VARIABILITY OF MORPHOLOGICAL AND CHEMICAL QUALITY CHARACTERISTICS IN FLOWERS OF MALE HOPS, HUMULUS LUPULUS L.

by

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INTRODUCTION

Hops are used almost entirely in the making of fermented malt beverages. It is a dioecious plant which produces annual aerial vines from perennial crowns. Only the female of the species is of commercial importance since its dried inflorescence constitutes the hop of commerce. The female inflorescence is a catkin or strobile, called a cone, with papery bracts and bracteoles. The value of the hop lies in its contents of bittering substances and essential oils which provide both flavor and aroma, as well as some preservative and protein coagulating properties. The bitter substances, made up principally of alpha- and beta-acids and related compounds, are produced in tiny granular glands which are borne on the surfaces of the bracts, bracteoles and seeds. These glands, called lupulin granules, contain also the essential oils.

Except for increasing the yield of hops due to the stimulation in strobile development, brought about by fertilization, the male hop is of little commercial importance. In parts of this country and in most of Europe, males are excluded from the hopyards so that seedless hops may be produced because they normally have a higher commercial value.

The value of the male plant, however, cannot be overlooked in a hop breeding program. The male contributes half of the inheritance and is just as important in a breeding program as the female.

The male hop flower is inconspicuous (Figure 1) and does not correspond morphologically with the inflorescence of the female. Hence, many difficulties in evaluating the male plants for their potential breeding value are encountered. Heretofore, the principal means of evaluating males for their breeding value has been through the use of expensive and time-consuming progeny tests. Only commercially important characters such as vigor and earliness, aside from those which are not commercially important, such as stem color or leaf shape, have lent themselves to phenotypic evaluation in the male. The potential for transmitting the bittering principle, essential oils and yield of strobiles has been evaluated only through progeny testing.

Any technique which would eliminate the need for progeny testing for quality factors in the early selection of males would be of great importance to a hop breeding program. Selection could be made first on the basis of phenotypic observation, and then a smaller number of lines could be used in crossing to test their breeding potential. Early elimination of many of the male lines on the basis of phenotype would allow better and more extensive testing of the few remaining ones for the same expenditures in time and money.

During the growing season of 1955, slight differences in aroma and stickiness of the flowers of different male hop lines were noticed while collecting pollen for crossing. Since it is a well known fact that both aroma and stickiness in female hops are due primarily to the occurrence of the resin glands on the female cones,

Figure 1. Panicle of male plant shedding pollen (about natural size).

these observations immediately suggested that this might be true also for the male flowers. Examination of the flowers did show the presence of resin glands (Figures 2 and 3). As a result, samples of male flowers were collected that season and submitted to the chemistry laboratory for evaluation in much the same manner as female cones are evaluated for chemical quality components. In addition a search was made of the literature, and it was found that resin glands had, in fact, been noted on male hop flowers many years before (Salmon and Wormald, 23, Vol. 11, p. 253). However, results of chemical analyses of male hop flowers had not been reported. This perhaps was due to the same difficulties encountered in this project in obtaining samples of sufficient size and the absence of a standard method for evaluating material having extremely low concentrations of alpha- and beta-acids.

Preliminary work in 1955 and 1956 indicated that the methods available for evaluating the commercial hop cones might be adaptable to the evaluation of low analysis male flowers. It was found that petroleum ether extracts of male hop flowers in weakly alkaline methanol solutions had ultra-violet light absorbing properties very similar to those of hops. This was very encouraging. However, it became apparent that the methods used in evaluating higher analysis hop cones could not be used on male hop flowers without modification. Such modifications were developed and later published by Likens and Brooks (21, vol. 63, p. 50-53).

Figure 2. Male flowers which have shed their pollen and separated from the plant. Resin glands are visible on the sepals and in the receptacle and dorsal furrows of the anthers (anthers are about 2.5 mm long).

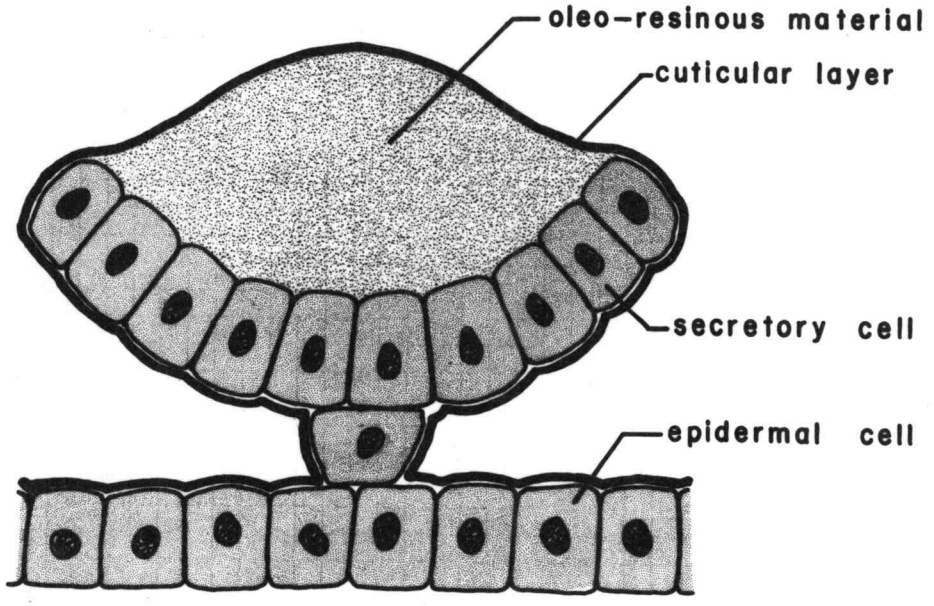


Figure 3. Diagrammatic sketch of resin gland (gland diameter is about 130 microns).

These investigations were designed to provide information on two aspects of phenotypic evaluation of male hops. Firstly, what is the extent of variability present in male hop lines for floral characters, particularly those having economic significance in female plants? How much of this variability is due to genotypic differences in a random group of lines, and how much is due to environment? Answers to these questions would not tell the hop breeder anything about transmissibility of such traits, but would provide fundamental knowledge of the male phenotype. Secondly, what is the nature of correlations between quality characters and other characters and among the quality factors themselves? Are such associations genetical or are they due to environmental influences? Are the associations brought about by the direct influence of one variable on another or are they brought about by correlated common causes? Answers to these questions would enable a hop breeder to more effectively carry out a selection program.

REVIEW OF LITERATURE

Fore and Sather (14, vol. 10, p. 20), Dark (5, p. 51) and Smith (28, p. 1218) have pointed out that the problem of hop breeding can be paralleled with that of breeding bulls for improving milk yields of dairy herds. Many of the cropping characters of the hop plant are sex-limited in that they are expressed only by the female plant. As a result only 50 per cent control has been exercised in breeding. Fifty per cent of the inheritance is supplied through the male line, and its important characters can be determined only by progeny testing.

Dark (6, p. 58-67) reviewed the available literature on hop genetics and listed four main disadvantages of hops as a subject for genetical study. One, a seedling hop usually does not flower until its second year, and some characters may not become stabilized until the fifth year. Two, separation of the sexes and commercial methods of propagation have produced heterozygous and unrelated males and females. Three, many of the commercially important characters cannot be measured objectively. Four, the habit of wind pollination natural to the plant creates technical difficulties in controlling cross-pollination.

Some of the earliest work on the study of variability in male hops was done by Wormald (30, vol. 7, p. 175-196). Male plants were found to vary in time of flowering, vine color, leaf color, length of lateral branches, in addition to the number of glands present per

unit of area on the leaves. The glands on the lower surfaces of the leaves varied from 120 to 190 microns in diameter. Full bloom occurred usually from 7 to 10 days after initial bloom, and several plants remained in bloom for two weeks. It was concluded that male hops exhibit definite variation, and selection for one or more characters would be feasible.

Salmon and Wormald (23, vol. 11, p. 244-248) studied the variation in seedlings of the European wild hop over a four-year period and found that plants varied in their date of flowering from year to year by as much as 10 to 12 days. Fore and Sather (14, vol. 10, p. 21) also studied flowering dates in male hops and reported that some plants began to shed pollen as early as June 15, but the latest plants began to shed towards the end of July. The total pollen shedding period varied from four to 52 days among plants with an average of 25 days per male plant. While it was not stated, it is possible that the 52-day pollen-shedding period for some plants included a first and second flowering. This phenomenon has been often observed by Brooks and others (3, p. 31-33) in several early genotypes. According to Hamaguchi (15, vol. 2, p. 119) hop vines in Japan begin flowering in the laterals produced near the center and then flowering proceeds upwards and downwards on the main vine. Since the flowers do not all mature at the same time, flowering extends over a several-day period. Ehara (11, vol. 10, p. 307) reported that a three-year-old Nagano male (H. lupulus) in Japan produced an average of 2,000 to 3,000 flowers per vine.

In 1915 Schmidt (24, vol. 11, p. 177-179) studied the amount of lupulin in plants raised from crosses and concluded that the transmission of lupulin percentage by the females is a complicated process. Usually the daughters were lower than the mothers in this respect, but it was easy to find plants which surpassed the mother. In this study the values for lupulin percentage included both total soft-resins and total hard-resins. Crosses were also made with an intermediate male plant (Schmidt, 25, vol. 11, p. 188-198) on female plants differing in time of flowering. Average flowering date was retarded in the offspring of early plants and accelerated in offspring of late plants. The offspring of intermediate mother plants exhibited little difference in that respect. This was not attributed entirely to paternal effect, because there was considerable alteration in flowering time in several cases. Later work by Schmidt (26, vol. 11, p. 330-332) indicated that male American plants transmitted their so-called wild-currant aroma to offspring from a mother plant which did not possess that aroma. It was stated that this particular aroma is never present in European hops and that it was transmitted to about three-fourths of the daughters of an Oregon Cluster female plant crossed with a wild Danish male. While the conclusions were based on data from a small number of plants, Schmidt's work furnished some of the first information regarding the inheritance of quality factors in hops. Additional work by Schmidt (27, vol. 13, p. 1-24) indicated that in crosses of plants differing in numbers of teeth on the leaves, the average number of teeth per leaf in the progenies was

intermediate between the parents.

Salmon and Wormald (23, vol. 11, p. 253) furnished the first report regarding the occurrence of resin glands on the flowers of male hops. On the average, 7 to 30 glands appeared on each perianth lobe, from 0 to 14 occurred in the dorsal furrows on the anthers, and there were from 0 to 10 on the receptacle from which the stamen filaments arose.

