic observations are not constant and fluctuate from one environment to another, genotype x environment (GE) interaction is said to be occurring. That is, genetic effects are not independent of the non genetic environmental effects. Statistical models define the GE interaction as a deviation from the expectations of genotype and environment (Sprague & Federer, 1951).

Trials conducted at a single environment may have GE interactions confounding comparisons between genotype effects. For this reason, field experiments are conducted over more than one environment in order to partition out GE effects. Sprague & Federer (1951) and Comstock & Moll (1963) have been chiefly responsible for the procedures used in the analysis of variance to investigate GE interactions.

Partitioning of variances associated with such interactions then, allows for more precise overall partitioning and therefore more precise interpretation of experiments.

Matzinger and Kempthorne (1956) outlined the procedure for analysing combining ability diallels over more than one environment following the procedures outlined by Rojas & Sprague (1952). In combined analyses a complex F test as outlined by Cochran & Cox (1955) is used where more than two mean squares are required to estimate the variance ratio. That is, the variance ratio is estimated by linear combinations of mean squares. Satterthwaite (1946) has presented the formula for calculating the degrees of freedom for the complex F test.

$$f' = \frac{[\ \Sigma\ a_n\ E(MS_n)]^2}{\Sigma\ \dfrac{a_n\ E(MS_n)^2}{f_n}}$$

where n = mean square index in numerator or denominator of the F test, a_n = constant = 1 (in the present case), and fn = degrees of freedom for the appropriate mean square (MS). Complex degrees of freedom are calculated for the numerator and denominator of the complex F test.

Due to their obvious importance to plant work, GE interactions have received considerable attention in the literature. Extensive reviews are available on the subject of GE interactions. (Comstock & Moll, 1963; Allard & Bradshaw 1964; Breese 1972; Knight 1970; Freeman 1973; Moll & Stuber 1974). Early reviews of GE interactions of Comstock & Moll (1963) and Allard & Bradshaw (1964) endeavoured to clarify the ways in which GE interactions are involved in quantitative genetics and plant breeding. Allard & Bradshaw state that consideration of genotypes and environments separately may provide the only reasonable means of gaining an insight into the nature and significance of GE interactions. They defined two types of buffering achieved by varieties; individual buffering, where the individuals of a variety are well buffered so that each member of the population is well adapted to a range of environments, and population buffering, where the variety can be made up of a number of genotypes each adapted to a somewhat different range of environments. Comstock and Moll (1963) reviewed the statistical approach of the analysis of variance of pooled experiments. Freeman (1973) and Moll & Stuber (1974) reviewed the use of regression methods in studying GE interactions. Yates & Cochran 1938, Finlay & Wilkinson

1963, Rowe & Andrew 1964, Eberhart & Russell 1966, Perkins & Jinks 1968, Johnson, Schafer & Schmidt 1968, Breese 1969, Baker 1969 and Freeman & Perkins 1971 have observed that the relation between performance of different genotypes in various environments and some measure of these environments is often linear or nearly so (Moll & Stuber 1974). Breese (1972), Moll & Stuber (1974) and Freeman (1973) emphasise the importance of the discovery of this linear relationship (between the differing responses of genotypes to environmental change) in the understanding of GE interaction to assist in plant breeding programmes.

Matzinger (1963) reviewed GE interaction estimates for some important self pollinating species. Matzinger concluded that highly significant second order interactions were common in the species examined (cotton, soybean & tobacco). Genotype x environment interaction studies on tobacco cultivar trials have been reported by Jones Matzinger & Collins (1960), Collins, Jones, Weybrew & Matzinger (1961), Povilaitis (1970b) and Gupton, Legg, Link & Ross (1974).

It can be concluded from these studies that although second order interactions were significant, they were small in comparison to the main genetic variance component. Jones _et_. _al_. (1960) Collins _et_. _al_. (1961) and Povilaitis (1970b) found little evidence of significant first order interactions with flue-cured tobacco. Gupton _et_. _al_. (1974) in a burley tobacco study found that four of the sixteen traits measured had significant cultivar x locations interations and one had significant cultivar x years interaction. They concluded that homozygous burley genotypes show little interaction with environment for one of the main characters, yield; while hybrid lines x locations interaction was significant.

Diallel studies of interactions between genotype and environment have not shown a consistent trend. Matzinger, Mann & Cockerham (1962) concluded there was a lack of GE interaction in their study on flue-cured tobacco. Gwynn (1966) found that gca x location component of variance was significant for all characters measured, but that the component was much smaller than the gca variance component. Legg, Collins & Litton (1970) found that in burley tobacco gca x years component was significant for six of the seven characters examined. Matzinger Wernsman & Ross (1971) found gca x years variance component to be significant for five of the seven characters measured while sca x years was significant for two. Fan and Aycock (1974), using Maryland cultivars as diallel parents, found that genetic x location effects were not significant for any character measured.

Conclusions that can be drawn from tobacco studies on GE interaction are that GE interactions are usually many times smaller than the main genetic component. The smallness of GE interactions compared with the main genetic component indicates considerable genetic stability of N. tabacum or little difference between locations or years. Jones et. al. (1960) and Collins et. al. (1961) conclude that valid information on tobacco cultivar measurements may be obtained from experiments conducted over fewer environments (sites and/or years) with their studies. However such conclusions must be drawn with caution as the amount of GE interaction variance estimated will depend on the broadness of the sampling. That is, the range of sites, years, genotypes and the type of characters to be examined.

1:2:4 HERITABILITY AND EXPECTED GENETIC ADVANCE

Heritability is defined as the portion of the observed phenotypic variance for which variability in heredity is responsible (Knight 1948). Expected genetic advance is a measure of the expected genetic gain from selecting a portion of a population (Falconer 1960; Allard 1960; Moll and Stuber 1974; Hanson 1963).

Heritability ratios take on many forms depending on the composition of the numerator and the denominator. The numerator can be altered to give estimates of broad sense heritability and narrow sense heritability (total genetic variance and additive genetic variance respectively). The denominator represents the phenotypic variation and can be split up in various ways. If all components of phenotypic variation are included in the denominator it may be called "full" heritability, while if any components are excluded from the denominator it may be called "restricted" heritability (Gordon, Byth & Balaam 1972).

Hanson (1963) discusses the need for a standard concept of heritability in plant breeding studies. The most common heritability estimate referred to in the literature, is narrow sense heritability (with restricted phenotype definition) where the individual plant is taken as the reference unit:-

$$h_N^{2\,\prime} = \frac{\sigma_A^2}{\sigma_G^2 + \sigma_{GE}^2 + \sigma^2} \quad \ldots\ldots\ldots\ldots \quad (1{:}2{:}3)$$

Hanson (1963) states that because plant work is frequently based on the total expression of individuals with a plot replicated within one or more environments, heritabilities should be defined as"the fraction of phenotypic variability for a defined reference unit expected to be transmitted to the progeny". That is, heritability for family means of r replications within e environments would be:-

$$\bar{h}_N^{2\,\prime} = \frac{\sigma_A^2}{\sigma_G^2 + \sigma_{GE/e}^2 + \sigma^2/_{re}} \qquad \ldots\ldots\ldots\ldots(1:2:4)$$

Expected genetic advance is commonly used in plant breeding programmes to compare the estimated genetic gains for various selection methods or comparing expected against actual genetic progress. (Hanson <u>et</u>. <u>al</u>. 1956; Moll & Robinson 1966; Matzinger & Wernsman 1968 a; Matzinger, Wernsman & Cockerham 1972; Moll <u>et</u>. <u>al</u>. 1975; Asay <u>et</u>. <u>al</u>. 1968; Frey 1968; Lebsock & Amaya 1969; Hill <u>et</u>. <u>al</u>. 1971; Empig <u>et</u>. <u>al</u>. 1970; Nasr <u>et</u>. <u>al</u>. 1972; Bhatt 1972; Legg <u>et</u>. <u>al</u>. 1965; Shelbourne 1969).

Expected genetic advance, ΔG, can be expressed in general terms as:-

$$\Delta G = i\,\sigma_P h^2$$

where i is the standardised selection differential, σ_P is the phenotypic standard deviation, and h^2 is the heritability estimate for the particular selection system. The heritability can be written as:

$$k\,\frac{\sigma_A^2}{\sigma_P^2}$$

where k is the fraction of the total additive genetic variance in the covariance of additive values for the particular relatives in question. For example k = 0.25 for selection amongst half-sib families (Falconer, 1960). Expected genetic advance formula for different selection procedures have been presented by Shelbourne (1969); Empig <u>et</u>. <u>al</u>. (1971); Sprague (1966); Legg <u>et</u>. <u>al</u>.(1965); and Falconer (1960).

Provided the distribution of phenotypic values is normal, the standardised selection differential, i, (also known as selection intensity) is:

$$\frac{S}{\sigma_P} = i = \frac{z}{p} \;,$$

from the mathematical properties of the normal distribution where S is selection differential = (mean of the selected population) - (mean of the base population), σ_P is the phenotypic standard deviation, z is the height of the ordinate at the point of truncation, and p is the proportion selected.

Therefore by standardising our selection response in terms of i, and assuming the phenotypic values of the character measured have a normal distribution within the base population, then i depends only on the proportion of the population in the selected group. Thus given only the proportion selected, p, we can find by how many standard deviations the mean of the selected individuals will exceed the mean of the population before selection; that is, the "i value" (Falconer 1960).

The values for i have been presented by Fisher & Yates (1953), Falconer (1960), Becker (1975), and Shelbourne (1969).

Expected genetic advance has been used in tobacco studies by Legg et. al. (1965) and Matzinger & Wernsman (1970). Matzinger and Wernsman (1968) found good agreement between predicted and actual gain over four cycles of mass selection for increased yield. However, comparisons between expected and observed responses for correlated characters have shown less consistency than the direct responses discussed above. For example, although Matzinger & Wernsman (1968 a) reported fairly good average agreement between observed and expected correlated responses, they were not linear over selection cycles. In another experiment Matzinger, Wernsman & Cockerham (1972), examing selection for increased alkaloids found that observed and expected correlated responses were in generally poor agreement, although yield did increase as expected. This indicates that the genetic correlation estimates between alkaloids and other characters were not very reliable.

1:2:5 SELECTION INDICIES

In practical plant breeding programmes, the objectives frequently require modification of several characters during the programme. Methods devised to select for multiple characters must take into account the correlations between the characters. Selection indicies are one of three ways of selecting for multiple characters, the other two being tandem selection and independent culling levels.

A selection index is used when a breeder simultaneously selects for more than one character. Smith (1936) and Hazel (1943) illustrate the procedure for constructing a selection index based on the economic weights and genetic parameters of the characters measured. The following information is required to construct an index:

(1) genotypic and phenotypic variances for each character considered in the index;

(2) genotypic and phenotypic covariances (or correlations) between each pair of characters;

(3) economic weights for each character.

Briefly, the aggregate genotypic value of the index is:

$$H = a_1 G_1 + a_1 G_2 + \ldots\ldots\ldots\ldots a_n G_n$$

where the a's refer to the relative economic values, the G's are the genotypic values of the character, and $i = 1, 2, \ldots\ldots n$ characters.

Normal simultaneous equations are used to obtain the b_i's which are the partial regression coefficients needed to construct a selection index, I.

$$I = b_1 X_2 + b_2 X_2 + \ldots\ldots\ldots\ldots X_n$$

where $X_1; X_2 \ldots\ldots\ldots X_n$ are the phenotypic values of the characters 1, 2, ...n; and $b_1, b_2, \ldots\ldots\ldots b_n$ are the coefficients for each character.

Usually more than two characters make up an index and the normal simultaneous equations used to solve for b can be represented by matrices. These matrices can be symbolized as:

$$P \underline{b} = G \underline{a}$$

where P is the variance - covariance matrix of phenotypic values, G is the variance covariance matrix of genotypic values, $\underline{b}$ is the vector of partial regression coefficients of the X's in the index,

$$I = \underline{b} X$$

and $\underline{a}$ is the vector of relative economic values.

By inverting the phenotypic matrix, the b's can be computed by

$$\underline{b} = P^{-1} G \underline{a} .$$

Hazel and Lush (1942) and Young (1961) have contrasted the genetic gain from the three selection methods for multiple characters and all conclude that the selection index has the greatest efficiency. However, for extensive use of selection indicies, computer facilities are required, and considerable note taking in the field is also needed. Consequently tandem selection and independent culling levels have been used as they require far less resources.

CHAPTER 2

MATERIALS AND METHODS

The thesis study was conducted in the climate rooms at Plant Physiology Division, DSIR, Palmerston North.

2:1 GENETIC MATERIAL

A five parent diallel cross, without parents or reciprocals, was used in the experiment. The five parents used represent a wide range of leaf type, chemical composition and country of origin.

All five parents have been tested in variety trials in New Zealand; three of the parents are grown commercially in New Zealand.

2:1:1 DESCRIPTION OF PARENTS:

Bright Yellow 103

Bright Yellow 103 is a Japanese flue-cured line, (Ohashi, Murai & Fujita, 1968) introduced into New Zealand in 1970 and possesses narrow leaf, high yields and good quality cured leaf and above average levels of reducing sugars. Bright Yellow 103 is not grown commercially in New Zealand.

Burley 1

Burley 1 is a United States air-cured cultivar (Heggestad & Clayton 1951) released in the 1950's because of its resistance to black root rot (Thielaviopsis basicola). Burley 1 is a moderate yielding cultivar, producing light coloured cigarette grades of leaf. Burley 1 is grown commercially in New Zealand.

Hicks

Hicks is a United States cultivar selected from an "old line" cultivar (Jones & Mann 1958). Hicks is probably the most widely grown flue-cured cultivar in the world, although lack of disease resistance is tending to limit its use today. Although its exact selection is not known, it seems likely that the original selection was heterogeneous. This has allowed tobacco plant breeders around the world to select amongst Hicks populations for locally adapted strains (Gordon & Byth, 1972). Hicks is one of the major commercially grown cultivars in New Zealand (Beatson 1976), producing moderate yields and good quality flue-cured leaf under New Zealand conditions. It is a medium leaf type, producing moderate nicotine and sugar levels.

Kutsaga 51

Kutsaga 51, a Rhodesian flue-cured cultivar (Raeber 1962) released in that country in the 1960's for its good quality and high yield, is a widely grown commercial cultivar in New Zealand (Beatson 1976) having good yield, good quality leaf, with moderate levels of nicotine and sugars. It is a medium leaf type cultivar and tends to be a late maturing line.

Virginia 815

Virginia 815 is a United States flue-cured cultivar, (Jones & Collins 1959) which is not grown commercially in New Zealand. It is a broad leaf cultivar producing moderate yields and moderate quality lemon grades of cured leaf. Virginia 815 leaf is low in nicotine and sugars, and produces a fast maturing leaf.

2:1:2 DIALLEL MATING DESIGN

A five parent diallel, containing only one set of F1's - Method 4 of Griffing(1956)- was used.

The crosses and genotype codes were as follows:

Code	(Female)		(Male)
12	Burley 1	x	Kutsaga 51
13	Burley 1	x	Hicks
14	Burley 1	x	Bright Yellow 103
15	Burley 1	x	Virginia 815
23	Kutsaga 51	x	Hicks
24	Kutsaga 51	x	Bright Yellow 103
25	Kutsaga 51	x	Virginia 815
34	Hicks	x	Bright Yellow 103
35	Hicks	x	Virginia 815
45	Bright Yellow 103	x	Virginia 815

2:2 TRIAL DESIGN

Three climate rooms were used, each room representing a separate environment. In addition to the ten F1 hybrids of the diallel, two parents, Hicks and Virginia 815, were used as controls.

A randomized complete block design with three replications per room was employed. Each plot was represented by a single plant, giving a total of thirty-six plants per room.

2:3 HANDLING THE PLANT MATERIAL

Seed of each cross was sown in flats in a glasshouse. The seedlings germinated well. The seedlings were watered daily and nutrient was applied once a week from week two following germination, and once every alternate day from the eighth week. Composition of the seedling nutrient solution is given in TABLE 2:1. Seedling flats were saturated with the nutrient solution. After six weeks, seedlings from each F1 were selected for evenness and transplanted into new flats. The final selection of plants for the experiment were taken from these seedlings.

The climate room phase was commenced at ten weeks from germination. Two plants were transplanted per pot and after three weeks this was reduced to one plant per plot. This ensured (1) no missing plots and (2) that each genotype was represented by relatively even plants.

2:4 CLIMATE ROOM CONDITIONS:

2:4:1 BIOLOGICAL:

Drums (40cm x 60cm) were used as containers, their large size ensuring adequate root growth. The potting mix used was comprised of 70% gravel: 15% peat: 15% vermiculite, this being a standard mix used in a wide range of biological experiments, devised by North Carolina State University.

A 'sequential' nutrient solution was used on the trial (Raper 1974), as flue-cured tobacco requires a declining N and K schedule to obtain ripe leaf. Six solutions were used during the trial, supplying high levels of N and K in the early stages of growth and eventually no N and K during the later stages of the experiment. The compositions of the six sequential nutrient solutions used are presented in TABLE 2:2. Also included in TABLE 2:2 is the schedule of dates when the six nutrient solutions were applied.

Each plot received 500×10^{-6} m^3 of solution per day, with the solutions being mixed daily from stocks. Appendix I shows the concentrations of stock chemicals and amounts used per 60×10^{-3} m^3 per day.