During the past half-dozen years considerable work has been done in England on the inheritance of resin characteristics in hops. Farrar and associates (13, p. 52-59) studied the correlation of gland number in male flowers with resin contents on cones from related females as well as resin and gland relationships in monoecious plants. It was suggested that any correlation between these two factors would facilitate breeding work, since a breeder could estimate the value of a male plant in this respect without the need for a laborious progeny test. The data from crosses involving low x high, low x low, high x low and high x high individuals showed that alpha-resin content was not correlated with gland number. There was some indication of a correlation between beta-resin with gland number, but this was not significant. Alpha- and beta-resin contents seemed to be inherited independently, while total soft-resin was simply the sum of the two. It appeared that the females transmitted alpha- and beta-resin contents, but total soft-resin content was not transmitted as such. The range in gland number among male plants was from 12 to 98, on the average. Neve (22, p. 43-49) studied variation within and

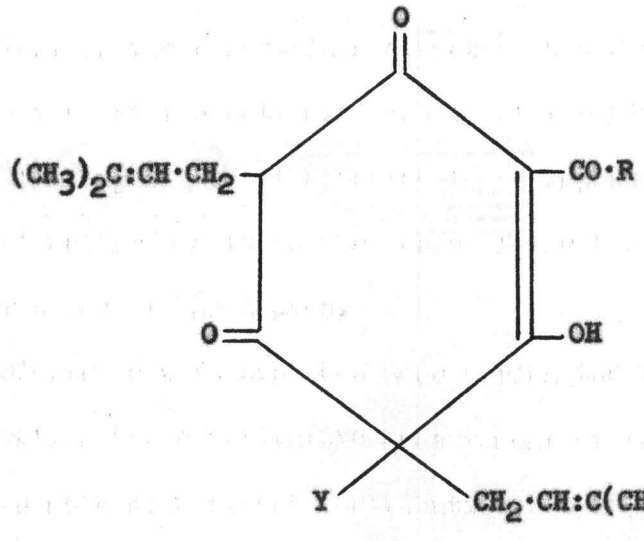
between families and provided the first information available on the transmission of resin characters by male hops. It was concluded that the genetic control of resin characters was effected by a large number of genes, each producing small effects. Since alpha- and beta-resin were correlated in only one family, it was assumed that these two components are normally inherited independently.

Since it is the female plant which has commercial importance in the industry, considerable work has been done to determine the variation in phenotypic expression of several characters. Davis (8, vol. 23, p. 23-24) pointed out that chemical yardsticks are needed against which structural features may be evaluated, and tried to correlate cone structure with chemical types. The purpose was to provide brewers with visual methods of determining quality factors without brewing trials or chemical analyses. Similarities of type among commercial varieties were noted. Keller and Likens (19, vol. 47, p. 518-521) reported estimates of heritability in female hops for several characters, among which were chemical quality components. Alpha-, beta-, and total-resin components varied considerably among genotypes, but there was some variability within genotypes. Smith (28, p. 1234-1235) also reported data on year to year deviations in sex expression, chemical composition and vigor. Davis (8, vol. 23, p. 19-24) showed that considerable variation existed within single plants for structural characteristics.

Dark and Tatchell (7, p. 67) attempted to find a means of evaluating the resin content of hop plants as indicated by the

density of resin glands on the lower surfaces of the leaves. Alpha-acid content in the cones, in most cases, was smaller at the top of the vines than at the bottom. Density of the glands on the leaves was just the opposite. It appeared to be impossible to predict resin content of mature plants on the basis of the resin gland density on the under sides of the leaves.

Considerable work has been done during the past decade on the identification and quantitative measurement of the constituents in hops which make significant contributions to beer flavor and aroma. With the aid of new analytical techniques, several new substances have been isolated and described. While new constituents are continually being found, the alpha- and beta-acids are the best understood both from the standpoint of chemical properties and utilization in the manufacturing of beer. Hudson (18, vol. 21, p. 183-184) has described the chemical structure of the humulones and lupulones (alpha- and beta-acids, respectively) as follows:



	Y	
R	OH	$CH_2\cdot CH:C(CH_3)_2$
$CH(CH_3)_2$	Cohumulone	Colupulone
$CH_2\cdot CH(CH_3)_2$	Humulone	Lupulone
$CH(CH_3)_2CH_2\cdot CH_3$	Adhumulone	Adlupulone

Humulones are isomerized to ischumulones during the boiling of the wort. All of the isohumulones are believed to be equally bitter and bacteriostatic. Neither lupulones nor their boiling products are appreciably soluble in wort or beer. So it is generally agreed that humulones are the main contributors to a hop's bittering power and bacteriostatic properties. The bittering power of hops has usually been taken in the trade to mean $(\alpha + \beta/9)$, whereas the value 10α has been meant to mean the preservative value.

Stewart (29, vol. 85, p. 30, 32, 35-36) summarized previous work by Riedl in Germany regarding the structure of humulone and lupulone.

Hop acids were synthesized from phloroglucinol and isovaleryl chloride which became phloroisovalerophenone on treatment. The latter substance was synthesized to either humulone or lupulone depending upon the treatment given it. DeWever and Emery (9, vol. 13, p. 160) analyzed several varieties of hops and found that beta-acid was formed before alpha-acid during female cone development and that alpha-acid was formed at the expense of beta-acid. It appeared that light favored the formation of alpha-acid. Work by Howard and Tatchell (16, vol. 62, p. 251-256) and by Fang and Bullis (12, vol. 21, p. 111) indicated that both alpha- and beta-acids appear simultaneously in the hop cone. Amounts of these components were present in the cones for a period of five or six weeks previous to normal harvest date.

Further work by Howard and Tatchell (17, vol. 63, p. 138-142) suggested that proportions of cohumulone in the alpha-acid and colupulone in the beta-acid were varietal characteristics and were affected by environment only to a minor extent. It was reported that this was also true in male flowers. To the writer's knowledge, this was the first account in the literature regarding analysis of male flowers for these two components. However, no description of sampling or analytical methods was given. It was not until the work of Likens and Brooks (21, vol. 63, p. 50-53) that a practical method of analyzing male flowers for hop-acid components became available.

MATERIAL AND METHODS

The field trial established in 1956 was composed of 20 male selections from the breeding block. Since quality characters of these lines were unknown, this group can be considered as a random sample in this respect except for one line which was selected for extreme earliness. No attention was paid to parentage of the lines, which resulted in having 17 different pedigrees represented among the 20 selections. The sample of 20 lines, therefore, can be considered a fairly random sample from a breeding population of hop lines. A list of the hop lines along with their pedigrees is given in Appendix Table 1.

The trial was established in single-hill plots replicated five times in a randomized block design. Only one rhizome was planted at each hill. Where the single rhizome failed to grow, the hill was replanted in 1957. The plots were grown in the hopyard located on the College East Farm. A planting plan of the trial is given in Appendix Table 2.

The culture of the plants was consistent with grower practice in the Willamette Valley. The height of trellis was 17½ feet and the spacing of the hills was 8 x 8 feet. Three strings were tied down at each hill, and two vines were trained up each string. The plots were irrigated on the average of twice each season, and fertilizer use and other cultural practices were consistent with good management. Data were taken in 1958 and 1959.

An attempt was made to obtain data for two years on five plants of each line, but this was not possible. Some replanting had to be done in 1957, and the replants were small in 1958. Since age of planting has some effect on the chemical composition of female hop plants, it seemed advisable to omit the young male plants from analysis in 1958. This fact, along with the occurrence of a few weak hills in 1959, made it necessary to reduce the number of replications from which data were obtained so that equal subclasses would be available for statistical analysis. As a result, the data from only three replications were used in the final summary. All measures of variability and covariability are based on this reduced number of replications.

Observations were made on date of flowering in both years. In 1958 an attempt was made to determine the day on which each plant was in full bloom. This perhaps was subject to some error, since the male hop plant flowers over a several day period, and it is somewhat difficult to determine the time when the greatest number of flowers on a plant are shedding pollen. As a result, the date on which the tip inflorescence came into bloom was recorded in 1959 to indicate the stage of full bloom. The plants bloomed seven days later in 1959 than in 1958, on the average, on the basis of these observations. Since female plants in a nearby trial reached the initial flower stage approximately eight days later, on the average, in 1959 than in 1958, both estimates of full bloom in the males are considered reliable. However, observations on the date the tip inflorescence

blooms can be made with less difficulty.

Individual hop flowers open at maturity, shed their pollen, and normally separate by abscission between the base of the calyx and the pedicle. Abscission usually occurs within one or two days after anthesis. In order to avoid obtaining stem material and collect a sample of uniform maturity, the method described by Likens and Brooks (21, vol. 63, p. 51-52) was employed. At the onset of anthesis, vegetable parchment bags were clipped over as many of the flowering branches as possible (Figure 4). After two or three days the branches were shaken lightly and the bags removed. The material in all bags taken from each plant was composited. Material collected in this manner consisted of sepals, anthers and pollen each of fairly uniform maturity (Figure 5). Usually it was necessary to re-bag the male plants a second or third time and composite the flowers from each to insure sufficient material. After collection the samples were dried at 50° C. under vacuum and stored in polyethylene bags at -20° C. until ready for analysis.

At the same time that samples were collected for chemical analysis, a smaller sample was collected for resin gland counts and flower weight measurements. Gland number was determined by counting the number of glands in the outer furrow of each anther, on the receptacle from which the stamen filaments arise, and on the outer sides of the sepals. Counts were made under a dissecting scope at a magnification of 15X. Samples containing 25 flowers were examined in 1958, and all of the resin glands on each flower were recorded.

Figure 4. Male plant which has been bagged for flower collection.

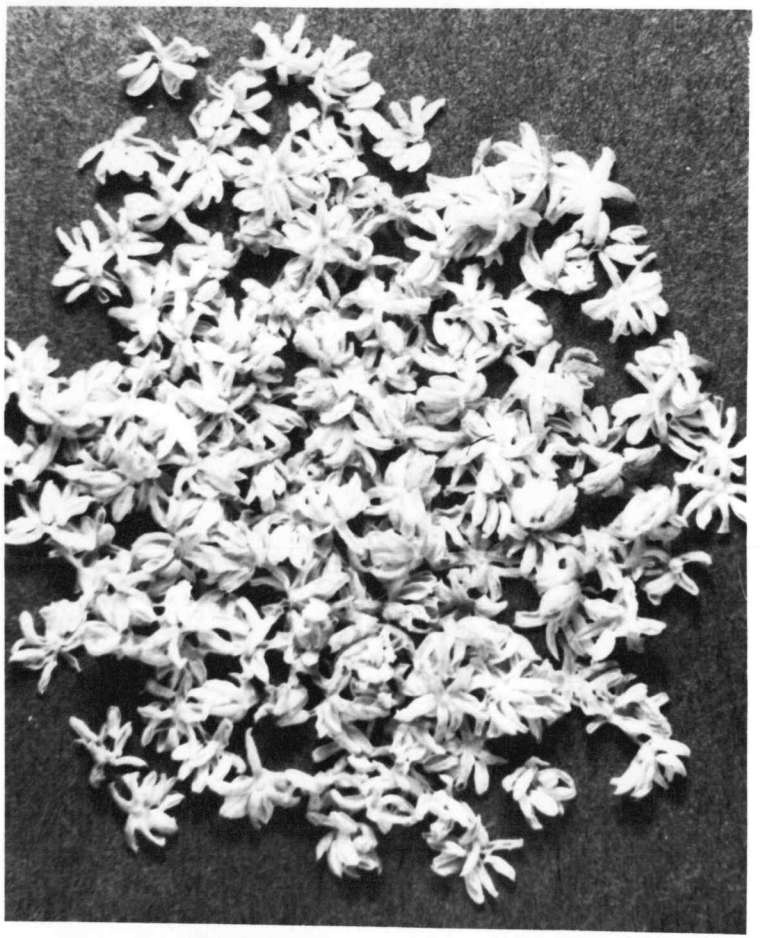


Figure 5. Small sample of male flowers collected as described in text.