Watering was commenced on Day 31. Each pot received 400 mls water daily, five hours before the light period commenced.

Flushing the pots to remove excess ions from the potting mix was done weekly, with two $\times 10^{-3}$ m^3 of water per pot.

The plants were topped when the first flower opened. Desuckering was conducted at fortnightly intervals where necessary. Harvesting of ripe leaves commenced on Day 58 and continued to the final harvest on Day 114.

2:4:2 ENVIRONMENTAL

The lighting system used consisted of 4 x 1000 W Sylvania "Metal-arc" high-pressure discharge lamps, together with 4 x 1000 W Philips tungsten iodide lamps. The photosynthetic light duration was 12 hours

TABLE 2:1 Seedling nutrient solution

Nutrient		$mg/10^{-3}\ m^3$
$Ca\ (NO_3)_2 \cdot 4H_2O$		944.60
$K\ H_2\ PO_4$		54.40
KNO_3		266.90
$NH_4\ NO_3$		320.00
$MgSO_4$		246.47
MICRO	H_3BO_3	5.148
	$MnCl_2 \cdot 4H_2O$	3.252
	$ZnSO_4 \cdot 7H_2O$	0.396
	$CuSO_4 \cdot 5H_2O$	0.144
	$H_2MoO_4 \cdot H_2O$	0.036
IRON *		0.48

* Iron as 0.48 ppm Fe or 3.154 $mg/10^{-3}\ m^3$ Fe EDTA.

TABLE 2:2 Composition and application schedule of the sequential nutrient solution for flue cured tobacco.

Sequential nutrient solutions (mg/10^{-3} m^3)

	A	B	C	D*	E *	F*
$Ca(NO_3)_2.4H_2O$	1063	1063	1181	998	498	-
$Ca(H_2PO_4)_2.H_2O$	126	126	-	126	126	126
$Ca\ (OH)_2$	-	-	-	20	177	333
KNO_3	1820	1213	506	-	-	-
$K\ H_2PO_4$	-	-	136	-	-	-
$K_2\ SO_4$	-	-	-	261	131	-
$NaNO_3$	2040	1105	170	-	-	-
$MgSO_4$	493	493	493	493	493	493
Iron EDTA	0.53	0.53	0.53	0.53	0.53	0.53
MICRO - H_3BO_3	4.29	4.29	4.29	4.29	4.29	4.29
- $MnCl_2.\ 4H_2O$	2.71	2.71	2.71	2.71	2.71	2.71
- $ZnSO_4.\ 7H_2O$	0.33	0.33	0.33	0.33	0.33	0.33
- $CuSO_4.\ 5H_2O$	0.12	0.12	0.12	0.12	0.12	0.12
- $H_2MoO_4.\ H_2O$	0.03	0.03	0.03	0.03	0.03	0.03
SCHEDULE	Day 2 - Day 14	Day 15 - Day 27	Day 28 - Day 40	Day 41 - Day 53	Day 54 - Day 66	Day 67 - end

* = pH adjusted to 6.2 - 6.5 with 5N $H_2\ SO_4$ before adding water so preventing Ca^{++} from precipitating out.

with an abrupt light-dark change. The light intensity of the 3 rooms is presented in TABLE 2:3.

A step-wise temperature treatment was imposed to approximate the natural seasonal progression of temperatures for New Zealand tobacco districts. After topping, the night temperatures were split up. The average minimum and maximum screen temperatures over the growing season at Motueka (1963-1974) are presented in FIGURE 2.1 (Tobacco Research Station unpublished data). Superimposed are the simplified day/night temperatures used in the experiment.

It should be pointed out that the step-wise seasonal change of temperature presented in FIGURE 2.1, is based on the normal progression of time. However the temperature treatments imposed in the experiment were based on morphological development time scale of the plants, as the plants grew slightly faster under field conditions. The temperature treatments imposed can be found in TABLE 2:5. All treatments were controlled within ± 0.5°C.

The relative humidity was maintained at 60% ± 5% day and 80% ± 5% night during the course of the experiment. Vapour pressure deficits in relation to temperatures are presented in TABLE 2:4.

Air flow down through the plants was 0.3 - 0.5 m. $sec.^{-1}$ as measured at the top of the plant canopy with an Alnor Instrument thermoanemometer.

The CO_2 level was monitored throughout the experiment on an infra red gas analyser and maintained at a level of 310-380 ppm.

2:5 CURING

An environmental cabinet 1m x 0.5m x 2m was set at 35°C and 85% humidity to colour the tobacco leaves. After the leaves had been coloured (2-4 days) they were removed and vacuum dried at 40°C.

2:6 MEASUREMENTS

Leaf length and leaf width measurements were taken on fresh leaves before curing. After curing, the leaves were allowed to equilibrate at 22°C and 50% humidity for 24 hours before weighing for cured (dry) weight.

The leaves were then ground individually, ready for measurement of reducing sugars and total nicotine alkaloids. An autoanalyser was used to measure reducing sugars and total nicotine alkaloids by the method of Harvey, Stahr & Smith (1969).

Growth measurements were taken also during the experiment, on days to flower, stem height and weight and number of leaves.

TABLE 2:3 Climate room light intensity
($W.m^{-2}$)

ROOM NO.	START *	END *	AVERAGE *
1	169.16	153.70	161
2	170.04	154.02	162
3	168.44	153.05	161

* The light intensity was measured at standard trolley height using an Eppley pyranometer and Schott RG 8 filter systems.

TABLE 2:4 Vapour pressure deficits

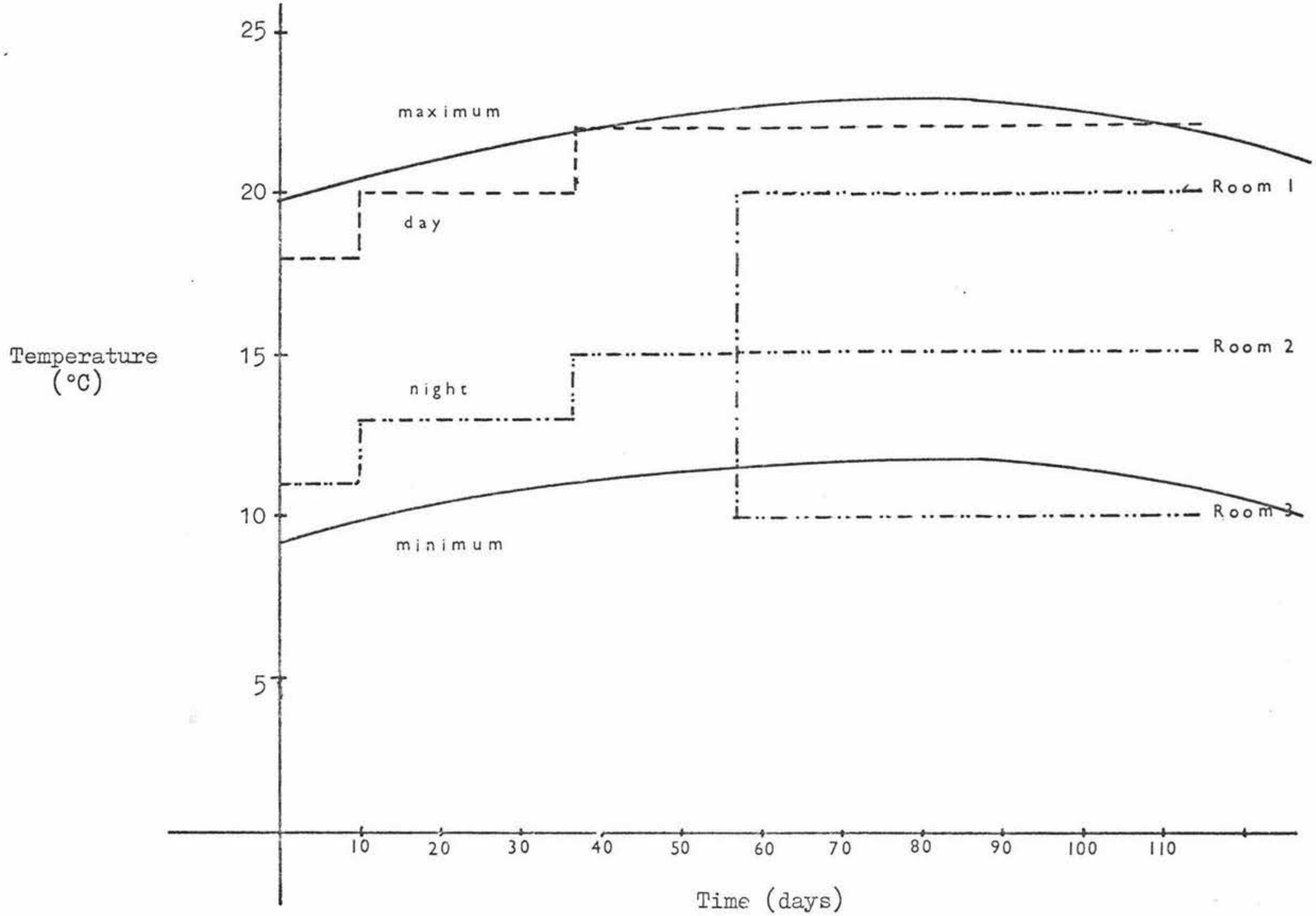
TEMPERATURE	V.P.D. m.b.
10	2
11	2
13	2
15	3
18	8
20	9
22	10

TABLE 2:5 Temperature treatments

Room	Day No.	Temperature (°C)	
		Day	Night
1	1 - 9	18	11
	10 - 35	20	13
	36 - 55	22	15
	56 - end	22	20
2	1 - 9	18	11
	10 - 35	20	13
	36 - 55	22	15
	56 - end	22	15
3	1 - 9	18	11
	10 - 35	20	13
	36 - 55	22	15
	56 - end	22	10

All temperatures were controlled within ± 0.5°C.

Figure 2.1 Seasonal progression of temperatures with climate room conditions superimposed



2:7 DATA HANDLING

For the statistical analysis, each character, measured on a "per leaf" basis, was averaged for each plant plot by dividing the total for each plant by the number of leaves per plant.

A computer program, AVENIC (Barrer, unpublished), was developed to average the individual leaf data and to calculate the derived characters, leaf area, specific leaf weight and leaf length:width ratio.

The averaged characters used in the diallel analysis were:

1. leaf width (cm)
2. leaf length (cm)
3. leaf area (cm^2)
4. leaf length:leaf width ratio
5. specific leaf weight (mg/cm^2)
6. yield (cured weight/plant) (gm)
7. cured weight/leaf (gm)
8. reducing sugars (%)
9. reducing sugars - top six leaves (%)
10. total nicotine alkaloids (TNA) (%)
11. days to flower (from transplanting)
12. stem weight (gm)
13. stem height (cm)
14. number of leaves/plant.

Leaf area was calculated as leaf length x width 0.6345 (after Suggs, Beeman & Splinter 1960). Specific leaf weight (SLW) was calculated as cured weight (mg)/leaf area (cm^2).

2:8 STATISTICAL ANALYSIS

A computer program DIANNE (Beatson & Gordon, unpublished) was used to analyse the data. This included diallel analysis of single environments after Griffing (1956) and pooled environments, estimation of variance components for single and pooled environments along with their respective standard error estimates (Crump 1946; Comstock & Moll 1963) and general combining ability (gca) and specific combining ability (sca) effects. Significance of estimated mean squares (F test), heritability estimates, correlation coefficients, approximate F' tests (Cochran & Cox 1955) calculation of degrees of freedom for F' test, (Satterthwaite 1946) homogeneity of error variances (Bartlett 1937) were also calculated by DIANNE.

2:8:1 SINGLE ENVIRONMENT

The procedures outlined by Griffing (1956) were used to analyse the diallel cross data. This procedure partitions the genetic variance into gca and sca components.

Griffing's Method 4 (no reciprocals, no parents or $\frac{1}{2}g\ (g-1)$ genotypes), Model II (random variables) was used.

The model for the diallel analysis is -

$$X_{ijk} = \mu + g_{i..} + g_{.j.} + s_{ij.} + b_{..k} + \varepsilon_{ijk}$$

where X_{ijk} is the $ijkth$ phenotypic observation

μ is the general population mean, $g_{i..}$ general combining ability effect of the ith parent, and $g_{.j.}$ gca effect for the jth parent, $S_{ij.}$ is the specific combining ability effect of the ijth cross, $b_{..k}$ is the kth block effect and ε_{ijk} is the residual variation associated with the ijkth observation.

The analysis of variance, including mean squares, degrees of freedom, expectations of mean squares and F ratios, is presented in TABLE 2:6. Standard deviation, coefficient of variation, standard error of parent means, standard error of cross means for single environments were estimated following the procedures of Griffing (1956).

Variance components and their standard errors were estimated for each character following the procedures of Crump (1946); Comstock and Moll (1963). The general formula used for standard errors of the variance estimates (after Crump 1946) is:

$$\sigma_{\sigma_x^2} = \sqrt{\frac{1}{n_x^2} \sum \frac{2\ (MS_i)^2}{f_i + 2}} \quad \ldots\ldots\ldots\ldots \ (2:8:1)$$

where σ_x^2 is the variance component estimate, n_x is the divisor appropriate to the estimator of σ_x^2, MS_i is the ith mean square used in estimating σ_x^2, and f_i is the degree of freedom of the ith mean square.

Formulae for estimating variance components and their standard errors can be found in Appendix II and Appendix III respectively.

Combining ability effects of each character were calculated from means.

1. Gca effects $$g_i = \frac{X_{i..}}{r(g-1)} - \frac{X_{...}}{rg\ (g-1)/2}$$

2. Sca effects $$S_{ij} = X_{ij.} - \frac{(X_{i..} + X_{.j.})}{rg\ (g-1)} + \frac{X_{...}}{rg\ (g-1)/2}$$

TABLE 2:6 Diallel analysis for single environments

SOURCE	df	MS	E(MS)	F ratio
BLOCKS	$r-1$	4	$\sigma^2 + (g(g-1)/2)\sigma_B^2$	MS4/MS1
GCA	$g-1$	3	$\sigma^2 + r\sigma_{sca}^2 + r(g-2)\sigma_{gca}^2$	MS3/MS2
SCA	$g(g-3)/2$	2	$\sigma^2 + r\sigma_{sca}^2$	MS2/MS1
ERROR	$[(r-1)\ (g(g-1)/2)]-1$	1	σ^2	
TOTAL	$[rg(g-1)/2]-1$			

where $r = 3$ $g = 5$

2:8:2 POOLED ANALYSIS

The mathematical model used for the pooled analysis of variance over environments was -

$$X_{ijkl} = \mu + g_{i...} + g_{.j..} + S_{ij..} + (ge)_{i..l} + (ge)_{.j.l} + (se)_{ij.l} + b_{..k(l)} + \varepsilon_{ijkl}$$

where X_{ijkl} is the ijklth phenotypic observation, μ is the population mean, $g_{i...}$ (and $g_{.j..}$) is the gca effect of the ith (jth) parent, $S_{ij..}$ is the sca effect of the ijth cross, $(ge)_{i..l}$ ($(ge)_{.j.l}$) is the interaction between the ith (jth) parent gca effect and the lth environment, $(se)_{ij.l}$ is the interaction between the sca effect of the ijth cross and the lth environment, $b_{..k(l)}$ is the kth block effect in the lth environment, and ε_{ijkl} is the residual variation associated with the ijklth observation.

The pooled analysis of variance is presented in TABLE 2:7 and includes mean squares, degrees of freedom, expectation of mean squares, F ratios. Standard deviation, coefficient of deviation, standard error of parent means and standard error of cross means were estimated following the procedures of Griffing (1956).

Variance components were estimated similarly to the single environments analysis following Crump (1946) and Comstock and Moll (1963). The standard errors were estimated from the formula presented in Equation (2:8:1). The variance component estimates and their respective standard errors formulae can be found in Appendix IV and Appendix V respectively.

Combining ability effects were estimated from means as follows -

1. gca effects $g_i = \dfrac{X_{i...}}{er\ (g-1)} - \dfrac{X_{....}}{erg\ (g-1)/2}$

2. sca effects $s_{ij} = X_{ij..} - \dfrac{(X_{i...} + X_{.j..})}{er\ (g-1)} + \dfrac{X_{....}}{erg\ (g-1)/2}$

2:8:3 SIGNIFICANCE OF ESTIMATES MEAN SQUARES

The null hypothesis that two variance estimates are the same may be tested by the F test. If they are equal the variance components by which the numerator differs from the denominator are equal to zero.

Where single mean squares could not be used in both numerator and denominator, the F' approximate test was used, where the variance ratios were estimated from linear combinations of mean squares (Cochran & Cox 1955). The degrees of freedom of the F' test were estimated from the

<u>TABLE 2:7</u> Analysis of variance: diallel x environments

SOURCE	D.F.	M.S.	EXPECTATION (M.S.)	V.R.
Environments	e-1	1	$\sigma^2 + r\sigma^2_{se} + r(g-2)\sigma^2_{ge} + [g(g-1)/2]\sigma^2_{b\langle e\rangle} + [rg(g-1)/2]\sigma^2_{e}$	(1 + 7)/(2 + 5)
Blocks within environments	e(r-1)	2	$\sigma^2 + [g(g-1)/2]\sigma^2_{b\langle e\rangle}$	2 / 7
G.c.a.	g-1	3	$\sigma^2 + r\sigma^2_{se} + r(g-2)\sigma^2_{ge} + re\sigma^2_{s} + re(g-2)\sigma^2_{g}$	(3 + 6)/(4 + 5)
S.c.a.	g(g-3)/2	4	$\sigma^2 + r\sigma^2_{se} + re\sigma^2_{s}$	4 / 6
G.c.a. x environments	(g-1)(e-1)	5	$\sigma^2 + r\sigma^2_{se} + r(g-2)\sigma^2_{ge}$	5 / 6
S.c.a. x environments	[g(g-3)/2](e-1)	6	$\sigma^2 + r\sigma^2_{se}$	6 / 7
Error	e(r-1)[(g(g-1)/2)-1]	7	σ^2	
Total	[erg(g-1)/2]-1			

Where $e = 3$ $g = 5$ $r = 3$

procedure of Satterthwaite (1946).

$$f' = \frac{\sum_n (a_n MS_n)^2}{\sum_n (a_n MS_n^2)/f_n}$$

where a_n is coefficient = 1 for F test, MS_n is the nth mean square used in the complex F' test, and f_n is the degree of freedom appropriate to the nth mean square.

The symbols used for significant differences in this study were

$NS = P > 0.10$ $(NS) = 0.10 \geq P > 0.05$ $* = 0.05 \geq P > 0.01$

$** = 0.01 \geq P > 0.005$ $*** = 0.005 \geq P > 0.001$ $**** = P \leq 0.001$

2:8:4 HOMOGENEITY OF ERROR VARIANCES

The chi-square test for homogeneity of error variances in the pooled analysis over 3 environments was used to examine the validity of pooling each character. The procedure followed was that of Steel & Torrie (1960) (after Bartlett 1937).

$$chi^2 = (PDFE.\ \ln PMS) - \sum_n (DFE_n.\ \ln MS_n)$$

$$\text{Correction factor} = 1 + \frac{1}{3\,(k-1)} \left[\frac{k}{DFE} - \frac{1}{PDFE} \right]$$

$$\text{Corrected}\quad chi^2 = \frac{\text{uncorrected } chi^2}{\text{correction factor}}$$

where DFE = degrees of freedom for error, PDFE = sum of the degrees of freedom for error pooled over all environments, ln = natural logarithm, PMS = pooled mean square for error, and k = the total number of DFE involved.

2:8:5 HERITABILITY

Broadsense full heritability and narrow sense (gca) restricted heritability were estimated for each character of the pooled analysis. Broadsense full heritability reflects the total genetic variability as a proportion of the total phenotypic variation. Narrow sense (gca) restricted heritability reflects the actual breeding potential available for the characters measured.

1. h^2_G (full) $= \sigma^2_G / \sigma^2_P$

where $\sigma^2_G = \sigma^2_{gca} + \sigma^2_{sca}$ (total genotypic variation)

and $\sigma^2_P = \sigma^2_e + \sigma^2_{gca\ x\ e} + \sigma^2_{sca\ x\ e} + \sigma^2_{gca} + \sigma^2_{sca} + \sigma^2_{b(e)} + \sigma^2$ (total phenotypic variation)

2. h^2_{gca} (narrow sense)

$$= \sigma^2_{gca} / (\sigma^2_{gca} + \sigma^2_{gca\ x\ e} + \sigma^2_{sca} + \sigma^2_{sca\ x\ e} + \sigma^2)$$

2:8:6 COVARIANCE ANALYSIS

Covariance analyses between pairs of characters were also calculated by DIANNE. The procedures followed the same form as the variance analysis. Mean product expectations of the covariance analysis are analogous to the mean square expectations for the analysis of variance.

The covariance components as such are not presented in the thesis study. * Instead, the covariance components were used to compute (via DIANNE) simple correlation coefficients between the various characters as follows:

$$r = \frac{\text{covariance }(x,y)}{\sqrt{[\text{variance }(x) \text{ x variance }(y)]}}$$

where r = correlation coefficient and x and y are any two of the characters examined.

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* covariance analysis data is available from the author, along with all types of correlation coefficients examined (only gca and phenotypic r values are presented in Chapter 3.)

FIGURE 2:2

Tobacco plants six weeks after transplanting

in the climate rooms.

CHAPTER 3

RESULTS AND DISCUSSION

3:1 HOMOGENEITY OF ERROR VARIANCES

Prior to pooling analyses for the three environments, a chi-square test for homogeneity of error variances, (Steel & Torrie, 1960) was carried out. The results of this test over all characters are presented in Table 3:1. Table 3:1 shows whether the three pooled environments have homogeneous (or heterogeneous) error variances. The right hand side of the table (where the three pooled environments were heterogeneous) gives the homogeneous environments, together with their chi-square values and probabilities.

Leaf width, length:width ratio, specific leaf weight, yield and weight/leaf had heterogeneous error variances over the three pooled environments. Homogeneous error variances were found for leaf width (environments two and three), length:width (two and three), specific leaf weight (one and two), yield (one and three) and weight/leaf (one, three).

One of the assumptions underlying the analysis of variance (Cochran 1947) is that variances amongst sub-sets of data are homogeneous. Several methods are available to test the homogeneity of variances (Bartlett, 1937; Bishop & Nair, 1939; and Stevens, 1936). Bartlett's chi-square test for homogeneity of error variances is the best known and most widely used. When the data is considered homogeneous the original sub-sets may be considered as random samples drawn from population with a common variance. Heterogeneous error mean squares will result in the probability of F values being different to that assumed. This results in significant differences being obtained more frequently than should be the case. The null hypothesis would be rejected more frequently than assumed at the significance level; Type I error rate would be increased. Cochran (1947) summarizes that "heterogeneity of errors may affect certain treatments or certain parts of the data to an unpredictable extent." Of the fourteen characters examined five were heterogeneous. Four of the five coefficients of variation for the heterogeneous pooled error variances were larger than their respective coefficients of variation for homogeneous pooled error variances. This indicates that their error variances would be biased upwards. In the fifth case (Specific leaf weight) error variances was under-estimated relative to that of the homogeneous environments.

Although some combined analyses were heterogeneous they are considered the best overall estimates (Cochran, 1947). The heterogeneous pooled data were noted as possibly biasing the components of variance and significance levels, but were used for comparative purposes with other characters.

3:2 COEFFICIENT OF VARIATION

The coefficients of variation for the pooled data are presented in TABLE 3:1. The coefficients of variation range from 3.71% for leaf length to 15.56% for reducing sugars.

The coefficients of variation are of the same order as those reported for single plant plot experiments on tobacco grown in artificial environments (Raper, Johnson & Downs, 1971; Raper & Johnson, 1971; Raper, 1971). Coefficients of variation in the order of 10-15% are common for agricultural experiments (Balaam, 1972). This means that the coefficients of variation presented in TABLE 3:1 are quite acceptable.

3:3 NIGHT TEMPERATURE INFLUENCE

The influence of late season night temperature differences is presented in TABLE 3:2. Thirteen of the fourteen characters measured were non significant.

Environment 2 (15°C) did not follow the same trends as the other two environments. This is borne out in the only significant environment estimate, plant height. The plants in room 2 were significantly ($P \leq 0.05$) shorter. No explanation can be given for the room abnormalities as the "room history" records were checked at the time and all appeared to be in order.

Of particular interest in this study was the influence of night temperatures on reducing sugars and the specific leaf weight. Experimental studies by Pearse (1961) indicated that reducing sugars may be increased by low night temperatures. In the same study the specific leaf weight was also increased by low night temperatures. Raper, Johnson & Downs (1971) found that cooler night coupled with cooler day temperatures increased specific leaf weights. There is an indication of increased level of reducing sugars under cool night and day temperatures in an experiment reported by Raper and Johnson (1971).

However, the above mentioned experiments differ from the present study in that the plants were subjected to the various temperature schedules from transplanting onwards, whereas this study, night

TABLE 3:2

Environmental means, pooled mean and variance ratio significance for environments

Character	Environmental means 20°	 15°	 10°	Pooled mean	Envir. F test Signif
Leaf width	21.9	22.4	21.9	22.1	NS
Leaf length	60.8	60.7	61.3	61.0	NS
Leaf area	875	892	880	882	NS
Leaf length: leaf width	3.01	2.92	3.30	2.99	NS
Specific leaf weight	6.01	6.35	6.10	6.16	NS
Yield	67.2	69.4	67.9	68.2	NS
Cured weight/leaf	4.56	4.97	4.63	4.72	NS
Reducing sugar %	13.6	14.4	13.7	13.9	NS
Reducing sugar % - top six leaves	22.5	23.1	22.9	22.8	NS
Total nicotine alkaloids %	1.10	1.20	1.12	1.14	NS
Days to flower	60.5	59.8	61.5	60.6	NS
Stem weight	81.6	78.0	82.6	80.7	NS
Stem height	117	109	121	116	*
Number of leaves/ plant	14.9	14.2	15.2	14.8	NS

where NS = $0.10 > P$

and * = $0.05 \geq P > 0.01$

LSD (0.05) for stem height $\geq$ 10 cms.

temperature influence was not imposed until topping time (day 56 from transplanting).

Another major difference between the studies reported above and the present one is that only one genotype was used in the earlier experiments, whereas the present study involved ten genotypes. Indications from TABLE 3:3 (a) are that there were significant genotype x environment interactions for specific leaf weight and reducing sugars. The differing responses of the genotypes in the three rooms may tend to mask any overall environmental influence that may otherwise be evident if only one genotype was used.

3:4 CHARACTER MEANS

Character means are presented in TABLE 3:2. The extrapolation of results from tobacco produced in artificial environments to the field environment situation is of some relevance. The main difference between the climate room study and field grown tobacco was that the number of leaves was reduced (14.8 versus approximately 18,(Beatson, unpublished))days to flower was reduced (60.6 versus approximately 80 days,(Beatson, unpublished)). This reduced yield per plant. The reasons for the early flowering and reduced number of leaves is not clear, but was probably due to the change over of the apex from vegetative to floral state in the seedling stage (i.e. before transplanting). Early flowering could also be due to F1 hybrids showing heterosis for decreased days to flower as shown in studies by Matzinger, Mann & Cockerham (1962), Chaplin (1966), Vandenberg & Matzinger (1970), and Fan & Aycock (1974). Early flowering in tobacco has been studied by many investigators (Kasperbauer, 1966, 1969, 1970; Kasperbauer & Lowe, 1966; Shinohara 1971; Hopkinson & Hannam, 1969; and Thomas, Anderson, Raper & Downs, 1975). Tobacco is said to be day neutral (Spector 1956). However evidence from flowering studies on tobacco indicate that tobacco is receptive to photoperiod and thermoperiod; before and immediately after transplanting.

The low value for TNA of 1.14% over the three environments was probably due to:

(1) the early curtailment of nitrogen in the nutrient solution; there are two nitrogen atoms in a molecule of nicotine (Elliot, 1975); and
(2) late topping of the plants; shorter interval for accumulating the topping-enhanced biosynthesis of nicotine (McCants & Woltz, 1967).

Average TNA levels for commercially grown flue-cured tobacco in New Zealand are around 2% (Rohrbach, pers. comm.).

Reducing sugars were somewhat lower than in field grown tobacco, this probably being caused by shading within the rooms. However the values in the top six leaves were more typical of New Zealand tobacco. (No shading occurred in the top of the plants).

3:5 COMPONENTS OF VARIANCE

Genetic components of variance, genotype - environment variance and error variance estimates are presented in TABLE 3:3 (a). The F test significance levels and the standard errors of the variance components are also included in TABLE 3:3 (a). The ratio of the variance components to error variance, is presented in TABLE 3:3 (b). The ratios to error indicate the relative importance of the variance components to the experimental error.

3:5:1 GENETIC VARIANCE

From TABLE 3:3 (a) it can be seen that twelve of the fourteen components examined, had significant F values ($P < 0.05$) for gca. In terms of the analysis of variance, the value of F obtained for gca has a probability of less than 5% for twelve of the characters. Hence the null hypothesis (of equal gca values) is rejected. Reducing sugar (top six leaves) and stem height were not significant for gca but were in the range $P = 0.05 - 0.10$. All fourteen gca estimates were greater than their respective standard errors.

Sca variance components were significant for six characters (Length: width ratio, specific leaf weight, yield, reducing sugar, top six reducing sugar and flowering.) Total nicotine alkaloids were in the range $P = 0.05$ to 0.10 (i.e. just non-significant). All significant estimates of sca were larger than their respective standard errors.

As shown in Chapter One, gca variance indicates the presence of additive genetic variance (some additive x additive epistatic variance as well) while sca variance represents dominance and epistatic variance.

The results of the thesis study indicate that gca variance (additive variance) is the main component of genetic variance present for the characters examined. This point is emphasised when the ratios to error in TABLE 3:3 (b) are examined. For seven of the characters a ratio of approximately one or more is obtained for gca variance whereas no estimates of sca variance were as large as error variance.

The predominance of additive genetic variance agrees with numerous studies on Nicotiana tabacum (Matzinger, Mann & Cockerham, 1962; Legg, Collins & Litten, 1970; Chaplin, 1966, 1967; Vandenberg & Matzinger,

TABLE 3:3 (a)

Estimates of components of variance, together with their standard errors and significances.

Character	$\hat{\sigma}^2_{gca}$	$\hat{\sigma}^2_{sca}$	$\hat{\sigma}^2_{gca \times e}$	$\hat{\sigma}^2_{sca \times e}$	$\hat{\sigma}^2$
Leaf width	1.5800 ***	-0.0772 NS	0.2220 NS	0.2393 NS	1.4673
SE	0.9903	0.1329	0.2303	0.3114	0.2773
Leaf length	3.1672 *	1.0567 NS	-0.1141 NS	0.3996 NS	5.1022
SE	2.1713	0.9816	0.3878	0.9157	0.9642
Leaf area	4314 ***	-458 NS	606 NS	437 NS	4515
SE	2652	283	620	842	853
Leaf length: leaf width	0.0347 *	0.0175 *	0.0013 NS	-0.0018 NS	0.0403
SF	0.0247	0.0116	0.0028	0.0054	0.0076
Specific leaf weight	0.2126 **	0.0563 *	0.0492 *	-0.0485 NS	0.3097
SE	0.1475	0.0405	0.0310	0.0297	0.0585
Yield	190.79 ***	24.18 **	2.32 NS	-2.26 NS	47.73
SE	116.25	15.47	3.59	6.33	9.02
Cured weight/ leaf	0.1958 ***	0.0003 NS	0.0147 NS	-0.0177 NS	0.3166
SE	0.1219	0.0198	0.0230	0.0410	0.0598
Reducing sugar %	3.2313 **	0.7215 ***	1.0249 ****	1.2618 NS	4.6776
SE	2.2319	0.4405	0.5043	0.3187	0.8840
Reducing sugar % - top six leaves	3.9640 (NS)	3.6102 ****	0.9689 *	-0.7867 NS	4.8625
SE	3.3024	2.0815	0.5691	0.4580	0.9189
Total nicotine alkaloids %	0.0893 ****	0.0059 (NS)	0.0023 NS	-0.0006 NS	0.0309
SE	0.0538	0.0051	0.0028	0.0044	0.0058
Days to flower	8.8759 ***	1.5753 **	1.1735 *	-1.9992 NS	8.9901
SE	5.7322	1.0288	0.6870	0.6975	1.6990
Stem weight	163.54 ***	11.62 NS	-9.13 NS	10.19 NS	91.66
SE	97.63	14.57	5.89	17.61	17.32
Stem height	15.5253 (NS)	3.8407 NS	-7.5296 NS	7.1082 NS	110.6568
SE	11.7743	11.5622	6.7841	19.2657	20.9122
Number of leaves	4.2673 ****	0.0444 NS	0.0253 NS	0.1815 NS	1.2148
SE	2.5155	0.1510	0.1270	0.2513	0.2296

where NS = $P > 0.10$

(NS) = $0.10 \geq P > 0.05$

* = $0.05 \geq P > 0.01$

** = $0.01 \geq P > 0.005$

*** = $0.005 \geq P > 0.001$

**** = $0.001 \geq P$

TABLE 3:3 (b)

Components of variance ratio to error

	$\hat{\sigma}^2_{gca}$	$\hat{\sigma}^2_{sca}$	$\hat{\sigma}^2_{gca\ x\ e}$	$\hat{\sigma}^2_{sca\ x\ e}$
Leaf width	1.08	-0.05	0.15	0.16
Leaf length	0.62	0.21	-0.02	0.08
Leaf area	0.96	-0.10	0.13	0.10
Leaf length: leaf width	0.86	0.44	0.03	-0.04
Specific leaf weight	0.69	0.18	0.16	-0.16
Yield	4.00	0.51	0.05	-0.05
Cured weight/leaf	0.62	0.00	0.05	-0.06
Reducing sugar %	0.69	0.15	0.22	-0.27
Reducing sugar % - top six leaves	0.82	0.74	0.20	-0.16
Total nicotine alkaloids %	2.89	0.19	0.07	-0.02
Days to flower	0.99	0.18	0.13	-0.22
Stem weight	1.78	0.13	-0.10	0.11
Stem height	0.14	0.03	-0.07	0.06
Number of leaves	3.51	0.04	0.02	0.15

1970; Gwynn, 1966; Povilaitis 1966, 1970 a, 1971; Lamprecht 1964, 1967, 1969, 1973; Lamprecht & Nuss, 1973; Lamprecht & van Wyk, 1969, 1971; Jones, Gupton & Terril, 1972; Matzinger, Wernsman & Ross, 1971; Aycock, 1972; Dean 1974; Gritton, Jones, Powerll & Matzinger, 1965; Fan & Aycock, 1974; Robinson, Mann & Comstock, 1954; Povilaitis, 1964; Matzinger, Mann & Robinson 1960; Legg & Collins 1971 a, 1971 b, 1974, 1975; Matzinger, 1968; Matzinger, Mann & Cockerham, 1966; Matzinger, Wernsman & Cockerham, 1972; Matzinger & Wernsman, 1970) and other self pollinating species estimates of genetic variance as well (Matzinger 1963). From a practical plant breeding point of view, breeding procedures which lead to the accumulation of genes acting additively for the characters under selection will be the best to follow. This is achieved by inbreeding lines to homozygosity after the creation of genetic variability in a base population (Matzinger, Mann & Cockerham, 1962). In Nicotiana tabacum this is most easily achieved by isolating homozygous lines from an F2 population via pedigree selection.