A study of the variances from the 1958 data indicated that almost as much precision could be gained by counting a fewer number of flowers, so only ten from each plant were examined in 1959.

Measurements were made of the diameters of the glands on 25-flower samples from each plant to furnish some measure of gland size. Each sample was shaken vigorously in the glass vial in which it had been stored and then sprinkled over a glass slide. The flower parts normally fell off of the slide when it was tilted on edge, but the glands, being sticky, adhered to the surface. This made it possible to examine the material under magnification (90X) without having the glands obscured by other floral material. Twenty glands were measured for each plant each year. The glands are not entirely circular in cross-section, and the measurement taken was the diameter of each gland across its widest axis. This was done to preclude any chance of not having a true cross-sectional measurement. Sometimes a gland was tilted on edge and the apparent diameter was smaller than the real diameter.

In addition to measuring gland diameter, an approximation of the volume of each gland was calculated. This was done using the measurement for gland diameter and substituting it in the formula for the volume of an oblate spheroid. In using this formula one-half of the measured diameter was taken as the radius of the major axis, and one-half of this value was taken as the radius of the minor axis. This was done because most glands appeared to resemble an oblate spheroid more than they did a sphere or a prolate spheroid. All

measurements were converted to cubic microns, and the results are reported on that basis. It is realized that this is an empirical approximation, but it is felt that it is a better measure of gland size than is the diameter. Surely true gland volume would vary by cubic progression rather than by linear progression.

The weight of flowers was also recorded on the samples obtained in 1958 and 1959. Samples containing 25 flowers from each plant were weighed on an electric balance to the nearest 0.1 milligram, and these values were later converted to micrograms per flower.

Alpha- and beta-acid were determined using the methods described by Likens and Brooks (21, vol. 63, p. 52-53). All extractions of male flowers were made with five mls of methanol per gram of sample, and the extractions were carried out in a 14,000 rpm sealed homogenizer for five minutes.

A 1:1 mixture of the methanol extract and two per cent (W/V) sulphuric acid was extracted three successive times with ten ml portions of petroleum ether. This was combined and made to volume. Aliquots were then diluted appropriately with 0.002 N NaOH in methanol for UV readings at 355 mμ, 325 mμ, and 275 mμ on a Beckman model DU spectrophotometer. The concentrations of alpha- or beta-acid were calculated from the regression equations of Alderton and others (1, vol. 26, p. 287):

(1) $C_{\alpha} = -51.56\ A_{355} + 73.79\ A_{325} - 19.07\ A_{275}$, or

(2) $C_{\beta} = 55.57\ A_{355} - 47.59\ A_{325} + 5.10\ A_{275}$,

where C is the concentration in mg/l, and the subscripts of A denote the wave length in mμ at which the absorbancy readings were taken. Calculations by this method give the results in percentages of alpha- and beta-acid on a weight basis. These percentages were later converted, using the information on flower weight, to the number of μg of either alpha- or beta-acid per flower, and all measures of variability and correlation were made using the absolute amounts per flower rather than the percentage concentrations. The amounts of alpha- and beta-acids were added together to give the values for total hop-acid.

Standard variance analyses were made on the data for two years from 20 lines on all seven of the variables measured. Following is a breakdown of the sources of variation, degrees of freedom and estimates of variance used in the analysis of variance:

Source	DF	MS is estimate of:
Total	119	
Genotypes	19	$V_E + 3V_{GY} + 6V_G$
Years	1	$V_E + 3V_{GY} + 60V_Y$
Genotypes x Years	19	$V_E + 3V_{GY}$
Plants in genotypes and years	80	V_E

Estimates of genetic variance compared to phenotypic variance were calculated after the method of Burton and DeVane (4, vol. 45, p. 478-481) and later used by Keller and Likens (19, vol. 47, p. 518-521) in estimating heritability values for female hop lines. Since the variance estimates calculated in the male hop line study were not

based on segregation in parent-progeny analysis, the term diversity was used in place of the term heritability (Brooks, 2, vol. 1, in press). Diversity estimates were made on the basis of single plant observations as well as on a multiple plant basis where three observations for two years or six observations per line were available. The formulae used for these calculations were as follows:

$$(1)\quad D_1 = \frac{V_G}{V_G + V_{GY} + V_E} \quad \text{or} \quad (2)\quad D_2 = \frac{V_G}{V_G + \frac{V_{GY}}{2} + \frac{V_E}{6}},$$

where D_1 is the diversity estimate based on a single plant observation, and D_2 is the diversity estimate based on a multiple plant basis. In addition, estimates were made regarding the gain that might be expected on the basis of selecting the upper five per cent of the population. Since a plant breeder, at least initially, must base his selection on individual plant performance, these estimates were calculated using variance estimates of single plants. The formula used for this calculation was as follows: $S = 2.06 \times D_1 \times \sqrt{V_{ph_1}}$, where S, D_1 and V_{ph_1} are the expected gain, the diversity estimate and the phenotypic variance based on a single plant, respectively. The value 2.06 is the expectation in the case of five per cent selection in large samples from a normally distributed population.

A plant breeder is interested not only in phenotypic correlations between variables in the material that he is examining, but he is also interested in the genotypic and environmental correlations. For example, the amount of genotypic correlation between two variables

may be overcome by a negative environmental correlation which would make it impossible to select for any two variables at the same time due to cancelling-out effects of environment. Conversely, a low negative genotypic correlation may be overcome by a high positive environmental correlation resulting in an overall selection for environmental effects rather than the desired genetic effects. Therefore, it seemed desirable to calculate the genotypic and environmental correlation coefficients. These were obtained by the following formulae:

$$(1)\quad r_{G_{1.2}} = \frac{Cov_{G_{1.2}}}{\sqrt{V_{G_1} \times V_{G_2}}} \quad \text{or} \quad (2)\quad r_{E_{1.2}} = \frac{Cov_{E_{1.2}}}{\sqrt{V_{E_1} \times V_{E_2}}},$$

where r_G is the genotypic correlation and r_E is the environmental correlation.

Correlation coefficients were computed on an Alwac III E for all possible combinations of the seven variables. Only total and phenotypic (line mean) correlations were computed in this manner. Genotypic and environmental correlations were computed later from the sums of cross products and correlation coefficients obtained from the Alwac III E. Following is a breakdown for the sources of covariance, degrees of freedom and estimates of mean product.

Source	DF	MP is estimate of:
Total	119	
Genotypes	19	$Cov_E + 3\ Cov_{GY} + 6\ Cov_G$
Years	1	$Cov_E + 3\ Cov_{GY} + 60\ Cov_Y$
Genotypes x Years	19	$Cov_E + 3\ Cov_{GY}$
Plants in genotypes and years	80	Cov_E

Path coefficient analysis was made according to Wright's methods as outlined by Dewey and Lu (10, vol. 51, p. 515) in studying yield components in crested wheatgrass. A path coefficient can be described as a standardized partial regression coefficient. Wright (31, vol. 20, p. 561) states that path coefficient analysis starts with the assumption that the direct influence along a given path can be measured by the standard deviation remaining in the effect after all other possible paths of influence are eliminated, while variation of the causes back of the given path is kept as great as ever, regardless of their relations to the other variables which have been made constant. Detailed discussions of the theory and application of path coefficient analysis are given by Wright (31, vol. 20, p. 557-585) and Li (20, p. 144-171).

Direct and indirect effects of any one variable on another were computed for phenotypic, genotypic and environmental effects using the appropriate correlation coefficients obtained above. Where all direct and indirect effects are taken into consideration in this type of analysis, the combined total effect will equal unity. If there are unknown factors which have direct and indirect effects, the combined effect of those measured will not equal unity. In each of the following path coefficient diagrams the single-arrowed lines indicate the direct effect of one variable on another, and the double-arrowed lines indicate the correlation between two variables.

The path diagram and association of all characters studied in the two years are shown in Figure 6. This diagram shows that total

hop-acid (T) is caused by alpha-acid (A) and beta-acid (B) which in turn are caused or influenced by resin gland number (N) and size (S), flower weight (W), and date of flowering (F). Further, resin gland number and size are influenced by flower weight and date of flowering. These directions of influence can be ascertained intuitively or by other means. For example, the direction must be from A and B to T, since T is the sum of A and B. Likewise, the direction would be from N and S to A and B, because the resins are contained in the glands. Similarly, A, B, N and S would be influenced by W and F rather than the other way around. The path diagram shows the direction of influence only; the sign of the influence is determined by the solution of the path coefficient from the appropriate correlation coefficients.

The plan shown in Figure 6 is too complex to comprehend as a single unit, and an attempt was made to simplify it by breaking it down into segments. Figure 7 shows the association of alpha- and beta-acids and their direct and indirect effects on total hop-acid content. This is a rigid cause and effect scheme since total hop-acid is the sum of alpha- and beta-acid. Equations (1) and (2) given with the figure indicate the direct and indirect effects for alpha- and beta-acid, respectively. The magnitudes and signs of the path coefficients were obtained by the simultaneous solution of those two equations. Equation (3) expresses the total effect of both variables on total hop-acid.

The path diagram and association of factors influencing alpha-,

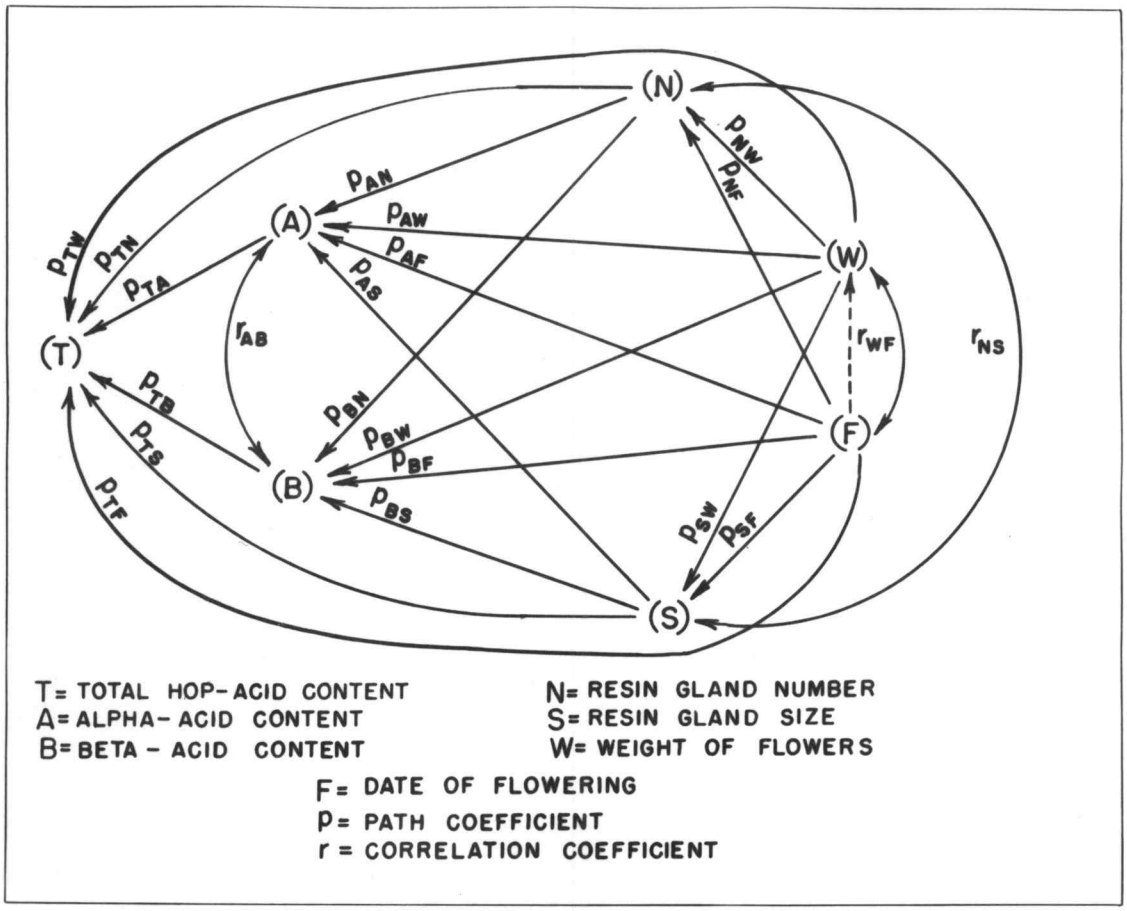


Figure 6. Path diagram and association of all characters studied in 1958 and 1959.

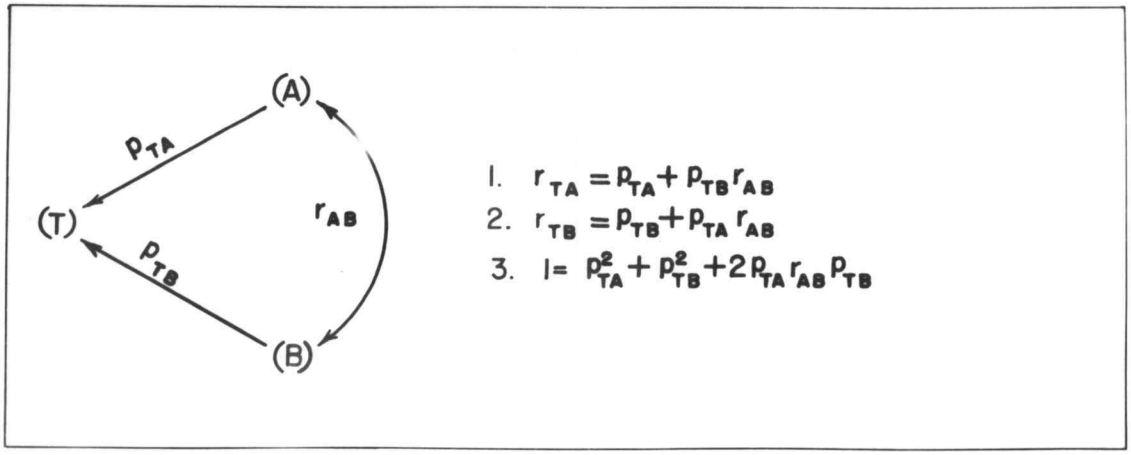


Figure 7. Path diagram and association of alpha-, beta-, and total hop-acid.

beta-, and total hop-acid is given in Figure 8. As before, the single-arrowed lines indicate direct effects and the double-arrowed lines indicate correlation between two variables. Equations (1), (2), (3) and (4) are those which were solved simultaneously for the determination of the various path coefficients. Equation (5) expresses a combination of all direct and indirect effects, known and unknown. If all effects are taken into consideration, the combined total of them will equal unity. However, unlike the case of alpha-, beta-, and total hop-acid, it became apparent that not all effects were taken into consideration. Therefore, the equation had to be enlarged to include the bracketed portion which indicates the influence of unknown causes (Z).

The third stage in the path coefficients analysis is indicated in Figure 9 where the direct and indirect effects of flowering date and flower weight on resin gland number and size are shown. Equations (1) and (2) were solved for the determination of the direct and indirect effects on resin gland number. Equations (3) and (4) were solved for the determination of the direct and indirect effects on resin gland size. Equation (5) expresses the overall association or correlation between resin gland number and size. Since combination of the known effects did not equal the calculated correlation coefficient, the bracketed portion of the equation was added to take into account the direct and indirect effects of an unknown variable or variables (Z) affecting the correlation coefficient between resin gland number and resin gland size.

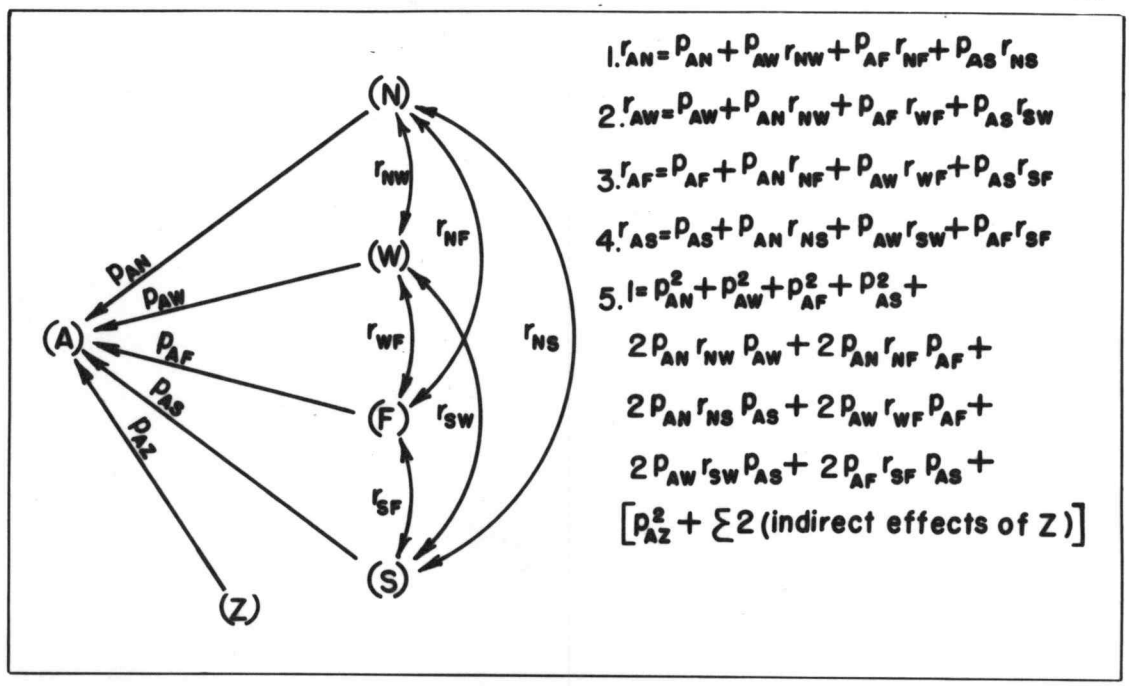


Figure 8. Path diagram and association of factors influencing alpha-, beta-, and total hop-acid (substitute B for A in the case of beta-acid and T for A in the case of total hop-acid).

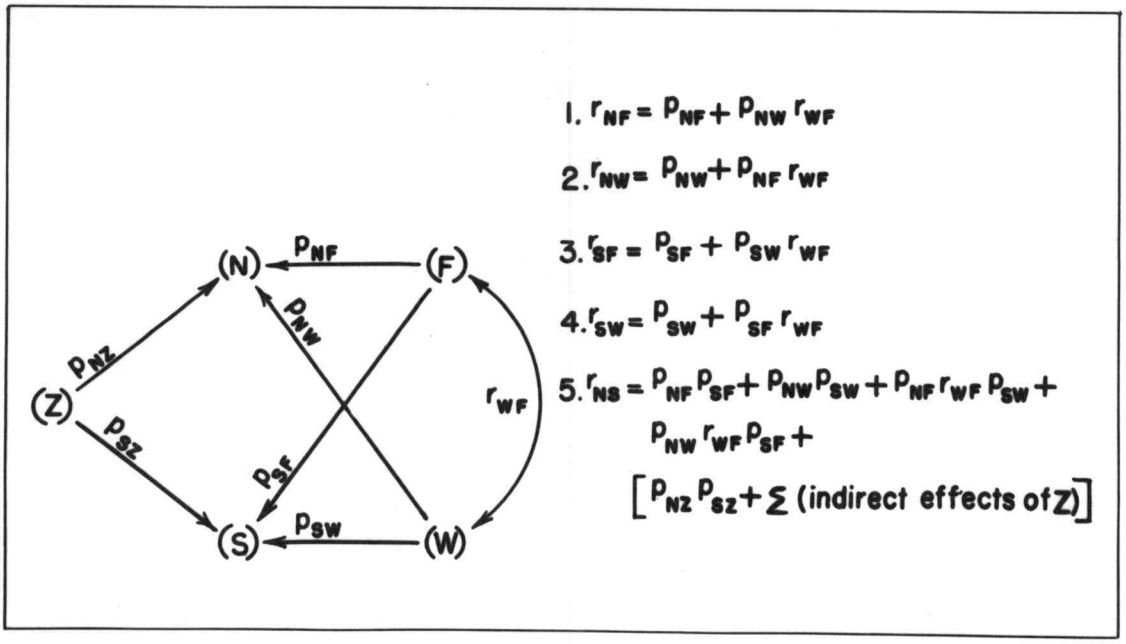


Figure 9. Path diagram and association of factors influencing resin gland number and size.

RESULTS AND DISCUSSION

Mean squares for the various sources of variation for all variables are given in Table 1. Average values for the 20 male lines are shown in Table 2. Significant differences among lines were shown for all of the characters studied. Significant genotype x year interactions were also shown for all seven variables. The mean squares for years for alpha-acid, beta-acid, total hop-acid, flower weight and flowering date were also significant.