3:5.2 GENOTYPE X ENVIRONMENT VARIANCE

General combining ability x environment components of variance were significant for specific leaf weight, reducing sugars, top six reducing sugars and flowering. However, all were considerably smaller than their respective gca components. This study also showed no significant sca x environment variances. The general lack of importance of genotype x environment interactions in this study is consistent with most other tobacco studies (Collins, Jones, Weybrew & Matzinger, 1961; Jones, Matzinger & Collins, 1960; Povilaitis, 1970 b, Gupton, Legg, Link & Ross, 1974; Matzinger, Mann & Cockerham, 1962; Legg, Collins & Litton, 1970; Gwynn, 1966; Matzinger, Wernsman & Ross, 1971; Fan & Aycock, 1974). This lack of GE interaction may mean that:

(1) genotypes of tobacco are stable over a range of environments;
(2) the sampling of genotypes and/or environments was not sufficient.

Lack of GE interaction, in plant breeding terms, means that genotypes can be examined over a smaller number of environments. However such conclusions must be drawn with caution, with regards to point (2) above.

Significant gca x environments interaction for reducing sugars and specific leaf weight indicate that temperature is influencing genotypes differentially. An anomaly appears to be the significant gca x environment interaction for flowering. Most of the genotypes were flowering before the different temperature treatments were imposed. This point has been raised earlier in section 3:4.

3:5:3 BLOCKS (WITHIN ENVIRONMENT) VARIANCE

The blocks (within environment) variance components along with their respective F test significance levels can be found in Appendix XII & XII Block effects were significant for six of the fourteen characters measured. In climate room experiments block effects would normally be very small because of the eveness of the conditions within a room. With tobacco, being such a large plant, "edge effects" may have given the block differences within the rooms. The highly (P = 0.001) significant block effect for number of leaves however is difficult to explain. Closer inspection of the results reveals that the "environment 2" room was the main cause of the significant pooled block effect for number of leaves per plant; ratios to error for Rooms 1, 2 and 3 were -0.07, 0.81, and 0.34 respectively. However from TABLE 3:1 (Homogeneity of error variances) it is evident that pooling of the three environments was valid.

The significant block effects then heightens the necessity for replication when conducting studies on tobacco in climate rooms as the analysis of variance allows for block effects to be partitioned out.

3:6 HERITABILITY

Estimates of broadsense full heritability and narrow sense (gca) restricted heritability, expressed as percentages are presented in TABLE 3:4.

Notable features are the high restricted gca heritabilities obtained for the commercially important characters, yield, total nicotine alkaloids and number of leaves. Most of the other characters measured have moderate restricted gca heritabilities in the range of 30-50%. Stem weight had a value of 61% and stem height 12%.

Broadsense full heritability values show a similar trend to narrow sense restricted values with the exception of leaf width, L:W ratio and top six reducing sugars, which have values 20-30% higher than gca restricted heritabilities.

Heritability estimates reflect the amount of genetic variation present that can be passed on to progeny. In plant breeding, there are no standard heritability measurements used. Genetic studies in N. tabacum have revealed that additive genetic variance is the principle genetic component. This study supported this evidence, as discussed previously. Therefore the heritability estimates of interest are the ones which will reflect gca variance as a portion of the total phenotypic variation. Phenotypic variation is made up of the following

TABLE 3:4

Heritability estimates

Character	h^2_G (full)*	h^2_{gca} (restricted)**
Leaf width	50.3	46.0
Leaf length	50.7	33.0
Leaf area	49.3	45.8
Leaf length: leaf width	59.6	37.7
Specific leaf weight	48.0	36.7
Yield	84.5	72.6
Cured weight/ leaf	38.7	38.4
Reducing sugar %	49.2	38.5
Reducing sugar % - top six leaves	60.2	31.4
Total nicotine alkaloids %	75.9	69.9
Days to flower	60.4	47.7
Stem weight	71.1	61.0
Stem height	14.2	12.0
Number of leaves/ plant	75.5	74.4

$* = \sigma^2_G / \sigma^2_P$

$** = \sigma^2_{gca} / (\sigma^2_{gca} + \sigma^2_{gca\ x\ e} + \sigma^2_{sca} + \sigma^2_{sca\ x\ e} + \sigma^2)$

where σ^2_G = genotypic variance and

σ^2_P = phenotypic variance

components: environment, genotype x environment interaction, genotype and residual error. The genotype is further partitioned by the diallel analysis into gca and sca variance. It is therefore evident that heritability estimates can be of many different types depending on what is included in the numerator and denominator. Two have been chosen for the thesis study. The first, broad sense full heritability, reflects the total genetic variability as a proportion of the total phenotypic variation. It is a "descriptive" statistic and indicates that proportion of the phenotypic variance which is attributable to consistentgenetic variation. The second, narrow sense "restricted" (gca) heritability reflects the amount of gca variance in relation to the phenotypic variance associated with genotype differences; that is, genetic variance, genotype x environment variance and error. This second type of heritability estimate reflects the breeding potential available under selfing for the various characters measured. The difference between h^2_G and h^2_{gca} values is due to (1) the influence of sca variance and/or (2) environment and block variation. Environment and block influence was very small so that the main difference between h^2_G and h^2_{gca}, where it occurred, was in sca variance.

Heritability estimates (mostly narrow sense) reported for tobacco studies indicate that yield, nicotines, number of leaves and days to flower have moderate to high values. (Matzinger, Mann & Cockerham, 1966; Matzinger, 1968; Matzinger, Wernsman & Cockerham, 1972; Legg & Collins, 1971a,1971b, 1975; Lamprecht, 1964, 1969, 1973; Lamprecht & van Wyk, 1969; Lamprecht & Nuss, 1973). The present values agree with these findings fairly well. On the basis of the present data, early generation selection on yield, number of leaves and TNA would result in considerable progress. Particular interest has been shown in the relatively high reducing sugar values of New Zealand flue-cured tobacco. Decreasing sugar values by plant breeding is one way of achieving a more acceptable level of reducing sugars in New Zealand tobacco. Reducing sugars, with heritability values of around 40% could feasably be decreased. In fact breeding lines with both low alkaloids (less than 0.5%) in conjunction with low sugars (10-15% or less) are being produced at the Tobacco Research Station (Beatson, unpublished data).

3:7 COMBINING ABILITY EFFECTS

As previously discussed the overwhelming evidence from the present study, and many other studies on tobacco, is that general combining ability variance (which is largely a measure of additive genetic variance) is the most important type of genetic variance present for the characters measured.

3:7:1 GENERAL COMBINING ABILITY EFFECTS

The gca effects of the fourteen characters for the five parents used in the diallel, are presented in TABLE 3:5.

For the leaf measurements (i.e. length, width and area) Kutsaga 51 shows the best gca as a parent. That is, if increased leaf size is the criterion for selection, Kutsaga 51 cross progeny would be expected to show the most promise.

Leaf length:leaf width ratio indicates that both Kutsaga 51 and Virginia 815 would be the most successful parents for breeding broad-leaf varieties, while Hicks and Bright Yellow 103 would be the best parents if narrowleafed lines were required.

Burley 1 and Virginia 815 had the best gca values for increasing specific leaf weight and yield while Kutsaga 51 and Hicks showed poor gca values. The better gca values for yield of Burley 1 and Virginia 815 largely resulted from the good gca values both of these parents had for number of leaves.

Burley 1, Bright Yellow 103, and Virginia 815 all had good gca values for increasing reducing sugars, while Kutsaga 51 and Hicks were good general combiners for decreasing reducing sugars. Bright Yellow 103 has a known history of producing high reducing sugar values and it is no surprise that as a parent it follows the same trends.

Based on previous evidence from Povilaitis (1971) on Flue x Burley crosses, the present results on Burley 1 are not in agreement. He concluded that burley cultivars when crossed with flue-cured cultivars gave decreased reducing sugar values.

Kutsaga 51 had the best gca value for TNA % while Burley 1 had the lowest value. This indicates that Kutsaga 51 would be a good parent for improving TNA values while Burley 1 could be used if lower TNA values was the object of a breeding programme.

Days to flower and number of leaves produced similar gca effects, with Burley 1 and Virginia 815 as parents producing more leaves and taking longer to flower.

Virginia 815 had the best gca value for stem height indicating that if taller plants were the breeding goal Virginia 815 progeny would give the best opportunity for selection, while Hicks and Kutsaga 51 would be more likely parents if shorter statured plants were desired.

In summary then, there appears to be considerable scope for

TABLE 3:5 General combining ability effects.

	Burley 1	K 51	Hicks	BY 103	Va 815
Leaf width	0.2903	1.3512	-1.0008	-0.9241	0.2834
Leaf length	-0.3301	2.1160	0.5702	-0.5987	-1.7573
Leaf area	14.4667	79.8556	-37.2556	-47.2556	-9.8111
Leaf length: leaf width	-0.0834	-0.1237	0.1916	0.1419	-0.1264
Specific leaf weight	0.4939	-0.4805	-0.1816	-0.0727	0.2409
Yield	16.0104	-7.7132	-8.4073	-5.6921	5.8021
Cured weight/ leaf	0.4433	0.1533	-0.2836	-0.3969	0.0439
Reducing sugar %	1.1688	-1.8565	-1.2759	0.6038	1.3599
Reducing sugar % - top six leaves	1.1510	-2.1329	-1.6868	0.9591	1.7096
Total nicotine alkaloids %	-0.2848	0.2554	0.1068	0.1175	-0.1949
Days to flower	2.1500	-3.1556	-1.7111	0.6222	2.0944
Stem weight	12.9354	-6.0779	-9.4635	-5.1310	7.7371
Stem height	-1.2722	-2.1333	-2.9111	1.0889	5.2278
Number of leaves/ plant	1.7944	-2.1778	-0.8444	0.1833	1.0444

selection amongst progeny of the five diallel parents for the characters measured. Also, the value of the combining ability analysis in choosing parents for crosses, was well demonstrated.

3:7:2 SPECIFIC COMBINING ABILITY EFFECTS

Specific combining ability effects (sca) were significant for six of the fourteen characters measured as previously discussed in section 3:5:1. They were leaf length:width ratio, specific leaf weight, yield, reducing sugars, top six reducing sugars, and flowering. TNA % was in the P = 0.05 - 0.10 range.

Sca effects for pooled environments for all fourteen characters can be found in Appendix XV . Comments will be confined to the six significant sca estimates plus TNA. The best crosses for each of the significant characters were as follows:

1. Length:width ratio
   - Burley 1 x Virginia 815 -0.2877 (broad) and
   - Burley 1 x Hicks 0.1253 (narrow)
2. Specific leaf weight
   - Burley 1 x Hicks 0.3424 (thickest) and
   - Kutsaga 51 x Hicks -0.5709 (thinnest)
3. Yield
   - Burley 1 x Virginia 815 12.5346 (largest)
4. Reducing Sugars
   - Burley 1 x Virginia 815 1.1257 (most) and
   - Kutsaga 51 x Hicks -2.1877 (least)
5. Top six reducing sugars
   - Burley 1 x Kutsaga 51 1.4562 (most) and
   - Kutsaga 51 x Hicks -4.2527 (least)
6. Days to flower
   - Burley 1 x Virginia 815 2.6 (longest) and
   - Kutsaga 51 x Virginia 815 -1.9833 (shortest)
7. TNA
   - Kutsaga 51 x Hicks 0.1665 (most) and
   - Burley 1 x Kutsaga 51 -0.1541 (least)

A major decision facing a plant breeder of self pollinating crops, in this case <u>N. tabacum</u>, is whether to develop homozygous cultivars or find specific desirable combinations of crosses (F1 hybrids). Considerable interest has been shown recently in the amount of heterosis in N. tabacum and its possible use in the production of F1 hybrids commercially. Many of these studies have shown that a small amount of heterosis is present for many commercial characters; heterosis being measured from the mid parent value. These heterosis studies include flue cured tobacco (Aycock, Mann & Matzinger, 1963; Chaplin, 1966, 1967; Matzinger & Mann, 1962; Mann, Jones & Matzinger, 1962; Matzinger, Mann & Cockerham, 1962; Povilaitis, 1966), Burley tobacco (Matzinger, Wernsmann & Ross, 1971; Legg, Collins & Litton, 1974), Maryland tobacco (Fan & Aycock, 1974), Burley x flue tobacco crosses (Povilaitis, 1971), Interspecific crosses (Matzinger & Wernsman, 1967), Tobacco Introductions x flue-cured crosses (Vandenberg & Matzinger, 1970), Oriental x flue cured crosses (Matzinger & Wernsman, 1968b), and Oriental tobacco (Marani & Sachs, 1966). However, in nearly every case it was concluded that plant breeders in N. tabacum can make more long term progress by developing pure-line varieties rather than isolating hybrids with good specific combining ability. Information from this study would agree with these conclusions.

Matzinger, Mann & Cockerham (1962) have suggested that tobacco hybrids may be used as a "stop gap" measure in temporary situations until more desirable pure lines can be developed. This suggestion is supported by Legg, Collins and Litton (1974) for burley tobacco, especially where resistance to a disease is urgently needed; in their case resistance to the common race of black shank (Phytophthora parasitica), which is simply inherited.

In recent years the anther culture technique has been refined considerably on N. tabacum and it is now possible to produce homozygous lines with relative ease within two or three years of crossing the parents (Kasperbauer & Collins 1972, 1974; Nakamura, Yamada, Kadotani, Itagaki & Oka, 1974). This technique then further strengthens the case for developing homozygous lines.

3:8 CORRELATION

General combining ability (gca) simple correlations and phenotypic simple correlations are presented in TABLES 3:6:1 and 3:6:2. General comparisons between gca and phenotypic simple correlations are that they are nearly always in the same direction.