Alpha-acid content ranged from 18.4 to 1.4 µg per flower and averaged 7.2 µg per flower for the two-year period. Beta-acid content ranged from 28.1 to 4.2 µg per flower and averaged 13.4 µg per flower for the two-year period. Total hop-acid which is the sum of alpha- and beta-acid averaged 20.6 µg for the two-year period and ranged from 46.1 to 7.1 µg per flower. The mean values for all three of these variables were higher in 1958 than they were in 1959.

Coefficients of variation for the acid contents were rather high, being 25 per cent for alpha-acid and 18 per cent for both beta-acid and total hop-acid. Considering that average values represent such dilute concentrations of these chemical components, any method of chemical analysis or any method of sampling would have to be extremely precise in order to produce low coefficients of variation.

Average resin gland number ranged from 16 to 61 for the two-year period. There was no apparent difference between 1958 and 1959 in resin gland number. Some lines were higher in 1958 than they were in 1959 and, conversely, some lines were higher in 1959 than they

TABLE 1.
Mean Squares for the Various Sources of Variation for All Variables.

Source of variation	DF	Alpha-acid	Beta-acid	Total Hop-acid	Gland Number	Gland Volume	Flower Weight	Flowering Date
Genotypes	19	148.3574*	278.4099*	647.4097*	961.83*	176,788*	94,227*	320.69*
Years	1	364.3570*	101.9370*	851.7340*	6.50	64	78,030*	1,801.90*
Genotypes x Years	19	11.0248*	17.2891*	36.8588*	150.33*	52,721*	15,393*	62.29*
Plants in genotypes and years	80	3.3381	5.5795	13.5972	26.22	16,188	4,635	16.69

* Significant at the one per cent level.

TABLE 2.
Mean Values for Chemical and Morphological Characters
Measured on 20 Male Hop Lines, 1958-59 Averages.

Male Line	Alpha-acid (µg/flower)	Beta-acid (µg/flower)	Total Hop-acid (µg/flower)	Gland number (glands/ flower)	Gland volume (μ^3/gland x 10^{-3})	Flower Weight (µg/flower)	Flowering date (days after 5/31)
106-S	6.4	13.1	19.6	16	710	1230	33
110-S	8.2	13.4	21.6	25	435	960	45
123	6.4	14.6	21.0	33	558	870	41
217	6.2	16.2	22.4	29	798	800	51
125	2.4	5.0	7.4	20	590	870	59
221	6.6	14.7	21.3	24	844	1080	51
324	7.9	5.9	13.8	24	502	860	50
224	5.0	4.2	9.1	18	468	830	47
317	10.4	25.2	35.6	52	740	860	58
319	2.6	4.5	7.1	16	487	790	54
322	2.9	16.6	19.5	30	975	1020	56
320	14.2	10.0	24.2	36	873	890	60
323	11.4	7.2	18.6	33	588	860	51
424	1.4	6.2	7.6	22	464	880	48
518	2.6	17.1	19.7	36	637	870	56
425-1	3.0	11.0	14.1	26	788	910	65
521	18.0	28.1	46.1	61	586	860	60
523	18.4	22.3	40.7	56	922	1160	56
417	4.8	16.9	21.7	31	899	1130	57
524	6.2	15.6	21.7	36	576	880	52
Mean	7.2	13.4	20.6	31	672	930	52
CV (%)	25	18	18	17	19	7	8

were in 1958. The differences in line means constitute about a five-fold difference between the lowest and the highest. Similarly, resin gland volume was essentially the same in 1958 and 1959 and averaged 672,000 μ^3 per gland for the two-year period. The absolute range in mean values for the two-year period was from 435,000 to 975,000 μ^3. This is a two-fold range and, although it is not as large a range as for some of the other variables, it appeared to be real. Resin gland diameter averaged 130 μ each year and for the two-year period. The smallest average diameter was 114 μ, and the largest average diameter was 146 μ.

Flower weight averaged 930 μg per flower over the two-year period and ranged from 790 to 1230. The entry having the largest flowers was 106-S and the entry having the smallest average flower weight was 319.

Average date of bloom was 52 days after May 31 and ranged from 33 days to 65 days after May 31. Line 106-S came into full bloom about July 3, on the average, whereas line 425-1 came into full bloom about August 4, on the average. This is approximately a month's difference between the earliest and the latest flowering lines. Average date of full bloom was July 19 in 1958 and July 26 in 1959.

Estimates of genetical variances and diversity ratios along with expected gains for selection are given in Table 3. Significant genetical variances were indicated for all seven of the characteristics studied. As a general rule, genotypic variances were quite high as compared to overall phenotypic variances. Genetical coefficients of variation ranged from 66 per cent for alpha-acid to

TABLE 3.
Genetical Estimates for Alpha-, Beta- and Total Hop-acid, Resin Gland Number and Size, Flower Weight, and Flowering Date For 20 Male Hop Lines (Average of 1958 and 1959).

Character	Mean	Genetical Variance	Genetical CV (%)	Diversity Ratio		Expected gain from Selection (± mean)
				Single Plant Basis	Multiple Plant Basis	
Alpha-acid content	7.2	22.889*	66	0.80	0.93	1.22
Beta-acid content	13.4	43.520*	49	0.82	0.94	0.92
Total hop-acid content	20.6	101.758*	49	0.83	0.94	0.92
Number of resin glands	31	135.25 *	38	0.67	0.84	0.65
Size of resin glands	672	20,678*	21	0.42	0.70	0.29
Weight of flowers	930	13,139*	12	0.62	0.84	0.19
Date of flowering	52	43.07 *	13	0.58	0.81	0.19

* Significantly greater than 0 at the one per cent level.

12 per cent for weight of flowers. Diversity ratio estimates ranged from 0.83 for total hop-acid content to 0.42 for the size of resin glands based on a single plant observation. Diversity estimates for the other characters were intermediate between these values. Calculations based on two years' observations on three plants increased the diversity ratios from 0.83 to 0.94 for total hop-acid content and from 0.42 to 0.70 for size of resin glands. These values indicate the ratio of genotypic variability compared to total phenotypic variability. They are quite high and indicate that permanent progress can be made by selecting for any one of the variables studied. Calculated expected gains from selection compared with the mean indicate that if the upper five per cent of the population were selected for high alpha-acid content the mean of those selected would be more than twice the mean of the unselected population. Likewise, beta-acid and total hop-acid would be essentially doubled by selection. An increase in the number of resin glands would be made to the extent of 65 per cent of the mean. Smaller gains would be realized for size of resin glands, weight of flowers, and date of flowering, on the basis of these estimates.

Simple correlation coefficients between variables are given in Table 4. Genotypic and environmental correlations are included in order to furnish a comparison of the correlations of genotype and environment with the phenotypic correlation. Most of the phenotypic and environmental correlation coefficients were positive, and many were significant. The phenotypic correlation coefficient between

flower weight and date of flowering was the only negative one, and it was not significant.

TABLE 4.
Phenotypic, Genotypic and Environmental Correlation Coefficients among Alpha-, Beta- and Total Hop-Acids, Resin Gland Number and Size, Flower Weight, and Flowering Date Based on Two-Year Averages for 20 Male Hop Lines. 1/

	Beta-acid	Total Hop-acid	Resin gland number	Resin gland size	Flower weight	Flowering date
Alpha-acid	.543*	.835**	.769**	.215	.160	.160
	(.560)	(.841)	(.834)	(.183)	(.162)	(.152)
	.542**	.843**	.288**	.187	.267*	.339**
Beta-acid		.916**	.837**	.456*	.289	.232
		(.920)	(.865)	(.467)	(.259)	(.208)
		.909**	.506**	.077	.526**	.173
Total hop-acid			.917**	.402	.266	.229
			(.961)	(.392)	(.261)	(.208)
			.467**	.142	.569**	.279*
Resin gland number				.311	.022	.448*
				(.253)	(.021)	(.468)
				.025	.167	.325**
Resin gland size					.561**	.394
					(.675)	(.368)
					.162	.254*
Flower weight						-.246
						(-.337)
						.004

1/ Upper values are the phenotypic correlations with 18 degrees of freedom, the values in parentheses are the genotypic correlations, and the lower values are the environmental correlations with 79 degrees of freedom (environmental effects include "plants in genotypes and years" only).

As a general rule, genotypic correlation coefficients were higher than the phenotypic correlations wherever a significant phenotypic correlation was found. Several of the genotypic correlations were smaller than their corresponding phenotypic correlation, but in every case the phenotypic correlation was not significant. However, a significant positive environmental correlation was shown in several instances, notably between alpha-acid and flower weight, alpha-acid and flowering date, beta-acid and flower weight, total hop-acid and flower weight, total hop-acid and flowering date, and resin gland size and flowering date. The data indicate in these instances that much of the phenotypic correlation was brought about by a correlation between environmental effects influencing the two variables. This means that environmental conditions which tend to increase one variable tend also to increase the other variable.

This was the case also in many instances where there was a significant phenotypic correlation between two variables. For example, in the case of alpha-acid and beta-acid, two variables which were significantly correlated phenotypically, there was the indication that environmental conditions which tend to increase alpha-acid also tend to increase beta-acid.

Where both the environmental correlation and the genotypic correlation are high and have the same sign, there would be little question that the phenotypic correlation would be significant. These effects might, however, tend to counteract each other. For example, in the case of flower weight and flowering date, there was a genotypic

correlation of -0.337. While there is no method available for testing the significance of genotypic correlations, a correlation of -0.337 is of sufficient size to indicate some genetical association. Almost complete lack of environmental association tended to counteract the genotypic association to the extent that the overall phenotypic correlation coefficient was not significant.

While there was no instance where a high genotypic correlation was counteracted by a high environmental correlation of opposite sign, an analysis of genotypic and environmental effects in this manner would be very informative to a plant breeder. It would indicate how much progress might be expected from selecting for two variables at a time or in selecting for one variable and hoping to bring about a change in a second variable simultaneously. Where environmental and genotypic correlations have the same sign, then some progress could be expected. However, where they have opposite signs, the influence of environment may counteract selection pressure in one direction to the extent that little gain could be expected.

It has been pointed out that the contents of alpha-acid and beta-acid were added together to give the value for total hop-acid. Hence, it is reasonable to assume that an increase in either alpha- or beta-acid would lead directly to an increase in total hop-acid. However, this relationship is not without variation. An increase in one of the components was not always associated with an increase in total hop-acid. Therefore, the relationship between either alpha-acid or beta-acid and total hop-acid was not a completely direct

effect. Part of the association between either component and total hop-acid was an indirect effect through the association of the components themselves. No data were obtained on the amount of possible common precursor of alpha- and beta-acid in the flowers, but it can be reasoned that the influence of precursor on total hop-acid would be a completely direct effect. However, since the ratio of alpha- to beta-acid formed from the precursor varies from plant to plant, then the total effect of either one of the components on total hop-acid is both direct and indirect.

Direct and indirect effects of alpha- and beta-acid on total hop-acid production are shown in Table 5. These relationships are broken down into both genetical and environmental effects which influenced the phenotypic association. It can be seen that the genotypic correlation of 0.841 between alpha-acid and total hop-acid was somewhat more than half due to a direct effect, and somewhat less than half due to an indirect effect through the association of alpha- and beta-acid. Similarly, beta-acid had a direct effect on total hop-acid production, but the genetical correlation between beta hop-acid and total hop-acid was partly made up through the association of alpha- and beta-acid. The same general picture was apparent for the environmental associations. This is reasonable considering that alpha- and beta-acid are formed from a common precursor, and any environmental influence which tends to push up the amount of precursor present in the flowers tends to push up both components.

TABLE 5.
Path Coefficient Analysis
Of the Influence of Alpha- and Beta-acids on Total Hop-acids.

	Pheno-typic	Geno-typic	Environ-mental 1/
Effect of alpha-acid on total hop-acid:			
Direct effect (p_{TA})	.480	.475	.496
Indirect effect via beta-acid (p_{TB})(r_{AB})	.355	.366	.347
Total (r_{TA})	.835**	.841	.843**
Effect of beta-acid on total hop-acid:			
Direct effect (p_{TB})	.655	.654	.640
Indirect effect via alpha-acid (p_{TA})(r_{AB})	.261	.266	.269
Total (r_{TB})	.916**	.920	.909**

1/ Includes "plants in genotypes and years" only.

Path coefficient analyses of factors influencing alpha-acid, beta-acid and total hop-acid are given in Tables 6, 7 and 8, respectively. A great deal of similarity is encountered in these three tables, as indeed it should be, since factors influencing alpha- and beta-acid through a common precursor should be similar in their effects. While some factors may have a greater or lesser effect on beta-acid than it would on alpha-acid, the direction of these effects should be the same in both cases. Some discrepancies are apparent, but these involve values of small magnitude and probably represent random variation about zero. Since factors affecting alpha- and beta-acid would also be affecting total hop-acid through their influence

TABLE 6.
Path Coefficient Analysis of Factors Influencing Alpha-acid.

	Pheno-typic	Geno-typic	Environ-mental 1/
Effect of gland number on alpha-acid:			
Direct effect (p_{AN})	.861	.976	.163
Indirect effect via gland size (p_{AS})(r_{NS})	-.019	.023	.001
Indirect effect via flower weight (p_{AW})(r_{NW})	.002	-.001	.038
Indirect effect via flowering date (p_{AF})(r_{NF})	-.075	-.164	.086
Total (r_{AN})	.769**	.834	.288**
Effect of gland size on alpha-acid:			
Direct effect (p_{AS})	-.062	.092	.079
Indirect effect via gland number (p_{AN})(r_{NS})	.268	.247	.004
Indirect effect via flower weight (p_{AW})(r_{SW})	.075	-.026	.037
Indirect effect via flowering date (p_{AF})(r_{SF})	-.065	-.130	.067
Total (r_{AS})	.215	.183	.187
Effect of flower weight on alpha-acid:			
Direct effect (p_{AW})	.134	-.039	.226
Indirect effect via flowering date (p_{AF})(r_{WF})	.042	.119	.001
Indirect effect via gland size (p_{AS})(r_{SW})	-.035	.062	.013
Indirect effect via gland number (p_{AN})(r_{NW})	.019	.020	.027
Total (r_{AW})	.160	.162	.267*
Effect of flowering date on alpha-acid:			
Direct effect (p_{AF})	-.168	-.352	.265
Indirect effect via flower weight (p_{AW})(r_{WF})	-.033	.013	.001
Indirect effect via gland size (p_{AS})(r_{SF})	-.024	.034	.020
Indirect effect via gland number (p_{AN})(r_{NF})	.385	.457	.053
Total (r_{AF})	.160	.152	.339**
Residual $[(p_{AZ})^2 + \Sigma 2$ (indirect effects of Z)]	.378	.230	.788

1/ Includes "plants in genotypes and years" only.

TABLE 7.
Path Coefficient Analysis of Factors Influencing Beta-acid.

	Pheno-typic	Geno-typic	Environ-mental 1/
Effect of gland number on beta-acid:			
Direct effect (p_{BN})	.865	1.076	.417
Indirect effect via gland size (p_{BS})(r_{NS})	.069	.316	0
Indirect effect via flower weight (p_{BW})(r_{NW})	.002	-.020	.076
Indirect effect via flowering date (p_{BF})(r_{NF})	-.099	-.507	.013
Total (r_{BN})	.837**	.865	.506**
Effect of gland size on beta-acid:			
Direct effect (p_{BS})	.224	1.249	-.018
Indirect effect via gland number (p_{BN})(r_{NS})	.269	.272	.010
Indirect effect via flower weight (p_{BW})(r_{SW})	.050	-.655	.075
Indirect effect via flowering date (p_{BF})(r_{SF})	-.087	-.399	.010
Total (r_{BS})	.456*	.467	.077
Effect of flower weight on beta-acid:			
Direct effect (p_{BW})	.090	-.971	.459
Indirect effect via flowering date (p_{BF})(r_{WF})	.054	.364	0
Indirect effect via gland size (p_{BS})(r_{SW})	.126	.843	-.003
Indirect effect via gland number (p_{BN})(r_{NW})	.019	.023	.070
Total (r_{BW})	.289	.259	.526**
Effect of flowering date on beta-acid:			
Direct effect (p_{BF})	-.221	-1.082	.040
Indirect effect via flower weight (p_{BW})(r_{WF})	-.022	.327	.002
Indirect effect via gland size (p_{BS})(r_{SF})	.088	.460	-.005
Indirect effect via gland number (p_{BN})(r_{NF})	.387	.503	.136
Total (r_{BF})	.232	.208	.173
Residual $[(p_{BZ})^2+\sum 2$ (indirect effects of Z)]	.199	-.037	.542

1/ Includes "plants in genotypes and years" only.

TABLE 8.
Path Coefficient Analysis of Factors Influencing Total Hop-acid.

	Pheno-typic	Geno-typic	Environ-mental 1/
Effect of gland number on total hop-acid:			
Direct effect (p_{TN})	.982	1.152	.326
Indirect effect via gland size (p_{TS})(r_{NS})	.059	.192	0
Indirect effect via flower weight (p_{TW})(r_{NW})	.001	-.011	.086
Indirect effect via flowering date (p_{TF})(r_{NF})	-.125	-.371	.055
Total (r_{TN})	.917**	.961	.467**
Effect of gland size on total hop-acid:			
Direct effect (p_{TS})	.190	.758	.008
Indirect effect via gland number (p_{TN})(r_{NS})	.305	.291	.008
Indirect effect via flower weight (p_{TW})(r_{SW})	.016	-.365	.083
Indirect effect via flowering date (p_{TF})(r_{SF})	-.109	-.292	.043
Total (r_{TS})	.402	.392	.142
Effect of flower weight on total hop-acid:			
Direct effect (p_{TW})	.029	-.542	.513
Indirect effect via flowering date (p_{TF})(r_{WF})	.069	.267	.001
Indirect effect via gland size (p_{TS})(r_{SW})	.107	.512	.001
Indirect effect via gland number (p_{TN})(r_{NW})	.021	.024	.054
Total (r_{TW})	.266	.261	.569**
Effect of flowering date on total hop-acid:			
Direct effect (p_{TF})	-.279	-.793	.169
Indirect effect via flower weight (p_{TW})(r_{WF})	-.007	.183	.002
Indirect effect via gland size (p_{TS})(r_{SF})	.075	.279	.002
Indirect effect via gland number (p_{TN})(r_{NF})	.440	.539	.106
Total (r_{TF})	.229	.208	.279*
Residual $[(p_{TZ})^2 + \sum 2$ (indirect effects of Z)]	.081	.635	.507

1/ Includes "plants in genotypes and years" only.

on the component constituents, an explanation of the data given in Table 8 would provide partial understanding of the data shown in Tables 6 and 7.

The overall association of resin gland number and total hop-acid content was a significant positive correlation. Most of the phenotypic correlation was made up from the direct effect of an increase in resin gland number which brought about an increase in total hop-acid. There was a slight negative effect of increasing gland number on total hop-acid indirectly through date of flowering. The same situation was true for the genetical effects, but these effects were much more pronounced. With respect to environmental correlation of gland number and total hop-acid, almost the entire positive significant correlation between these two factors was included in the direct effect of gland number on total hop-acid content.

These data suggest that genetical effects which tended to increase resin gland number also tended to directly increase total hop-acid. Coupled with this was a fairly well pronounced negative effect of decreasing total hop-acid indirectly through date of flowering. It appears that resin gland number and total hop-acid were either genetically linked or biologically inseparable. The latter seems reasonable since the acids are produced in the glands, and logically an increase in gland number should bring about an increase in total hop-acid in its overall effect. The interesting thing about this was the moderate negative influence of resin gland

number on total hop-acid via flowering date. This phenomenon suggests a modification of the interpretation of the effects of gland number on acid production. Restated--resin gland number was strongly associated with total hop-acid within any single date of flowering. Going from early to later flowering genotypes, the association between resin gland number and total hop-acid became weaker. This interpretation is substantiated by a negative association between flowering date and total acid of -0.793, which was the direct effect of flowering date on total hop-acid production. Therefore, total acid was increased as gland number was increased, but the increase in total acid was modified by the effect of flowering date. An increase in flowering date, on the other hand, brought about an increase in resin gland number, but tended to decrease the amount of total acid produced in these glands.

The overall phenotypic correlation of gland size and total hop-acid was not significant. Neither were the environmental effects correlated to any significant extent. While the overall genetical association of gland size and total hop-acid was low, an inspection of the breakdown of direct and indirect effects is very interesting. Here it is shown that the direct effect of gland size was to bring about an increase in total hop-acid (0.758). In addition, there was a slight positive, indirect effect via gland number. These two positive effects were partially counteracted by moderate negative effects, whereby an increase in either flower weight or flowering date tended to reduce the correlation between gland size and total

hop-acid. As a result, the genetical correlation between gland size and total hop-acid was only 0.392.

Flower weight was not significantly associated with total hop-acid in the phenotype. The direct genotypic effect of flower weight on total hop-acid was negative in direction and moderately strong (-0.542). This in turn was counteracted to a large extent by a moderately strong positive, indirect effect through gland size. As a result, the overall genetical correlation was rather low. A breakdown of the environmental correlation is somewhat different. The direct effect of flower weight on total hop-acid accounted for almost all of the significant positive correlation between these two variables. Environmental conditions which tended to increase flower weight appeared to increase total hop-acid (or perhaps it was the other way around). The indirect environmental effects through the other variables were very slight.

Although the inter-relationships of flowering date, gland number and total hop-acid have already been discussed, one or two points need further clarification. In the first place, a genetically controlled delay in flowering date tended to decrease total hop-acid content directly. Were it not for the fact that a delay in flowering date also tended to increase gland number, the overall effect of delayed flowering would have decreased total hop-acid. Indirect effects through the other variables were of sufficient magnitude to more than overcome the direct negative influence of flowering date on total hop-acid production resulting in a positive, though non-significant, correlation coefficient.