For the characters measured, twenty-two of the ninety-one gca correlation coefficients were significant, with a further nine in the $P = 0.05 - 0.10$ range. General combining ability correlations are of main interest because they measure the overall association between two

TABLE 3:6:1

General combining ability

simple correlations

X1	leaf width
X2	leaf length
X3	leaf area
X4	leaf length:leaf width
X5	specific leaf weight
X6	yield
X7	cured weight/leaf
X8	reducing sugar %
X9	top six leaves - reducing sugar %
X10	total nicotine alkaloids %
X11	days to flower
X12	stem weight
X13	stem height
X14	number of leaves/plant

significance levels

*	$0.05 \geq P > 0.01$
**	$0.01 \geq P > 0.005$
***	$0.005 \geq P > 0.001$
****	$P \leq 0.001$

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14
X1		0.40	0.96 **	0.91 *	0.13	0.23	0.78	-0.18	-0.18	-0.01	-0.21	0.31	0.01	-0.24
X2			0.64	0.01	-0.76	-0.54	0.13	-0.92 *	-0.92 *	0.73	-0.91 *	-0.59	-0.82	-0.84
X3				-0.76	0.30	0.07	0.73	-0.42	-0.43	0.19	-0.43	0.11	-0.27	-0.43
X4					-0.25	-0.54	-0.83	-0.22	-0.22	0.38	0.20	-0.64	-0.34	-0.15
X5						0.93 *	0.42	0.89 *	0.84	-0.98 ***	0.94 *	0.90 *	0.42	0.98 ***
X6							0.72	0.77	0.71	-0.95 *	0.81	0.98 ***	0.32	0.87
X7								0.17	0.11	-0.52	0.21	0.72	-0.08	0.27
X8									0.99 ****	-0.84	0.99 ****	0.80	0.72	0.95 *
X9										-0.78	0.97 ***	0.75	0.77	0.91 *
X10											-0.90 *	-0.94 *	-0.46	-0.93 *
X11												0.83	0.66	0.98 ***
X12													0.46	0.85
X13														0.51
X14														

TABLE 3:6:2

Phenotypic simple
correlations

X1	leaf width
X2	leaf length
X3	leaf area
X4	leaf length:leaf width
X5	specific leaf weight
X6	yield
X7	cured weight/leaf
X8	reducing sugar %
X9	top six leaves - reducing sugar %
X10	total nicotine alkaloids %
X11	days to flower
X12	stem weight
X13	stem height
X14	number of leaves/plant

significance levels

*	$0.05 \geq P > 0.01$
**	$0.01 \geq P > 0.005$
***	$0.005 \geq P > 0.001$
****	$P \leq 0.001$

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14
X1		0.37 ****	0.93 ****	-0.85 ****	0.11	0.22 *	0.68 ****	-0.23 *	-0.24 *	0.13	-0.34 ***	0.17	-0.24 *	-0.37 ****
X2			0.68 ****	0.11	-0.46 ****	-0.31 ***	0.16	-0.62 ****	-0.52 ****	0.47 ****	-0.61 ****	-0.20	-0.13	-0.58 ****
X3				-0.63 ****	-0.23 *	0.10	0.61 ****	-0.40 ****	-0.37 ****	0.24 *	-0.47 ****	0.11	-0.21 *	-0.47 ****
X4					-0.10	-0.39 ****	-0.63 ****	-0.03	0.04	0.13	0.02	-0.27 **	0.22 *	0.08
X5						0.79 ****	0.57 ****	0.82 ****	0.71 ****	-0.55 ****	0.45 ****	0.62 ****	0.05	0.55 ****
X6							0.65 ****	0.64 ****	0.56 ****	-0.72 ****	0.52 ****	0.84 ****	0.08	0.69 ****
X7								0.29 **	0.17	-0.14	-0.14	0.41 ****	-0.31 ***	-0.07
X8									0.87 ****	-0.54 ****	0.60 ****	0.53 ****	0.19	0.60 ****
X9										-0.58 ****	0.58 ****	0.56 ****	0.30 ****	0.61 ****
X10											-0.76 ****	-0.76 ****	-0.32 ****	-0.84 ****
X11												0.59 ****	0.48 ****	0.86 ****
X12													0.49 ****	0.72 ****
X13														0.43 ****
X14														

characters caused by genetic linkage and/or pleiotropy. Of particular interest are the gca correlations between the economically important characters measured. The negative correlation (-0.95) between yield and TNA is in the same direction as other estimates from tobacco populations (Legg, Matzinger & Mann, 1965; Matzinger & Mann, 1963; Matzinger, Mann & Cockerham, 1966; Matzinger, Mann & Robinson, 1960; Matzinger & Wernsmann, 1970; Matzinger, 1968; Legg & Collins 1971 (a), 1971 (b), 1975). The negative gca correlation of -0.95 in this study is somewhat higher than most other reported genetic correlations for the same traits, a typical value would be approximately -0.5 (Mann, Matzinger & Wernsman, 1972). Under the present marketing system, it is desirable to have high yields (for the producer) and a reasonable nicotine level in commercial varieties. Therefore, in a breeding programme selection for increased yields and maintaining nicotines at an acceptable level, simultaneous selection for both must be practiced. Cultivars available today reflect the success of selecting in opposition to the genetic correlation. The exact nature of the negative genetic association between these two traits is not well understood. Genes for high yield may be located on chromosomes adjacent to genes for low nicotines. This would be linkage and would tend to be transferred to their offspring. Recombination would tend to overcome this negative association. The negative correlation could arise from physiological relationships whereby a single gene may control similar biochemical pathways which lead to the eventual expression of yield and nicotines.

For other important characters the correlations are in general agreement with the tobacco studies previously mentioned. Of particular interest in the study is the relationships between reducing sugars and various characters. Reducing sugars are negatively correlated to leaf width and TNA ($P = 0.05 - 0.10$), and positively correlated with specific leaf weight (SLW), days to flower and number of leaves. If selection for low sugars was the aim of a breeding programme then TNA, SLW and days to flower may be favourably altered. However it should be mentioned that many tobacco studies have shown reducing sugars to be positively correlated to both yield and quality. The gca correlation between reducing sugars and yield was not significantly different from zero but was of sufficient magnitude (0.77) to agree with other studies. It seems then that decreasing reducing sugars may have some beneficial effects but simultaneous selection on additional characters such as yield, quality, TNA and number of leaves must also be carried out, if the programme is to have any success.

In a breeding programme, selection occurs on phenotypes but is effective only if it changes genotypes. Therefore phenotypic correlations are important to the breeder. The important characters, yield and TNA have a correlation of -0.72, which agrees with their gca correlation in direction. Reducing sugars have a negative phenotypic correlation with leaf width and TNA as is the case with gca correlations. In addition reducing sugars have a positive correlation with SLW, days to flower and number of leaves, again agreeing with gca correlations. Yield has a positive correlation with SLW, days to flower and number of leaves.

In conclusion then gca and phenotypic simple correlations are in good agreement.

CHAPTER 4

GENERAL DISCUSSION

4:1 CLIMATE ROOM STUDIES ON TOBACCO:-

A number of difficulties were encountered in this study and these will be discussed in relation to recommendations for future tobacco climate room studies. Early flowering, manual nutrient application, over-crowding of plants, lack of curing facilities, and inconsistent rooms were the main causes for concern.

4:1:1 EARLY FLOWERING:-

Early flowering has already been discussed in Chapter 3. It could not be established whether early flowering was due to pre- or post-transplant conditions, or both. However as the early post-transplant climate room conditions were approximately simulating those found in New Zealand field conditions, it is suspected that pre-transplant conditions may have contributed to the early flowering.

The problem of early flowering in tobacco has been studied by Kasperbauer (1970) (1973) (1966) (1969) Kasperbauer & Lowe (1966), Hopkinson & Hannam (1969) and Thomas, Anderson, Raper & Downs (1975). Although N. tabacum is day neutral it has been demonstrated that it is sensitive to both temperature and photoperiod. Thomas et. al. (1975) suggests that the cultivar used in their study would more appropriately be classified as "preferentially ambiphotoperiodic" rather than "day neutral".

There are two alternatives to lessen the possibility of early flowering caused by pre-transplant conditions:

(i) grow the seedlings under suitable controlled light and temperature conditions.

(ii) grow the seedlings in appropriate field conditions and season, and time the climate room work to commence when normal field transplanting takes place.

Growing the seedlings under controlled conditions would be the most convenient. From the literature it would seem that the best conditions for the production of seedlings are long days (12 hrs) and warm conditions (20-25°C). Once tobacco seedlings have reached the 5-6 leaf stage they have reached a minimum leaf area adequate for the apex to respond to a floral stimulus. This is called "ripeness to flower" (Hopkinson & Hannam 1969). The "commital to flower" stage takes place next and under normal commercial tobacco seedling production this stage is effectively delayed until after transplanting by what is termed "hardening off". This involves clipping the seedlings(reducing leaf area), removing protective seedbed covers at night and keeping the watering of the seedlings to a minimum.

4:1:2 NUTRIENT APPLICATION:-

Because successful flue-cured tobacco production requires a declining plane of N and K nutrition, a special nutrient schedule had to be used and applied manually every day. A more convenient arrangement would have been to apply the nutrient solution via the capillary tubes provided for the automatic watering. This would have involved some sort of heading tank whereby the nutrient solutions could be mixed fresh and fed straight to the capillary tubes.

4:1:3 OVERCROWDING:-

One of the outstanding features of the tobacco plant is its enormous leaf area. A mature plant with 18 leaves can have a total leaf area of more than 2.5 m^2 (Garner 1946). This leads to difficulties in climate rooms as only a small number of plants can be housed in comparison to most other species (e.g. grasses, cereals). Some of the lower leaves of the plants in the climate rooms ripened prematurely because they were shaded. A better number to use per room would be about 18 plants, if they are to be harvested. This would give similar spacing to field grown tobacco where plants are grown at 0.38-0.61m spacing within rows and 1.07-1.22m between rows. Eighteen plants per room would also allow easier harvesting without damaging leaves on adjacent plants. Despite the difficulties of overcrowding experienced in the climate rooms, the experiment was quite successful from a plant breeding point of view.

4:1:4 CURING FACILITIES:-

Flue-curing tobacco is an art in itself. There are three distinct phases in flue-curing leaf once it has been harvested ripe. Stage one involves keeping the temperature between 33-38°C and under high humidity 80-95% whereby the enzymatic degradation of starch to sucrose and other soluble carbohydrates takes place. This is called yellowing. This process can take up to 3-4 days but is usually complete within 60 hours of commencement. The second stage is colour fixing whereby the enzymes are "killed" by increasing the temperature from around 38°C up to 57°C. The final stage is midrib drying which involves taking the temperature up to 70-80°C and maintaining it until the midrib is dry. The whole flue-curing process takes about 5-7 days. Unfortunately no curing facilities were available at Plant Physiology Division so a "simulated cure" was performed, by placing the leaves in a climate chamber at 35°C held at 85% humidity (see chapter 2). As the leaves yellowed they were removed and vac uum dried at 40°C. This process resulted in reasonably good flue-cured tobacco being produced. However the facilities are not very convenient for flue-curing and perhaps a better procedure would have been to examine starch and nicotine levels in freshly harvested green leaves. This would be far more straight forward and less prone to mistakes (i.e. a curing cabinet failure).

4:1:5 SUMMARY

This study brought to notice some recommendations which should be taken into careful consideration before future tobacco climate room studies are commenced.

(i) produce the tobacco seedlings under controlled conditions of light and temperature;

(ii) a more automated nutrient application would lessen the arduous task of the daily manual nutrient application;

(iii) future studies on tobacco should have a maximum of 18 plants per room if the leaf is to be harvest for further analysis;

(iv) tobacco studies should be organised so that analyses can be conducted on green leaf rather than cured leaf.

Climate room work on tobacco lends itself to studies involving pre-transplant and early post-transplant conditions; that is, while the plants are small and easily handled. Climate room work could also prove very useful in the detailed examination of important diseases of tobacco in New Zealand such as Verticillium wilt and Sclerotinia/Botrytis complex.

4:2 LATE SEASON NIGHT TEMPERATURES:-

The overall effect of varying late season night temperatures on the genotypes studied was negligible. This has been discussed in Chapter 3. However some G X E interaction was present for reducing sugars and specific leaf weight, indicating that the plants did respond differently to temperature differences both chemically and physically.

The lack of overall effect of night temperature differences could have been due to one or more of the following reasons:

1. The temperature differences were not wide enough. That is, instead of 10° 15° and 20°C they should have ranged from lower than 10° to higher than 20°C;

2. The temperature treatments were imposed too late to affect the development of the tobacco plants. If this is correct then one could conclude that as the plants get older they are less affected by environmental changes;

3. There were too many genotypes present to obtain a clear picture of the real effect of temperature differences between rooms.

Recommendations for future studies on the factors affecting the development of flue-cured tobacco can be made:

1. Repeat the experiment using only one genotype, taking into consideration the points raised in section 4:1:5;
2. Examine the effect of having a wider range of late season night temperatures;
3. Examine the effect of night temperatures imposed at different stages of growth. For example impose a night temperature of 10°C at transplanting, at the 10 leaf stage, and at flowering;
4. Varying day and night length from transplanting time or later could be another useful study on factors affecting the development of flue-cured tobacco. Because of the lattitude of New Zealand (42°S) it has been postulated by Weybrew and Woltz (1975) that the relatively high sugars in New Zealand tobacco could be due to our longer days (more photosynthesis) coupled with shorter cooler nights (less respiration).

4:3 PLANT BREEDING ASPECTS:-

The thesis study brought to notice some important points in conducting tobacco breeding studies in climate rooms. Some of these points have been discussed earlier in Chapter 4.

These results were in general agreement with other tobacco diallel studies. That is, there is a predominance of additive genetic variance present for the characters measured. The single plant plots gave comparable coefficients of variation to field plots involving around 30 plants/plot.

The study then demonstrates that tobacco breeding studies in climate rooms can be carried out successfully, and that such results may have practical applications.

As mentioned previously in Chapter 4, the serious limitation involving tobacco breeding climate room studies is the lack of room to test a large number of genotypes, due to the large size of the plant at maturity. Future studies which required harvesting of the leaf should be more spaced out than in the present experiment. A convenient number of plants would be 18 or 20; say, 3 replications of 6 genotypes, or 4 replications of 5 genotypes.

CONCLUSIONS:-

1. The influence of controlled variations in night temperature applied after flowering took place in flue-cured tobacco genotypes was negligible on the fourteen characters measured, only one being significant. However genotype x environment interactions did occur for some of the characters examined.
2. The genetic analysis of the diallel indicates that general combining ability variance is the main component present for the fourteen characters measured. This agrees with numerous tobacco diallel studies carried out by other workers. As general combining ability is largely a measure of additive genetic variance, this indicates that selection for homozygous pure lines is the best breeding method to follow.
3. Although general combining ability variance estimates were large for all characters, specific combining ability variance estimates were significant for six of these characters.
4. Four estimates of general combining ability x environments were significant, but in all cases the variance component estimates were smaller than their respective variance estimates.
5. Many of the characters examined had moderate to high narrow sense heritability values, indicating that considerable progress can be made by selecting for these characters in segregating populations.
6. There is considerable divergence among the five diallel parents for general combining ability effects of the characters examined. Kutsaga 51 and Burley 1 appear to be good general combiners for most characters.
7. General combining ability and phenotypic correlations are in good agreement. This indicates that selection on the phenotype for specific characters will result in a similar change in direction of the genotype. The correlations presented in this work show similar trends to those found by other workers for the same characters.
8. A number of recommendations are made on the conduct of future tobacco climate room studies. These include different approaches to the study of night temperature effects, and ways to produce plants which are more akin to field grown tobacco.

APPENDIX

APPENDIX I

Concentrations of stock chemicals

Stock		Solutions ***					
Compound	Conc	A	B	C	D	E	F
		mls stock/60 litres					
$Ca(NO_3)_2$	1 M	270	270	300	253.5	126.6	...
$Ca(H_2PO_4)_2.H_2O$	0.05 M	600	600	...	600	600	600
$Ca(OH)_2$	1 M	...	...	...	16.5	143.4	270
KNO_3	1 M	1080	720	300	...	...	...
KH_2PO_4	1 M	...	...	60	...	...	...
K_2SO_4	0.25 M	...	...	...	360	180	...
$Na\ NO_3$	2 M	720	390	60	...	...	...
$Mg\ SO_4$	1 M	120	120	120	120	120	120
MICRO *		60	60	60	60	60	60
IRON **		60	60	60	60	60	60

* Micro Stock	g/litre stock
H_3BO_3	4.29
$Mn\ CL_2.4H_2O$	2.71
$Zn\ SO_4.7H_2O$	0.33
$Cu\ SO_4.5H_2O$	0.12
$H_2MoO_4.H_2O$	0.03

** Fe at 80 ppm. stock as Fe EDTA

*** each plant received 500 mls of the current sequential nutrient solution daily.

APPENDIX II

Variance component estimates for single environments *

Source	Variance component estimates
Blocks	$(MS_4 - MS_1) / [g(g-1)/2]$
Gca	$(MS_3 - MS_2) / r(g-2)$
Sca	$(MS_2 - MS_1) / r$
ERROR	MS_1

where $r = 3$ $g = 5$

* Variance component estimates were derived from mean square expectations from the analysis of variance (after Comstock & Moll 1963).

APPENDIX III

Standard error estimates for single environments *

$$\text{S.E. gca} = \sqrt{\frac{1}{[r(g-2)]^2}\left[\frac{2\,(\text{MS gca})^2}{(g-1)+2} + \frac{2\,(\text{MS sca})^2}{[g(g-3)/2]+2}\right]}$$

$$\text{S.E. sca} = \sqrt{\frac{1}{r^2}\left[\frac{2\,(\text{MS sca})^2}{[g(g-3)/2]+2} + \frac{2\,(\text{MS error})^2}{[(r-1)((g(g-1)/2)-1)]+2}\right]}$$

$$\text{S.E.}_B = \sqrt{\frac{1}{[g(g-1)/2]^2}\left[\frac{2\,(\text{MS}_B)^2}{(r-1)+2} + \frac{2\,(\text{MS error})^2}{[(r-1)((g(g-1)/2)-1)]+2}\right]}$$

$$\text{S.E. error} = \sqrt{\frac{2\,(\text{MS error})^2}{[(r-1)((g(g-1)/2)-1)]+2}}$$

where $r = 3$ and $g = 5$

* after Crump 1946

APPENDIX IV
Variance components for pooled environments

Source	Variance component estimates*
Environments	$[(MS_1 + MS_7) - (MS_2 + MS_5)] / (rg\ (g-1)/2)$
Blocks within environments	$(MS_2 - MS_7) / (g\ (g-1)/2)$
Gca	$[(MS_3 + MS_6) - (MS_4 + MS_5)] / (re\ (g-2))$
Sca	$(MS_4 - MS_6) / (re)$
Gca x environment	$(MS_5 - MS_6) / (r\ (g-2))$
Sca x environment	$(MS_6 - MS_7) / r$
Error	MS_7

where $r = 3$
$e = 3$
$g = 5$

* Variance component estimates were derived from mean square expectations from the analysis of variance for pooled environments (after Comstock & Moll 1963)

APPENDIX V

Standard error estimates for pooled environments *

$$\mathrm{S.E.}_{E} = \sqrt{\frac{1}{[rg(g-1)/2]^2}\left[\frac{2\,(MS_1)^2}{(e-1)+2} + \frac{2\,(MS_2)^2}{[e(r-1)]+2} + \frac{2\,(MS_5)^2}{[(g-1)(e-1)]+2} + \frac{2\,(MS_7)^2}{[e(r-1)((g(g-1)/2)-1)]+2}\right]}$$

$$\mathrm{S.E.}_{B(E)} = \sqrt{\frac{1}{[g(g-1)/2]^2}\left[\frac{2\,(MS_2)^2}{[e(r-1)]+2} + \frac{2\,(MS_7)^2}{[e(r-1)((g(g-1)/2)-1)]+2}\right]}$$

$$\mathrm{S.E.}_{gca} = \sqrt{\frac{1}{[re(g-2]^2}\left[\frac{2\,(MS_3)^2}{(g-1)+2} + \frac{2\,(MS_4)^2}{[g(g-3)/2]+2} + \frac{2\,(MS_5)^2}{[(g-1)(e-1)]+2} + \frac{2\,(MS_6)^2}{[(g(g-3)/2)(e-1)]+2}\right]}$$

$$\mathrm{S.E.}_{sca} = \sqrt{\frac{1}{(re)^2}\left[\frac{2\,(MS_4)^2}{[g(g-3)/2]+2} + \frac{2\,(MS_6)^2}{[(g(g-3)/2(e-1)]+2}\right]}$$

$$\mathrm{S.E.}_{gca\ x\ e} = \sqrt{\frac{1}{[r(g-2)]^2}\left[\frac{2\,(MS_5)^2}{[(g-1)(e-1)]+2} + \frac{2\,(MS_6)^2}{[(g(g-3)/2)(e-1)]+2}\right]}$$

$$\mathrm{S.E.}_{sca\ x\ e} = \sqrt{\frac{1}{r^2}\left[\frac{2\,(MS_6)^2}{[(g(g-3)/2)(e-1)]+2} + \frac{2\,(MS_7)^2}{[e(r-1)((g(g-1)/2-1)]+2}\right]}$$

$$\mathrm{S.E.\ error} = \sqrt{\frac{2\,(MS_7)^2}{[e(r-1)((g(g-1)/2)-1)]+2}}$$

Where $r = 3$
$g = 5$
$e = 3$

* after Crump 1946

APPENDIX VI

Analysis of variance for environment 1 (20°C)

(1) Leaf width

Source	DF	Mean Square	F-Test	Signif
Blocks	2	1.5989	0.58	NS
Gca	4	19.7682	4.16	(NS)
Sca	5	4.7512	1.72	NS
ERROR	18	2.7598		

Standard deviation = 1.6613

Coeff of variation = 0.0758

S.E. parent means = 0.4796

S.E. Cross means = 0.9591

(2) Leaf length

Source	DF	Mean Square	F-Test	Signif
Blocks	2	3.1271	0.76	NS
Gca	4	33.8909	4.15	(NS)
Sca	5	8.1714	1.98	NS
ERROR	18	4.1180		

Standard deviation = 2.0293

Coeff of variation = 0.0334

S.E. parent means = 0.5858

S.E. cross means = 1.1716

(3) Leaf area

Source	DF	Mean Square	F-Test	Signif
Blocks	2	2723.7000	0.44	NS
Gca	4	59416.6445	6.58	*
Sca	5	9035.2444	1.47	NS
ERROR	18	6144.9222		

Standard deviation = 78.3896

Coeff of variation = 0.0896

S.E. parent means = 22.6291

S.E. cross means =45.2582

(4) Leaf length

Source	DF	Mean Square	F-Test	Signif
Blocks	2	0.0696	0.86	NS
Gca	4	0.4258	3.17	NS
Sca	5	0.1341	1.66	NS
ERROR	18	0.0808		

Standard deviation = 0.2843 Coeff of variation = 0.0944
S.E. parent means = 0.0821 S.E. cross means = 0.1641

(5) Specific leaf weight

Source	DF	Mean Square	F-Test	Signif
Blocks	2	0.6007	2.42	NS
Gca	4	3.3985	5.63	*
Sca	5	0.6032	2.43	(NS)
ERROR	18	0.2482		

Standard deviation = 0.4982 Coeff of variation = 0.0828
S.E. parent means = 0.1438 S.E. cross means = 0.2876

(6) Yield

Source	DF	Mean Square	F-Test	Signif
Blocks	2	95.8134	2.84	(NS)
Gca	4	2199.1326	14.36	**
Sca	5	153.1828	4.53	**
ERROR	18	33.7927		

Standard deviation = 5.8131 Coeff of variation = 0.0865
S.E. parent means = 1.6781 S.E. cross means = 3.3562

(7) Cured weight/leaf

Source	DF	Mean Square	F-Test	Signif
Blocks	2	0.3629	2.01	NS
Gca	4	2.8128	7.44	*
Sca	5	0.3781	2.09	NS
ERROR	18	0.1809		

Standard deviation = 0.4253 Coeff of variation = 0.0934
S.E. parent means = 0.1228 S.E. cross means = 0.2456

(8) Reducing sugar %

Source	DF	Mean Square	F-Test	Signif
Blocks	2	11.5089	2.09	NS
Gca	4	40.6591	9.79	*
Sca	5	4.1512	0.75	NS
ERROR	18	5.5091		

Standard deviation = 2.3471 Coeff of variation = 0.1729
S.E. parent means = 0.6776 S.E. cross means = 1.3551

(9) Reducing sugar % - top six leaves

Source	DF	Mean Square	F-Test	Signif
Blocks	2	7.6557	1.20	NS
Gca	4	70.2898	5.03	(NS)
Sca	5	13.9734	2.19	NS
ERROR	18	6.3767		

Standard deviation = 2.5252 Coeff of variation = 0.1122
S.E. parent means = 0.7290 S.E. cross means = 1.4579

(10) Total nicotine alkaloids %

Source	DF	Mean Square	F-Test	Signif
Blocks	2	0.0067	0.27	NS
Gca	4	0.8353	12.76	**
Sca	5	0.0654	2.60	(NS)
ERROR	18	0.0252		

Standard deviation = 0.1588

Coeff of variation = 0.1437

S.E. parent means = 0.0458

S.E. cross means = 0.0917

(11) Days to flower (from transplanting)

Source	DF	Mean Square	F-Test	Signif
Blocks	2	0.0333	0.01	NS
Gca	4	49.2556	8.27	*
Sca	5	5.9556	1.24	NS
ERROR	18	4.8111		

Standard deviation = 2.1934

Coeff of variation = 0.0363

S.E. parent means = 0.6332

S.E. cross means = 1.2664

(12) Stem height

Source	DF	Mean Square	F-Test	Signif
Blocks	2	0.5333	0.00	NS
Gca	4	223.0333	1.20	NS
Sca	5	186.1667	1.26	NS
ERROR	18	148.0519		

Standard deviation = 12.1677

Coeff of variation = 0.1037

S.E. parent means = 3.5125

S.E. cross means = 7.0250

(13) Number of leaves

Source	DF	Mean Square	F-Test	Signif
Blocks	2	0.5333	0.34	NS
Gca	4	29.9667	17.29	**
Sca	5	1.7333	1.10	NS
ERROR	18	1.5704		

Standard deviation = 1.2531   Coeff of variation = 0.0839

S.E. parent means = 0.3618   S.E. cross means = 0.7235

(14) Stem weight

Source	DF	Mean Square	F-Test	Signif
Blocks	2	87.1004	0.99	NS
Gca	4	1725.5204	7.41	*
Sca	5	232.8881	2.65	(NS)
ERROR	18	87.7533		

Standard deviation = 9.3677   Coeff of variation = 0.1148

S.E. parent means = 2.7042   S.E. cross means = 5.4084

APPENDIX VII

Analysis of variance for environment 2 (15°C)

(1) Leaf width

Source	DF	Mean Square	F-Test	Signif
Blocks	2	3.3317	3.56	*
Gca	4	14.8148	23.74	***
Sca	5	0.6240	0.67	NS
ERROR	18	0.9353		

Standard deviation = 0.9671 Coeff of variation = 0.0431

S.E. parent means = 0.2792 S.E. cross means = 0.5584

(2) Leaf length

Source	DF	Mean Square	F-Test	Signif
Blocks	2	0.1664	0.03	NS
Gca	4	48.2913	10.21	*
Sca	5	4.7289	0.76	NS
ERROR	18	6.2222		

Standard deviation = 2.4944 Coeff of variation = 0.0411

S.E. parent means = 0.7201 S.E. cross means = 1.4402

(3) Leaf area

Source	DF	Mean Square	F-Test	Signif
Blocks	2	3827.2333	0.87	NS
Gca	4	37908.1444	19.46	***
Sca	5	1947.8111	0.44	NS
ERROR	18	4393.3815		

Standard deviation = 66.2826　　Coeff of variation = 0.0743
S.E. parent means = 19.1341　　S.E. cross means = 38.2683

(4) Leaf length: leaf width

Source	DF	Mean Square	F-Test	Signif
Blocks	2	0.0731	5.24	*
Gca	4	0.3343	9.23	*
Sca	5	0.0362	2.60	(NS)
ERROR	18	0.0140		

Standard deviation = 0.1181　　Coeff of variation = 0.0405
S.E. parent means = 0.0341　　S.E. cross means = 0.0682

(5) Specific leaf weight

Source	DF	Mean Square	F-Test	Signif
Blocks	2	1.6082	2.69	(NS)
Gca	4	3.0491	16.09	**
Sca	5	0.1895	0.32	NS
ERROR	18	0.5971		

Standard deviation = 0.7727　　Coeff of variation = 0.1216
S.E. parent means = 0.2231　　S.E. cross means = 0.4461

(6) Yield

Source	DF	Mean Square	F-Test	Signif
Blocks	2	111.3781	1.28	NS
Gca	4	1669.2888	34.20	***
Sca	5	48.8076	0.56	NS
ERROR	18	86.8633		

Standard deviation = 9.3200 Coeff of variation = 0.1343
S.E. parent means = 2.6905 S.E. cross means = 5.3809

(7) Cured weight/leaf

Source	DF	Mean Square	F-Test	Signif
Blocks	2	2.6253	4.07	*
Gca	4	1.8046	8.49	*
Sca	5	0.2125	0.33	NS
ERROR	18	0.6452		

Standard deviation = 0.8032 Coeff of variation = 0.1616
S.E. parent means = 0.2319 S.E. cross means = 0.4637

(8) Reducing sugars %

Source	DF	Mean Square	F-Test	Signif
Blocks	2	24.3601	4.15	*
Gca	4	33.1621	16.40	**
Sca	5	2.0216	0.34	NS
ERROR	18	5.8658		

Standard deviation = 2.4219 Coeff of variation = 0.1676
S.E. parent means = 0.6992 S.E. cross means = 1.3983

(9) Reducing sugar % - top six leaves

Source	DF	Mean Square	F-Test	Signif
Blocks	2	19.6223	4.14	*
Gca	4	55.9486	4.81	(NS)
Sca	5	11.6293	2.46	(NS)
ERROR	18	4.7349		

Standard deviation = 2.1760 Coeff of variation = 0.0942
S.E. parent means = 0.6282 S.E. cross means = 1.2563

(10) Total nicotine alkaloids %

Source	DF	Mean Square	F-Test	Signif
Blocks	2	0.0674	1.81	NS
Gca	4	0.9454	77.14	****
Sca	5	0.0123	0.33	NS
ERROR	18	0.0372		

Standard deviation = 0.1930 Coeff of variation = 0.1612
S.E. parent means = 0.0557 S.E. cross means = 0.1114

(11) Days to flower (from transplanting)

Source	DF	Mean Square	F-Test	Signif
Blocks	2	10.8000	0.88	NS
Gca	4	116.8111	16.12	**
Sca	5	7.2444	0.59	NS
ERROR	18	12.2074		

Standard deviation = 3.4939 Coeff of variation = 0.0584
S.E. parent means = 1.0086 S.E. cross means = 2.0172

(12) Stem height

Source	DF	Mean Square	F-Test	Signif
Blocks	2	506.1000	5.17	*
Gca	4	79.6111	0.60	NS
Sca	5	132.1778	1.35	NS
ERROR	18	97.9148		

Standard deviation = 9.8952
Coeff of variation = 0.0908
S.E. parent means = 2.8565
S.E. cross means = 5.7130

(13) Number of leaves

Source	DF	Mean Square	F-Test	Signif
Blocks	2	10.0333	9.06	***
Gca	4	57.1333	19.26	***
Sca	5	2.9667	2.68	(NS)
ERROR	18	1.1074		

Standard deviation = 1.0523
Coeff of variation = 0.0739
S.E. parent means = 0.3038
S.E. cross means = 0.6076

(14) Stem weight

Source	DF	Mean Square	F-Test	Signif
Blocks	2	155.0094	1.17	NS
Gca	4	1272.9539	12.54	**
Sca	5	101.5326	0.77	NS
ERROR	18	132.2308		

Standard deviation = 11.4992
Coeff of variation = 0.1474
S.E. parent means = 3.3195
S.E. cross means = 6.6390

APPENDIX VIII

Analysis of variance for environment 3 (10°C)

(1) Leaf width

Source	DF	Mean Square	F-Test	Signif
Blocks	2	1.6028	2.27	NS
Gca	4	19.9336	41.07	***
Sca	5	0.4854	0.69	NS
ERROR	18	0.7068		

Standard deviation = 0.8407 Coeff of variation = 0.0384

S.E. parent means = 0.2427 S.E. cross means = 0.4854

(2) Leaf length

Source	DF	Mean Square	F-Test	Signif
Blocks	2	4.9735	1.00	NS
Gca	4	28.6642	1.85	NS
Sca	5	15.5132	3.12	*
ERROR	18	4.9665		

Standard deviation = 2.2286 Coeff of variation = 0.0363

S.E. parent means = 0.6433 S.E. cross means = 1.2867

(3) Leaf area

Source	DF	Mean Square	F-Test	Signif
Blocks	2	5990.7000	1.99	NS
Gca	4	48878.1111	20.56	***
Sca	5	2376.8444	0.79	NS
ERROR	18	3007.6630		

Standard deviation = 54.8422 Coeff of variation = 0.0623

S.E. parent means = 15.8316 S.E. cross means = 31.6631

(4) Leaf length:leaf width

Source	DF	Mean Square	F-Test	Signif
Blocks	2	0.0166	0.64	NS
Gca	4	0.4739	5.15	(NS)
Sca	5	0.0920	3.54	*
ERROR	18	0.0260		

Standard deviation = 0.1613　　Coeff of variation = 0.0532

S.E. parent means = 0.0466　　S.E. cross means = 0.0931

(5) Specific leaf weight

Source	DF	Mean Square	F-Test	Signif
Blocks	2	0.0718	0.86	NS
Gca	4	1.6192	7.84	*
Sca	5	0.2066	2.46	(NS)
ERROR	18	0.0838		

Standard deviation = 0.2896　　Coeff of variation = 0.0475

S.E. parent means = 0.0836　　S.E. cross means = 0.1672

(6) Yield

Source	DF	Mean Square	F-Test	Signif
Blocks	2	83.3089	3.70	*
Gca	4	1686.2052	12.18	*
Sca	5	138.4812	6.15	***
ERROR	18	22.2530		

Standard deviation = 4.7458　　Coeff of variation = 0.0699

S.E. parent means = 1.3700　　S.E. cross means = 2.7400

(7) Cured weight/leaf

Source	DF	Mean Square	F-Test	Signif
Blocks	2	0.6394	5.17	*
Gca	4	1.8609	9.20	*
Sca	5	0.2023	1.63	NS
ERROR	18	0.1237		

Standard deviation = 0.3518 Coeff of variation = 0.0760

S.E. parent means = 0.1015 S.E. cross means = 0.2031

(8) Reducing sugars %

Source	DF	Mean Square	F-Test	Signif
Blocks	2	1.1792	0.44	NS
Gca	4	50.2662	16.77	**
Sca	5	2.9971	1.13	NS
ERROR	18	2.6581		

Standard deviation = 1.6304 Coeff of variation = 0.1193

S.E. parent means = 0.4706 S.E. cross means = 0.9413

(9) Reducing sugar % - top six leaves

Source	DF	Mean Square	F-Test	Signif
Blocks	2	22.3614	6.43	**
Gca	4	46.9489	3.26	NS
Sca	5	14.3967	4.14	*
ERROR	18	3.4760		

Standard deviation = 1.8644 Coeff of variation = 0.0813

S.E. parent means = 0.5382 S.E. cross means = 1.0764

(10) Total nicotine alkaloids %

Source	DF	Mean Square	F-Test	Signif
Blocks	2	0.2719	8.97	***
Gca	4	0.8326	13.28	**
Sca	5	0.0627	2.07	NS
ERROR	18	0.0303		

Standard deviation = 0.1741 Coeff of variation = 0.1551

S.E. parent means = 0.0503 S.E. cross means = 0.1005

(11) Days to flower (from transplanting)

Source	DF	Mean Square	F-Test	Signif
Blocks	2	13.4333	1.35	NS
Gca	4	128.4222	12.90	**
Sca	5	9.9556	1.00	NS
ERROR	18	9.9519		

Standard deviation = 3.1547 Coeff of variation = 0.0513

S.E. parent means = 0.9107 S.E. cross means = 1.8213

(12) Stem height

Source	DF	Mean Square	F-Test	Signif
Blocks	2	108.3000	1.26	NS
Gca	4	343.7000	3.06	NS
Sca	5	112.1667	1.30	NS
ERROR	18	86.0037		