With respect to the residual factor or factors (Z), it can be noted that the combined direct and indirect effects of these unknown factors on the phenotypic expression of total hop-acid either were small or were cancelled out. This is apparent in the small value for the residual unknown effects. In the case of the genetical correlations, however, this was not true. A residual value of 0.635 indicates that less than half of the effects were accounted for in the path coefficient analysis. The same is true regarding the environmental correlations. Only about half of the effects were accounted for in this analysis. This makes the path coefficient analysis much less rigid than would be desired. It is possible that the residual value is made up of a multitude of many variables, each having small effects. However, this analysis was not complete enough to indicate what these variables might be.

A path coefficient analysis of the influence of flowering date and flower weight on resin gland number and size is given in Table 9. The influence of date of flowering on gland number was almost all direct for the phenotypic, genetical, and environmental correlations. Flower weight, on the other hand, had little effect, direct or indirect, on resin gland number.

Both date of flowering and flower weight had direct positive effects on gland size. This was true particularly for genetical correlations which tended to counteract weak indirect effects via the other variable in each case. Although the correlation coefficients for environment between date of flowering and gland size and

TABLE 9.
Path Coefficient Analysis of the Influence of Flowering Date and Flower Weight on Resin Gland Number and Size.

	Pheno-typic	Geno-typic	Environ-mental 1/
Effect of flowering date on gland number:			
Direct effect (p_{NF})	.482	.536	.324
Indirect effect via flower weight (p_{NW})(r_{WF})	-.034	-.068	.001
Total (r_{NF})	.448*	.468	.325**
Effect of flower weight on gland number:			
Direct effect (p_{NW})	.141	.202	.166
Indirect effect via flowering date (p_{NF})(r_{WF})	-.119	-.181	.001
Total (r_{NW})	.022	.021	.167
Effect of flowering date on gland size:			
Direct effect (p_{SF})	.567	.672	.253
Indirect effect via flower weight (p_{SW})(r_{WF})	-.173	-.304	.001
Total (r_{SF})	.394	.368	.254*
Effect of flower weight on gland size:			
Direct effect (p_{SW})	.700	.902	.161
Indirect effect via flowering date (p_{SF})(r_{WF})	-.139	-.227	.001
Total (r_{SW})	.561**	.675	.162
Residual $\left[(p_{NZ})(p_{SZ})+\sum(\text{indirect effects of Z})\right]$ 2/	.062	-.081	-.084

1/ Includes "plants in genotypes and years" only.

2/ Factors influencing r_{NS}.

flower weight and gland size were small, they were almost entirely composed of the direct effects of these two variables on gland size.

The overall phenotypic, genetical and environmental correlation coefficients between resin gland number and resin gland size are composed of several effects. These are (1) the direct and indirect effects of both flowering date and flower weight on gland size and gland number, (2) all unknown effects which tend to make flowering date and flower weight correlated, and (3) any other unknown variables which tend to make gland number and gland size correlated, irrespective of their correlations through flower weight and flowering date. It would appear from these data that all three correlation coefficients between gland size and gland number were accounted for by the effects of flower weight and flowering date. This is borne out by relatively small values for the residual effects of unknown and known variables. However, there may be variables which influenced the correlation coefficient between gland number and gland size which were rather large in their effect, but which were cancelled out either by their indirect effects or by the direct and indirect effects of other unknown variables.

The calculated genetic path coefficients for the overall relationship of all seven variables is given in Figure 10. This diagram shows that total hop-acid was influenced moderately by alpha-acid content and strongly by beta-acid content. In turn, alpha-acid content was influenced strongly by resin gland number and moderately by date of flowering. The direct effects of flower weight and gland

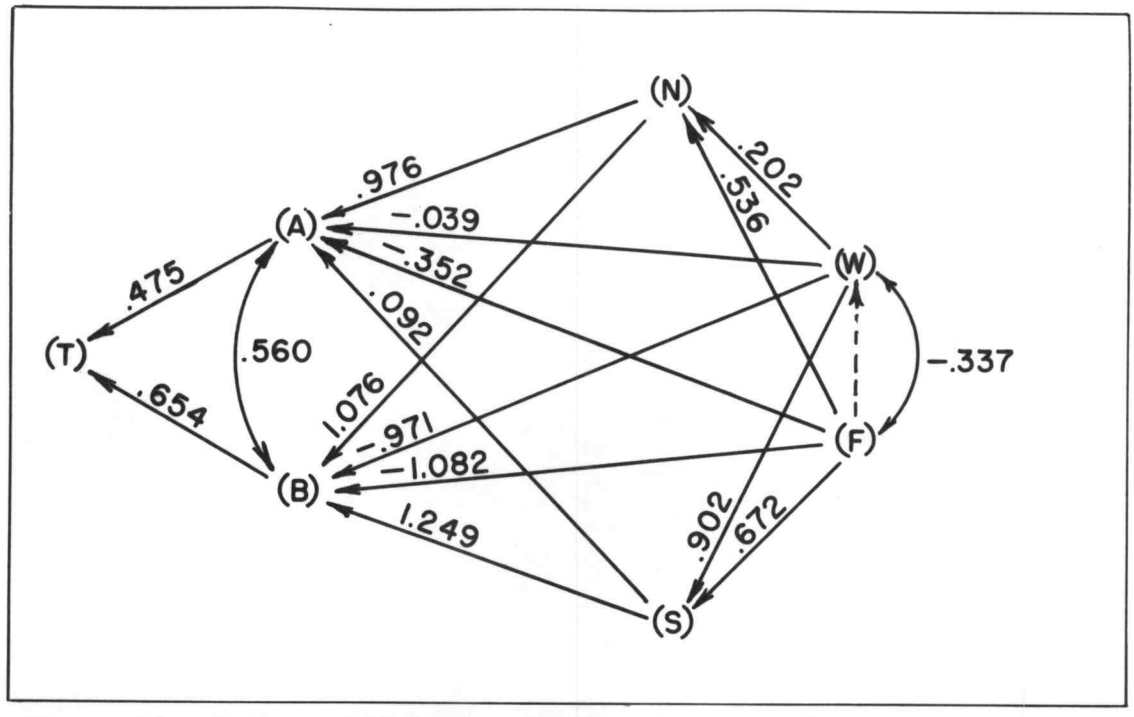


Figure 10. Path coefficient analysis of genetical effects.

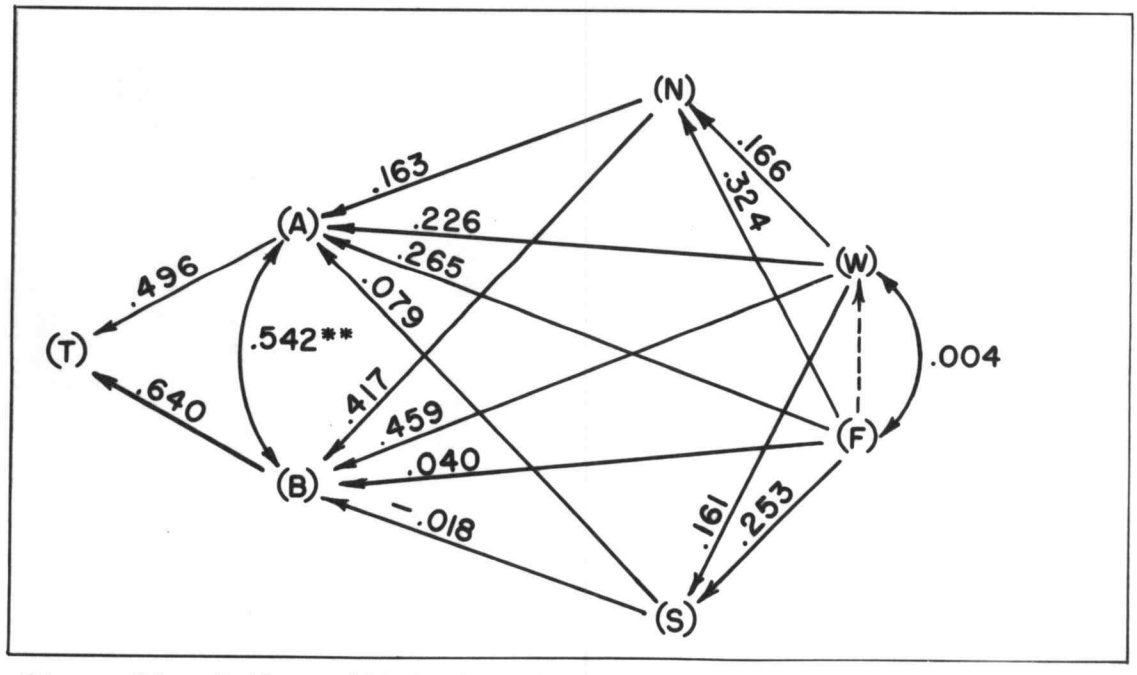


Figure 11. Path coefficient analysis of environmental effects.

size were of little consequence. On the other hand, resin gland number, flower weight, date of flowering, and gland size all had strong direct effects on beta-acid production. The direct effects of resin gland number and resin gland size were positive in nature, and those for flower weight and date of flowering were negative in nature. Both flower weight and date of flowering had direct positive influence on resin gland number and size. The influence of flower weight on gland number was slight.

These data suggest that selection for high resin gland number would lead to increases in alpha- and beta-acids, and in turn total hop-acid content. Selection for an increase in gland size would lead to an increase in beta-acid, but would have little effect on the content of alpha-acid. Selection for later flowering would lead to decreases in beta-acid and alpha-acid in material in which the size and number of glands remained unchanged. Likewise, selection for increased flower size would lead to decreases in both alpha- and beta-acids, but only the beta-acid would be affected very greatly. On the other hand, selection for either an increase in flower weight or delayed maturity would bring about increases in gland size and number.

Although it was not possible to determine the direct effect of date of flowering on flower weight, it is reasonable to assume that such does exist. In this case, the correlation coefficient was -0.337, and it is assumed that selection for delayed maturity would result in smaller flowers.

A path coefficient analysis of the overall environmental effects is given in Figure 11. The data indicate that environmental conditions which tended to increase resin gland number, flower weight, flowering date, and gland size, also tended to increase the content of alpha-acid. All of these effects, however, were small. Environmental conditions which tended to increase resin gland number and flower weight tended to increase beta-acid to a moderate degree also. Environmental effects which tended to delay maturity or to increase gland size had little effect on beta-acid. Likewise, environmental conditions which affected flowering date had little effect on flower weight.

The results reported here will be of importance to hop breeders all over the world. Almost every paper written about hop breeding in the past 50 years has pointed out that several important characters of the hop plant are sex-limited. Each has pointed out the difficulties of selecting the male parent for crosses involving selection for economic traits. A method for phenotypically evaluating male hop lines without progeny tests has been long desired.