Standard deviation = 9.2738 Coeff of variation = 0.0768

S.E. parent means = 2.6771 S.E. cross means = 5.3542

(13) Number of leaves

Source	DF	Mean Square	F-Test	Signif
Blocks	2	4.3000	4.45	*
Gca	4	34.4778	35.26	***
Sca	5	0.9778	1.01	NS
ERROR	18	0.9667		

Standard deviation = 0.9832
Coeff of variation = 0.0647
S.E. parent means = 0.2838
S.E. cross means = 0.5676

(14) Stem weight

Source	DF	Mean Square	F-Test	Signif
Blocks	2	0.9030	0.02	NS
Gca	4	1641.8189	12.00	*
Sca	5	136.8188	2.49	(NS)
ERROR	18	55.0077		

Standard deviation = 7.4167
Coeff of variation = 0.0898
S.E. parent means = 2.1410
S.E. cross means = 4.2820

APPENDIX IX

Variance component estimates, standard errors, and ratios to error for environment 1 (20°C)

(1) Leaf width

Source	Estimate	S.E.	Ratio to error
Blocks	-0.1161	0.1428	-0.04
Gca	1.6686	1.2991	0.60
Sca	0.6638	0.8951	0.24
ERROR	2.7598	0.8727	

(2) Leaf length

Source	Estimate	S.E.	Ratio to error
Blocks	-0.0991	0.2566	-0.02
Gca	2.8577	2.2276	0.69
Sca	1.3511	1.5193	0.33
ERROR	4.1180	1.3022	

(3) Leaf area

Source	Estimate	S.E.	Ratio to error
Blocks	-342.1222	273.5924	-0.06
Gca	5597.9333	3849.1681	0.91
Sca	963.4407	1735.2706	0.16
ERROR	6144.9222	1943.1950	

(4) Leaf length: leaf width

Source	Estimate	S.E.	Ratio to error
Blocks	-0.0011	0.0055	-0.01
Gca	0.0324	0.0285	0.40
Sca	0.0178	0.0254	0.22
ERROR	0.0808	0.0256	

(5) Specific leaf weight

Source	Estimate	S.E.	Ratio to error
Blocks	0.0353	0.0432	0.14
Gca	0.3106	0.2209	1.25
Sca	0.1183	0.1106	0.48
ERROR	0.2482	0.0785	

(6) Cured weight/plant

Source	Estimate	S.E.	Ratio to error
Blocks	6.2021	6.8588	0.18
Gca	227.3278	141.3675	6.73
Sca	39.7967	27.5247	1.18
ERROR	33.7927	10.6862	

(7) Yield

Source	Estimate	S.E.	Ratio to error
Blocks	0.0182	0.0263	0.10
Gca	0.2705	0.1818	1.50
Sca	0.0657	0.0700	0.36
ERROR	0.1809	0.0572	

(4) Leaf length: leaf width

Source	Estimate	S.E.	Ratio to error
Blocks	-0.0011	0.0055	-0.01
Gca	0.0324	0.0285	0.40
Sca	0.0178	0.0254	0.22
ERROR	0.0808	0.0256	

(5) Specific leaf weight

Source	Estimate	S.E.	Ratio to error
Blocks	0.0353	0.0432	0.14
Gca	0.3106	0.2209	1.25
Sca	0.1183	0.1106	0.48
ERROR	0.2482	0.0785	

(6) Yield

Source	Estimate	S.E.	Ratio to error
Blocks	6.2021	6.8588	0.18
Gca	227.3278	141.3675	6.73
Sca	39.7967	27.5247	1.18
ERROR	33.7927	10.6862	

(7) Cured weight/leaf

Source	Estimate	S.E.	Ratio to error
Blocks	0.0182	0.0263	0.10
Gca	0.2705	0.1818	1.50
Sca	0.0657	0.0700	0.36
ERROR	0.1809	0.0572	

(8) Reducing sugar %

Source	Estimate	S.E.	Ratio to error
Blocks	0.6000	0.8322	0.11
Gca	4.0564	2.6199	0.74
Sca	-0.4526	0.9404	-0.08
ERROR	5.5091	1.7421	

(9) Reducing sugar % - top six leaves

Source	Estimate	S.E.	Ratio to error
Blocks	0.1279	0.5777	0.02
Gca	6.2574	4.5848	0.98
Sca	2.5322	2.5788	0.40
ERROR	6.3767	2.0165	

(10) Total nicotine alkaloids %

Source	Estimate	S.E.	Ratio to error
Blocks	-0.0019	0.0009	-0.07
Gca	0.0855	0.0537	3.39
Sca	0.0134	0.0120	0.53
ERROR	0.0252	0.0080	

(11) Days to flower (from transplanting)

Source	Estimate	S.E.	Ratio to error
Blocks	-0.4778	0.1522	-0.10
Gca	4.8111	3.1795	1.00
Sca	0.3815	1.1761	0.08
ERROR	4.8111	1.5214	

(12) Stem height

Source	Estimate	S.E.	Ratio to error
Blocks	-14.7519	4.6820	-0.10
Gca	4.0963	18.0820	0.03
Sca	12.7049	36.6579	0.09
ERROR	148.0519	46.8181	

(13) Number of leaves

Source	Estimate	S.E.	Ratio to error
Blocks	-0.1037	0.0624	-0.07
Gca	3.1370	1.9251	2.00
Sca	0.0543	0.3504	0.03
ERROR	1.5704	0.4966	

(14) Stem weight

Source	Estimate	S.E.	Ratio to error
Blocks	-0.0653	6.7552	-0.00
Gca	165.8480	111.5530	1.89
Sca	48.3783	42.5131	0.55
ERROR	87.7533	27.7500	

APPENDIX X

Variance component estimates, standard errors, and ratios to error for environment 2 (15°C)

(1) Leaf width

Source	Estimate	S.E.	Ratio to error
Blocks	0.2396	0.2374	0.26
Gca	1.5768	0.9511	1.69
Sca	-0.1038	0.1486	-0.11
ERROR	0.9353	0.2958	

(2) Leaf length

Source	Estimate	S.E.	Ratio to error
Blocks	-0.6056	0.1971	-0.10
Gca	4.8403	3.1106	0.78
Sca	-0.4978	1.0678	-0.08
ERROR	6.2222	1.9676	

(3) Leaf area

Source	Estimate	S.E.	Ratio to error
Blocks	-56.6148	304.2045	-0.01
Gca	3995.5926	2434.5586	0.91
Sca	-815.1901	578.7123	-0.19
ERROR	4393.3815	1389.3092	

(4) Leaf length: leaf width

Source	Estimate	S.E.	Ratio to error
Blocks	0.0059	0.0052	0.42
Gca	0.0331	0.0216	2.37
Sca	0.0074	0.0066	0.53
ERROR	0.0140	0.0044	

(5) Specific leaf weight

Source	Estimate	S.E.	Ratio to error
Blocks	0.1011	0.1153	0.17
Gca	0.3177	0.1959	0.53
Sca	-0.1359	0.0714	-0.23
ERROR	0.5971	0.1888	

(6) Yield

Source	Estimate	S.E.	Ratio to error
Blocks	2.4515	8.3409	0.03
Gca	180.0535	107.1242	2.07
Sca	-12.6852	12.6278	-0.15
ERROR	86.8633	27.4686	

(7) Cured weight/leaf

Source	Estimate	S.E.	Ratio to error
Blocks	0.1980	0.1868	0.31
Gca	0.1769	0.1164	0.27
Sca	-0.1442	0.0778	-0.22
ERROR	0.6452	0.2040	

(8) Reducing sugars %

Source	Estimate	S.E.	Ratio to error
Blocks	1.8494	1.7325	0.32
Gca	3.4601	2.1307	0.59
Sca	-1.2814	0.7156	-0.22
ERROR	5.8658	1.8549	

(9) Reducing sugar % - top six leaves

Source	Estimate	S.E.	Ratio to error
Blocks	1.4887	1.3956	0.31
Gca	4.9244	3.6550	1.04
Sca	2.2981	2.1313	0.49
ERROR	4.7349	1.4973	

(10) Total nicotine alkaloids %

Source	Estimate	S.E.	Ratio to error
Blocks	0.0030	0.0049	0.08
Gca	0.1037	0.0607	2.78
Sca	-0.0083	0.0045	-0.22
ERROR	0.0372	0.0118	

(11) Days to flower (from transplanting)

Source	Estimate	S.E.	Ratio to error
Blocks	-0.1407	0.8557	-0.01
Gca	12.1741	7.5058	1.00
Sca	-1.6543	1.8226	-0.14
ERROR	12.2074	3.8603	

(12) Stem height

Source	Estimate	S.E.	Ratio to error
Blocks	40.8185	35.9204	0.42
Gca	-5.8407	9.3653	-0.06
Sca	11.4210	25.7130	0.12
ERROR	97.9148	30.9634	

(13) Number of leaves

Source	Estimate	S.E.	Ratio to error
Blocks	0.8926	0.7103	0.81
Gca	6.0185	3.6693	5.43
Sca	0.6198	0.5413	0.56
ERROR	1.1074	0.3502	

(14) Stem weight

Source	Estimate	S.E.	Ratio to error
Blocks	2.2779	11.7313	0.02
Gca	130.1579	81.8824	0.98
Sca	-10.2327	22.8373	-0.08
ERROR	132.2308	41.8150	

APPENDIX XI

Variance component estimates, standard errors, and ratios to error for environment 3 (10°C)

(1) Leaf width

Source	Estimate	S.E.	Ratio to error
Blocks	0.0896	0.1155	0.13
Gca	2.1609	1.2791	3.06
Sca	-0.0738	0.1141	-0.10
ERROR	0.7068	0.2235	

(2) Leaf length

Source	Estimate	S.E.	Ratio to error
Blocks	0.0007	0.3852	0.00
Gca	1.4612	2.0567	0.29
Sca	3.5156	2.8132	0.71
ERROR	4.9665	1.5705	

(3) Leaf area

Source	Estimate	S.E.	Ratio to error
Blocks	298.3037	434.1526	0.10
Gca	5166.8074	3138.7183	1.72
Sca	-210.2728	529.0153	-0.07
ERROR	3007.6630	951.1065	

(4) Leaf length: leaf width

Source	Estimate	S.E.	Ratio to error
Blocks	-0.0009	0.0014	-0.04
Gca	0.0424	0.0309	1.63
Sca	0.0220	0.0166	0.85
ERROR	0.0260	0.0082	

(5) Specific leaf weight

Source	Estimate	S.E.	Ratio to error
Blocks	-0.0012	0.0057	-0.01
Gca	0.1570	0.1046	1.87
Sca	0.0409	0.0379	0.49
ERROR	0.0838	0.0265	

(6) Yield

Source	Estimate	S.E.	Ratio to error
Blocks	6.0786	5.9337	0.27
Gca	171.9693	108.4823	7.64
Sca	38.6527	24.7877	1.72
ERROR	22.5230	7.1224	

(7) Cured weight/leaf

Source	Estimate	S.E.	Ratio to error
Blocks	0.0516	0.0454	0.42
Gca	0.1843	0.1200	1.49
Sca	0.0262	0.0391	0.21
ERROR	0.1237		

(8) Reducing sugars %

Source	Estimate	S.E.	Ratio to error
Blocks	-0.1479	0.1184	-0.06
Gca	5.2521	3.2295	1.98
Sca	0.1130	0.6031	0.04
ERROR	2.6581	0.8406	

(9) Reducing sugar % - top six leaves

Source	Estimate	S.E.	Ratio to error
Blocks	1.8885	1.5850	0.54
Gca	3.6169	3.1308	1.04
Sca	3.6402	2.5912	1.05
ERROR	3.4760	1.0992	

(10) Total nicotine alkaloids %

Source	Estimate	S.E.	Ratio to error
Blocks	0.0242	0.0193	0.80
Gca	0.0855	0.0535	2.82
Sca	0.0108	0.0116	0.36
ERROR	0.0303	0.0096	

(11) Days to flower (from transplanting)

Source	Estimate	S.E.	Ratio to error
Blocks	0.3481	1.0007	0.03
Gca	13.1630	8.2595	1.32
Sca	0.0012	2.0608	0.00
ERROR	9.9519	3.1471	

(12) Stem height

Source	Estimate	S.E.	Ratio to error
Blocks	2.2296	8.1266	0.03
Gca	25.7259	23.0328	0.30
Sca	8.7210	21.9452	0.10
ERROR	86.0037	27.1968	

(13) Number of leaves

Source	Estimate	S.E.	Ratio to error
Blocks	0.3333	0.3056	0.34
Gca	3.7222	2.2125	3.85
Sca	0.0037	0.2018	0.00
ERROR	0.9667	0.3057	

(14) Stem weight

Source	Estimate	S.E.	Ratio to error
Blocks	-5.4105	1.7407	-0.10
Gca	167.2222	105.6357	3.04
Sca	27.2704	25.0577	0.50
ERROR	55.0077	17.3950	

APPENDIX XII
Analysis of variance for pooled environments

(1) Leaf width

Source	Mean Square	F-Test	Signif	Test DF
Envir.	2.7811	0.67	NS	(5,14)
Blk (E)	2.1778	1.48	NS	
Gca	46.1495	8.52	***	(4,12)
Sca	1.4901	0.68	NS	
Gca-E	4.1835	1.91	NS	
Sca-E	2.1852	1.49	NS	
ERROR	1.4673			

Standard deviation = 1.2113 Coeff of Variation = 0.0548

S.E. parent means = 0.2019 S.E. cross means = 0.4038

(2) Leaf length

Source	Mean Square	F-Test	Signif	Test DF
Envir.	3.4971	1.07	NS	(11,14)
Blk (E)	2.7556	0.54	NS	
Gca	100.2985	5.06	*	(5,8)
Sca	15.8114	2.51	NS	
Gca-E	5.2739	0.84	NS	
Sca-E	6.3010	1.23	NS	
ERROR	5.1022			

Standard deviation = 2.2588 Coeff of Variation = 0.0371

S.E. parent means = 0.3765 S.E. Cross means = 0.7529

(3) Leaf area

Source	Mean Square	F-Test	Signif	Test DF
Envir.	2324.6779	0.44	NS	(15,13)
Blk (E)	4180.5444	0.93	NS	
Gca	123642.1927	9.97	***	(4,10)
Sca	1706.7371	0.29	NS	
Gca-E	11280.3537	1.94	NS	
Sca-E	5826.5815	1.29	NS	
ERROR	4515.3222			

Standard deviation = 67.1961

Coeff of variation = 0.0762

S.E. parent means = 11.1994

S.E. cross means = 22.3987

(4) Leaf length:leaf width

Envir	0.1140	1.55	NS	(4,13)
Blk (E)	0.0531	1.32	NS	
Gca	1.1410	4.92	*	(4,7)
Sca	0.1927	5.53	*	
Gca-E	0.0465	1.33	NS	
Sca-E	0.0348	0.87	NS	
ERROR	0.0403			

Standard deviation = 0.2007

Coeff of variation = 0.0672

S.E. parent means = 0.0334

S.E. cross means = 0.0669

(5) Specific leaf weight

Source	Mean Square	F-Test	Signif	Test DF
Envir	0.9271	0.90	NS	(4,13)
Blk (E)	0.7602	2.45	*	
Gca	6.8539	5.49	**	(4,12)
Sca	0.6710	4.09	*	
Gca-E	0.6065	3.70	*	
Sca-E	0.1641	0.53	NS	
ERROR	0.3097			

Standard deviation = 0.5565 Coeff of variation = 0.0904

S.E. parent means = 0.0928 S.E. cross means = 0.1855

(6) Yield

Source	Mean Square	F-Test	Signif	Test DF
Envir	39.0183	0.55	NS	(9,12)
Blk (E)	96.8335	2.03	(NS)	
Gca	5430.8942	17.08	***	(4,7)
Sca	258.5889	6.32	**	
Gca-E	61.8662	1.51	NS	
Sca-E	40.9414	0.86	NS	
ERROR	47.7263			

Standard deviation = 6.9084 Coeff of variation = 0.1014

S.E. parent means = 1.1514 S.E. cross means = 2.3028

(7) Cured weight/leaf

Source	Mean Square	F-Test	Signif	Test DF
Envir	1.4889	1.12	NS	(3,10)
Blk (E)	1.2092	3.82	***	
Gca	5.6862	8.98	***	(4,13)
Sca	0.2662	1.01	NS	
Gca-E	0.3961	1.50	NS	
Sca-E	0.2634	0.83	NS	
ERROR	0.3166			

Standard deviation = 0.5627 Coeff of variation = 0.1193

S.E. parent means = 0.0938 S.E. cross means = 0.1876

(8) Reducing sugar %

Envir	6.8950	0.52	NS	(6,13)
Blk (E)	12.3494	2.64	*	
Gca	103.8542	5.98	**	(4,13)
Sca	7.3855	8.28	***	
Gca-E	10.1168	11.34	****	
Sca-E	0.8922	0.19	NS	
ERROR	4.6776			

Standard deviation = 2.1628 Coeff of variation = 0.1556

S.E. parent means = 0.3605 S.E. cross means = 0.7209

(9) Reducing sugar % - top six leaves

Source	Mean Square	F-Test	Signif	Test DF
Envir	2.9480	0.28	NS	(13,13)
Blk (E)	16.5464	3.40	**	
Gca	150.7415	3.32	(NS)	(4,8)
Sca	34.9943	13.98	****	
Gca-E	11.2229	4.48	*	
Sca-E	2.5026	0.51	NS	
ERROR	4.8625			

Standard deviation = 2.2051 Coeff of variation = 0.0965

S.E. parent means = 0.3675 S.E. cross means = 0.7350

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(10) Total nicotine alkaloids %

Source	Mean Square	F-Test	Signif	Test DF
Envir	0.0715	0.62	NS	(4,11)
Blk (E)	0.1153	3.73	***	
Gca	2.5145	19.33	****	(4,10)
Sca	0.0823	2.83	(NS)	
Gca-E	0.0494	1.70	NS	
Sca-E	0.0291	0.94	NS	
ERROR	0.0309			

Standard deviation = 0.1759 Coeff of variation = 0.1540

S.E. parent means = 0.0293 S.E. cross means = 0.0586

(11) Days to flower (from transplanting)

Source	Mean Square	F-Test	Signif	Test DF
Envir	22.9333	1.48	NS	(4,14)
Blk (E)	8.0889	0.90	NS	
Gca	267.3815	8.80	***	(4,12)
Sca	17.1704	5.74	**	
Gca-E	13.5537	4.53	*	
Sca-E	2.9926	0.33	NS	
ERROR	8.9901			

Standard deviation = 2.9984 Coeff of variation = 0.0495

S.E. parent means = 0.4997 S.E. cross means = 0.9995

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(12) Stem height

Envir	1090.0111	4.46	*	(2,10)
Blk (E)	204.9778	1.85	NS	
Gca	517.9148	2.82	(NS)	(6,9)
Sca	166.5481	1.26	NS	
Gca-E	64.2148	0.49	NS	
Sca-E	131.9815	1.19	NS	
ERROR	110.6568			

Standard deviation = 10.5194 Coeff of variation = 0.0909

S.E. parent means = 1.7532 S.E. cross means = 3.5065

(13) Number of leaves

Source	Mean Square	F-Test	Signif	Test DF
Envir	7.4778	1.25	NS	(3,11)
Blk (E)	4.9556	4.08	***	
Gca	117.6037	28.79	****	(4,12)
Sca	2.1593	1.23	NS	
Gca-E	1.9870	1.13	NS	
Sca-E	1.7593	1.45	NS	
ERROR	1.2148			

Standard deviation = 1.1022 Coeff of variation = 0.0745

S.E. parent means = 0.1837 S.E. cross means = 0.3674

(14) Stem weight

Envir	174.2603	2.20	NS	(5,11)
Blk (E)	81.0043	0.88	NS	
Gca	4560.1526	17.55	***	(4,7)
Sca	226.7760	1.86	NS	
Gca-E	40.0703	0.33	NS	
Sca-E	122.2318	1.33	NS	
ERROR	91.6639			

Standard deviation = 9.5741 Coeff of variation = 0.1186

S.E. parent means = 1.5957 S.E. cross means = 3.1914

APPENDIX XIII

Variance component estimates, their standard errors, and ratio to error

(1) Leaf width

Source	Estimate	S.E.	Ratio to error
Envir	-0.0704	0.0979	-0.05
Blk (E)	0.0711	0.1124	0.05
Gca	1.5800	0.9903	1.08
Sca	-0.0772	0.1329	-0.05
Gca-E	0.2220	0.2303	0.15
Sca-E	0.2393	0.3114	0.16
ERROR	1.4673	0.2773	

(2) Leaf length

Envir	0.0190	0.1270	0.00
Blk (E)	-0.2347	0.1682	-0.05
Gca	3.1672	2.1713	0.62
Sca	1.0567	0.9816	0.21
Gca-E	-0.1141	0.3878	-0.02
Sca-E	0.3996	0.9157	0.08
ERROR	5.1022	0.9642	

(3) Leaf area

Envir	-287.3633	192.2057	-0.06
Blk (E)	-33.4778	225.7739	-0.01
Gca	4314.1364	2652.1562	0.96
Sca	-457.7605	283.0706	-0.10
Gca-E	605.9747	619.7117	0.13
Sca-E	437.0864	842.3725	0.10
ERROR	4515.3222	853.3157	

(4) Leaf length: leaf width

Source	Estimate	S.E.	Ratio to error
Envir	0.0018	0.0029	0.05
Blk (E)	0.0013	0.0028	0.03
Gca	0.0347	0.0247	0.86
Sca	0.0175	0.0116	0.44
Gca-E	0.0013	0.0028	0.03
Sca-E	-0.0018	0.0054	-0.04
ERROR	0.0403	0.0076	

(5) Specific leaf weight

Envir	-0.0043	0.0269	-0.01
Blk (E)	0.0451	0.0385	0.15
Gca	0.2126	0.1475	0.69
Sca	0.0563	0.0405	0.18
Gca-E	0.0492	0.0310	0.16
Sca-E	-0.0485	0.0297	-0.16
ERROR	0.3097	0.0585	

(6) Yield

Envir	-2.3985	2.0956	-0.05
Blk (E)	4.9107	4.9250	0.10
Gca	190.7919	116.2496	4.00
Sca	24.1831	15.4698	0.51
Gca-E	2.3250	3.5916	0.05
Sca-E	-2.2617	6.3308	-0.05
ERROR	47.7263	9.0194	

(7) Cured weight/leaf

Source	Estimate	S.E.	Ratio to Error
Envir	0.0067	0.0409	0.02
Blk (E)	0.0893	0.0608	0.28
Gca	0.1958	0.1219	0.62
Sca	0.0003	0.0198	0.00
Gca-E	0.0147	0.0230	0.05
Sca-E	-0.0177	0.0410	-0.06
ERROR	0.3166	0.0598	

(8) Reducing sugar %

Envir	-0.3631	0.3040	-0.08
Blk (E)	0.7672	0.5238	0.16
Gca	3.2313	2.2319	0.69
Sca	0.7215	0.4405	0.15
Gca-E	1.0249	0.5043	0.22
Sca-E	-1.2618	0.3187	-0.27
ERROR	4.6776	0.8840	

(9) Reducing sugar % - top six leaves

Envir	-0.6653	0.3314	-0.14
Blk (E)	1.1684	0.8324	0.24
Gca	3.9640	3.3024	0.82
Sca	3.6102	2.0815	0.74
Gca-E	0.9689	0.5691	0.20
Sca-E	-0.7867	0.4580	-0.16
ERROR	4.8625	0.9189	

(10) Total nicotine alkaloids %

Source	Estimate	S.E.	Ratio to Error
Envir	-0.0021	0.0027	-0.07
Blk (E)	0.0084	0.0058	0.27
Gca	0.0893	0.0538	2.89
Sca	0.0059	0.0051	0.19
Gca-E	0.0023	0.0028	0.07
Sca-E	-0.0006	0.0044	-0.02
ERROR	0.0309	0.0058	

(11) Days to flower (from transplanting)

Envir	0.3427	0.5953	0.04
Blk (E)	-0.0901	0.4387	-0.01
Gca	8.8759	5.7322	0.99
Sca	1.5753	1.0288	0.18
Gca-E	1.1735	0.6870	0.13
Sca-E	-1.9992	0.6975	-0.22
ERROR	8.9901	1.6990	

(12) Stem height

Envir	31.0492	25.9450	0.28
Blk (E)	9.4321	10.4601	0.09
Gca	15.5235	11.7743	0.14
Sca	3.8407	11.5622	0.03
Gca-E	-7.5296	6.7841	-0.07
Sca-E	7.1082	19.2657	0.06
ERROR	110.6568	20.9122	

(13) Number of leaves

Source	Estimate	S.E.	Ratio to Error
Envir	0.0583	0.1970	0.05
Blk (E)	0.3741	0.2488	0.31
Gca	4.2673	2.5155	3.51
Sca	0.0444	0.1510	0.04
Gca-E	0.0253	0.1270	0.02
Sca-E	0.1815	0.2513	0.15
ERROR	1.2148	0.2296	

(14) Stem weight

Source	Estimate	S.E.	Ratio to Error
Envir	4.8283	4.4026	0.05
Blk (E)	-1.0660	4.4051	-0.01
Gca	163.5384	97.6344	1.78
Sca	11.6160	14.5651	0.13
Gca-E	-9.1290	5.8912	-0.10
Sca-E	10.1893	17.6074	0.11
ERROR	91.6639	17.3229	

APPENDIX XIV

Table of means for pooled environments

(1) Leaf width

Male/Fem	1	2	3	4	5
1					
2	24.0533				
3	20.7522	22.8744			
4	21.2833	22.8222	19.6189		
5	23.4311	24.0133	21.1100	20.9378	
Parents	22.3800	23.4408	21.0889	21.1656	22.3731
General =	22.0897				

(2) Leaf length

Male/Fem	1	2	3	4	5
1					
2	63.7656				
3	61.8233	64.0656			
4	60.6822	62.8189	59.6378		
5	56.2511	61.6567	60.5967	58.3089	
Parents	60.6306	63.0767	61.5308	60.3619	59.2033
General =	60.9607				

(3) Leaf area

Male/Fem	1	2	3	4	5
1					
2	1004.7778				
3	842.6667	947.7778			
4	855.7778	926.2222	755.5556		
5	883.6667	969.6667	834.0000	802.4444	
Parents	896.7222	962.1111	845.0000	835.0000	872.4444
General =	882.2556				

(4) Leaf length: leaf width

Male/Fem	1	2	3	4	5
1					
2	2.8089				
3	3.2189	2.9311			
4	3.0922	2.9633	3.4033		
5	2.4878	2.7433	3.1544	3.0500	
Parents	2.9019	2.8617	3.1769	3.1272	2.8589
General =	2.9853				

(5) Specific leaf weight

Male/Fem	1	2	3	4	5
1					
2	6.1367				
3	6.8100	4.9222			
4	6.4778	5.7333	5.8878		
5	7.1722	5.9067	6.2744	6.2311	
Parents	6.6492	5.6747	5.9736	6.0825	6.3961
General =	6.1552				

(6) Yield

Male/Fem	1	2	3	4	5
1					
2	77.3556				
3	78.8211	43.7089			
4	77.9967	57.9056	49.9611		
5	102.5067	62.8156	66.5178	64.0067	
Parents	84.1700	60.4464	59.7522	62.4675	73.9617
General =	68.1596				

(7) Cured weight/leaf

Male/Fem	1	2	3	4	5
1					
2	5.4500				
3	4.9989	4.4656			
4	4.6944	4.6744	3.8478		
5	5.5011	5.0544	4.4244	4.0667	
Parents	5.1611	4.9111	4.4342	4.3208	4.7617
General =	4.7178				

(8) Reducing sugar %

Male/Fem	1	2	3	4	5
1					
2	13.4144				
3	14.1156	8.5789			
4	15.1878	12.7656	13.8922		
5	17.5533	13.4111	13.9056	16.1656	
Parents	15.0678	12.0425	12.6231	14.5028	15.2589
General =	13.8990				

(9) Reducing sugar % - top six leaves

Male/Fem	1	2	3	4	5
1					
2	23.3189				
3	22.8100	14.7722			
4	24.3378	22.1656	22.8244		
5	25.5156	22.5900	24.2244	25.8867	
Parents	23.9956	20.7117	21.1578	23.8036	24.5542
General =	22.8446				

(10) Total nicotine alkaloids %

Male/Fem	1	2	3	4	5
1					
2	0.9582				
3	0.9246	1.6704			
4	0.9758	1.6716	1.4292		
5	0.5690	1.2883	0.9699	0.9602	
Parents	0.8569	1.3971	1.2485	1.2592	0.9469
General =	1.1417				

(11) Days to flower (from transplanting)

Male/Fem	1	2	3	4	5
1					
2	60.3333				
3	60.5556	54.4444			
4	62.6667	57.4444	59.7778		
5	67.4444	57.5556	60.7778	65.0000	
Parents	62.7500	57.4444	58.8889	61.2222	62.6944
General =	60.6000				

(12) Stem height

Male/Fem	1	2	3	4	5
1					
2	116.5556				
3	109.0000	105.4444			
4	110.3333	115.3333	116.5556		
5	121.7778	116.8889	120.1111	124.8889	
Parents	114.4167	113.5556	112.7778	116.7778	120.9167
General =	115.6889				

(13) Number of leaves

Male/Fem	1	2	3	4	5
1					
2	14.4444				
3	16.0000	10.6667			
4	16.8889	12.6667	13.8889		
5	19.0000	12.6667	15.2222	16.4444	
Parents	16.5833	12.6111	13.9444	14.9722	15.8333
General =	14.7889				

(14) Stem weight

Male/Fem	1	2	3	4	5
1					
2	93.0344				
3	86.3711	54.8556			
4	86.3500	71.5367	61.2967		
5	108.9144	79.1900	82.5511	83.2211	
Parents	93.6675	74.6542	71.2686	75.6011	88.4692
General =	80.7321				

APPENDIX XV

Table of effects (gca, sca) for pooled environments

(1) Leaf width

(Sca)	1	2	3	4	5	Gca
1						0.2903
2	0.3222					1.3512
3	-0.6270	0.4344				-1.0008
4	-0.1726	0.3055	-0.5459			-0.9241
5	0.7677	0.2891	-0.2623	-0.5112		0.2834

(2) Leaf length

(Sca)	1	2	3	4	5	Gca
1						-0.3301
2	1.0190					2.1160
3	0.6226	0.4187				0.5702
4	0.6504	0.3409	-1.2943			-0.5987
5	-2.6221	0.3373	0.8232	-0.2957		-1.7573

(3) Leaf area

(Sca)	1	2	3	4	5	Gca
1						14.4667
2	28.2000					79.8556
3	-16.8000	22.9222				-37.2556
4	6.3111	11.3667	-42.1889			-47.2556
5	-3.2444	17.3667	-1.1889	-22.7444		-9.8111

(4) Leaf length: leaf width

(Sca)	1	2	3	4	5	Gca
1						-0.0834
2	0.0306					-0.1237
3	0.1253	-0.1222				0.1916
4	0.0484	-0.0402	0.0845			0.1419
5	-0.2877	0.0081	0.1039	0.0492		-0.1264

(5) Specific leaf weight

(Sca)	1	2	3	4	5	Gca
1						0.4939
2	-0.0320					-0.4805
3	0.3424	-0.5709				-0.1816
4	-0.0987	0.1313	-0.0131			-0.0727
5	0.2822	-0.0089	0.0599	-0.0923		0.2409

(6) Yield

(Sca)	1	2	3	4	5	Gca
1						16.0104
2	0.8987					-7.7132
3	3.0584	-8.3302				-8.4073
4	-0.4813	3.1512	-4.0991			-5.6921
5	12.5346	-3.4329	0.9634	-4.2629		5.8021

(7) Cured weight/leaf

(Sca)	1	2	3	4	5	Gca
1						0.4433
2	0.0956					0.1933
3	0.1214	-0.1619				-0.2836
4	-0.0697	0.1603	-0.1894			-0.3969
5	0.2961	0.0994	-0.0536	-0.2981		0.0439

(8) Reducing sugar %

(Sca)	1	2	3	4	5	Gca
1						1.1688
2	0.2032					-1.8565
3	0.3237	-2.1877				-1.2759
4	-0.4838	0.1193	0.6654			0.6038
5	1.1257	0.0087	-0.0774	0.3029		1.3599

(9) Reducing sugar % - top six leaves

(Sca)	1	2	3	4	5	Gca
1						1.1510
2	1.4562					-2.1329
3	0.5012	-4.2527				-1.6868
4	-0.6168	0.4948	0.7076			0.9591
5	-0.1896	0.1687	1.3571	0.3734		1.7096

(10) Total nicotine alkaloids %

(Sca)	1	2	3	4	5	Gca
1						-0.2848
2	-0.1541					0.2554
3	-0.0391	0.1665				0.1068
4	0.0014	0.1569	0.0632			0.1175
5	-0.0930	0.0861	-0.0838	-0.1041		-0.1949

(11) Days to flower (from transplanting)

(Sca)	1	2	3	4	5	Gca
1						2.1500
2	0.7389					-3.1556
3	-0.4833	-1.2889				-1.7111
4	-0.7056	-0.6222	0.2667			0.6222
5	2.6000	-1.9833	-0.2056	1.6833		2.0944

(12) Stem height

(Sca)	1	2	3	4	5	Gca
1						-1.2722
2	4.2722					-2.1333
3	-2.5056	-5.2000				-2.9111
4	-5.1722	0.6889	2.6889			1.0889
5	2.1333	-1.8944	2.1056	2.8833		5.2278

(13) Number of leaves

(Sca)	1	2	3	4	5	Gca
1						1.7944
2	0.0389					-2.1778
3	0.2611	-1.1000				-0.8444
4	0.1222	-0.1278	-0.2389			0.1833
5	1.3722	-0.9889	0.2333	0.4278		1.0444

(14) Stem weight

(Sca)	1	2	3	4	5	Gca
1						12.9354
2	5.4449					-6.0779
3	2.1671	-10.3351				-9.4635
4	-2.1865	2.0135	-4.8409			-5.1310
5	7.5099	-3.2012	3.5454	-0.1171		7.7371

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