The problem can be compared to the selection of sires in a dairy breeding program for improving milk and butterfat yields in dairy herds. In such a program, objective selection of a sire has been made possible only through the utilization of progeny tests. Dairy breeders have also long desired a method of phenotypically evaluating a sire in order to make initial selection for milk producing potential. Initial selection on the basis of phenotypic performance would

considerably reduce the time and effort that must be expended in progeny testing a large number of individuals. If a method were developed by which a dairy bull could be evaluated directly for milk and butterfat production, it would immediately have world-wide significance.

The time is approaching when brewers will be universally concerned with the contents of the various acid components in hops. The results reported here regarding the variability of these components in male hop flowers and their relationships with other characters provide practical information basic to hop breeding. Probably alpha-acid will continue to receive greatest consideration.

Work of this nature is not completed. Phenotypic evaluation of a male is of no value if the characters evaluated are not transmitted to its progeny to a significant degree. Work by European investigators has shown that female parents transmit resin contents. One study of transmissibility of acid contents by male parents has been made, but the results are not extensive. While attempts have been made to measure transmissibility of resin content by males high in resin gland number, only negative results have been obtained. Now that resin gland number has been shown to be highly correlated with resin content in the same individual, it remains to be seen if resin content itself is transmitted by the males. Such a study is currently in progress at Corvallis, Oregon.

Unfortunately, several characters which are of great importance to the hop breeder still appear to be sex-limited with little hope of

isolating superior males without the use of progeny tests. Among these are yield, resistance to shattering during harvest, storage-ability of the hop cones, as well as many other quality factors. At the present time there is no way of isolating a superior male on the basis of phenotypic evaluation of these characters. This does not mean, however, that prospects for the future are hopeless. There seems to be little doubt that eventually hop investigators will develop means of evaluating male lines for most of the characters which are of commercial importance in the female hop.

A complete understanding of the formation of acid components in female hop cones during maturation is not available. Because of the similar structural formulae for alpha- and beta-acids, it has been assumed that these two components have similar paths of synthesis. It is assumed also that acid synthesis in male flowers would parallel acid synthesis in female hops. Farrar and others (13, p. 58-59) have stated that if limiting reactions occur after divergence then alpha- and beta-acid would be independent because of an excess of a common precursor at the point of divergence. If limiting reactions occur before divergence, the amount of total hop-acid will be controlled, and the amounts of alpha-acid and beta-acid will be influenced by their rates after divergence.

While the study reported here was not designed to provide answers to this question, the data provide some information on the subject. Not only are alpha- and beta-acid dependent to a significant degree, but part of the correlation of either component with

total hop-acid is made up through the indirect effect of one component through the other. Therefore, these two components must have similar paths of synthesis until late in their formation. Lack of perfect correlation between alpha- and beta-acid indicates the occurrence of limiting reactions after divergence of the common paths.

SUMMARY AND CONCLUSIONS

An experimental trial involving 20 male hop lines was undertaken in 1956. It was designed to measure variability in several characters, particularly those concerned with quality components important in the female cones and to study their relationships with other characters. The data obtained from this trial in 1958 and 1959 were used to calculate estimates of genetic, phenotypic and environmental variability for these characters. Inter-relationships of characters were studied by means of path coefficient analysis.

Significant differences among lines were shown for all seven of the characters studied. Six- to 13-fold differences were found for alpha-, beta-, and total hop-acid contents. The range in gland number was approximately five-fold. The range in gland volume was about 80 per cent of the mean, and the range in flower weight was about half of the mean. Approximately a 30-day range in flowering date was found.

Genetic diversity ratios varied from moderate to quite high. For alpha-, beta- and total hop-acid they were from 0.80 to 0.83 on the basis of single plant observations. For number of resin glands the ratio was 0.67, and for size of resin glands it was 0.42. The diversity ratio for weight of flowers was 0.62 and for date of flowering was 0.58 on the basis of single plant observations. Expected gains from selection calculated on the basis of single plant observations indicated that alpha-acid, beta-acid and total hop-acid would be essentially doubled by selecting the upper five per cent of

the population. For number of resin glands, an increase of 65 per cent of the mean would be expected on the same basis. Expected gains from selection for size of resin glands, weight of flowers, and date of flowering were much less, but the data suggested that significant gains would be expected.

Alpha- and beta-acid contents were directly associated with total hop-acid content and were significantly correlated with each other. Each component was also found to be indirectly associated with total hop-acid content through the other variable. This was true for both the genetical and environmental associations.

Both alpha-acid and beta-acid, and in turn total hop-acid, were directly influenced by resin gland number, whereby an increase in resin gland number increased the chemical components. This was true for both the genotypic and environmental effects. An increase in resin gland size was found to bring about an increase in beta-acid content through its genetical effect but not through its environmental effect. Gland size appeared to have no effect on alpha-acid content. An increase in flower weight tended to increase beta-acid and alpha-acid through its environmental effect, but the genetical effect of flower weight on beta-acid was one of reducing its amount. Flowering date had a direct decreasing effect on both alpha- and beta-acid through its genetic association, but tended to increase alpha-acid through its environmental association. It appeared that lines possessing genes for late flowering were inherently low in alpha-acid and beta-acid, but environmental conditions associated with late

flowering tended to increase alpha-acid content. Similarly, the association of high resin gland number with late flowering also tended to increase hop-acid content. The path coefficient analysis of the effects of these variables on hop-acid components was fairly rigid for the phenotypic associations, but both genetical and environmental effects were only partially accounted for.

The correlation coefficient between resin gland number and resin gland size appeared to be almost entirely accounted for by the effects of date of flowering and flower weight. This was true for the phenotypic as well as the genetical and environmental associations. An increase in date of flowering tended to increase resin gland number and resin gland size. The indirect effect of flowering date on gland number through flower weight was of little consequence, but it exerted a moderately strong negative influence on gland size via flower weight. Flower weight had little influence on the number of resin glands, but it did exert a positive genetic effect and in turn a phenotypic effect on gland size. The genotypic correlation between date of flowering and flower weight was negative. The environmental association between these two variables was very slight.

The data reported in this study are of importance to the hop breeder and illustrate the amount of variability present and thus the expected gain from selection for several characters. Of particular significance, the data demonstrate for the first time that phenotypic selection for factors of hop quality can be made among male hop lines.

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APPENDIX

APPENDIX TABLE 1.

Pedigrees and Identification of the 20 Male Hop Lines.

Entry	Source	Accession No.	Pedigree
1	106-S	C 19170 M	Unknown x 54-17 (East Kent Golding x (Early Green x Kent Golding-S)
2	110-S	C 19173 M	Streisselspalt x 73-28 (Late Cluster-S)
3	123	C 19009 M	Fuggle x Fuggle-S
4	217	C 19036 M	Late Clusters x Fuggle-S
5	125	C 52045 M	(East Kent Golding x (Early Green x Kent Golding-S) x 521 (Early green x Unknown-S)
6	221	C 19037 M	Fuggle-S (51-31) x Fuggle-S
7	324	C 19048 M	Fuggle-S (51-31) x Red Vine-S
8	224	C 19039 M	Fuggle-S (51-31) x Red Vine-S
9	317	C 19041 M	Early Green x Unknown-S
10	319	C 19043 M	Belgian Burvine x Fuggle-S
11	322	C 19046 M	Late Cluster-S x Fuggle-S
12	320	C 19044 M	Fuggle x Fuggle-S
13	323	C 19047 M	Elsasser x Fuggle-S
14	424	I 19007 M	Brewers Favourite-S
15	518	I 19005 M	Late Cluster-S
16	425-I	C 51101 M	308-2 (Fuggle-S (51-31) x (Landhopfen-S x (Golden Cluster x Fuggle-S) x (Landhopfen-S x (Golden Cluster x Fuggle-S)
17	521	C 19058 M	Early Green x Unknown-S
18	523	C 19060 M	East Kent Golding x Bavarian-S
19	417	C 19050 M	Fuggle x Fuggle-S
20	524	C 19061 M	Late Grape x Fuggle-S

APPENDIX TABLE 2.
Planting Plan of Male Hop Line Study in 1956.

Entry	Old Breeding Block No.	Accession No.	Hill number in replication I	II	III	IV	V
1	106-S	C 19170 M	112	220	306	413	516
2	110-S	C 19173 M	101	207	301	403	515
3	123	C 19009 M	103	215	302	416	519
4	217	C 19036 M	116	204	309	401	508
5	125	C 52045 M	113	213	308	404	520
6	221	C 19037 M	119	202	303	405	517
7	324	C 19048 M	120	212	320	418	512
8	224	C 19039 M	105	209	318	408	507
9	317	C 19041 M	109	218	310	410	505
10	319	C 19043 M	117	216	305	417	504
11	322	C 19046 M	110	201	317	411	509
12	320	C 19044 M	106	208	311	415	513
13	323	C 19047 M	114	214	307	409	506
14	424	I 19007 M	111	219	304	407	502
15	518	C 19005 M	102	211	312	406	503
16	425-1	C 51101 M	108	203	314	402	514
17	521	C 19058 M	107	205	315	414	511
18	523	C 19060 M	104	217	316	412	510
19	417	C 19050 M	115	206	313	419	518
20	524	C 19061 M	118	210	319	420	501

APPENDIX TABLE 3.
Mean Values for Chemical and Morphological Characters Measured on 20 Male Lines, 1958 and 1959.

Entry	Male Line	Alpha-acid (µg/flower) 1958	1959	Ave.	Beta-acid (µg/flower 1958	1959	Ave.	Total hop-acid (µg/flower) 1958	1959	Ave.	Gland number (glands/flower) 1958	1959	Ave.	Gland volume ($\mu^3 x 10^3$/gland) 1958	1959	Ave.	Flower weight (µg/flower) 1958	1959	Ave.	Flowering date (days after 5/31) 1958	1959	Ave.
1	106-S	6.2	6.7	6.4	12.5	13.7	13.1	18.7	20.4	19.6	11	21	16	652	766	710	1200	1250	1230	31	35	33
2	110-S	10.2	6.1	8.2	15.4	11.4	13.4	25.6	17.5	21.6	22	28	25	385	484	435	1070	840	960	45	44	45
3	123	7.9	4.9	6.4	15.2	13.8	14.6	23.1	18.7	21.0	31	35	33	594	521	558	890	850	870	41	42	41
4	217	8.0	4.4	6.2	19.1	13.2	16.2	27.1	17.5	22.4	35	23	29	832	763	798	790	800	800	52	50	51
5	125	2.8	1.9	2.4	5.8	4.3	5.0	8.6	6.2	7.4	19	21	20	463	716	590	900	840	870	49	68	59
6	221	8.7	4.4	6.6	18.5	10.9	14.7	27.2	15.3	21.3	27	20	24	957	730	844	1200	960	1080	49	52	51
7	324	8.4	7.4	7.9	5.8	6.1	5.9	14.2	13.4	13.8	21	27	24	473	530	502	930	790	860	44	56	50
8	224	5.3	4.7	5.0	4.2	4.1	4.2	9.5	8.8	9.1	19	16	18	425	510	468	860	790	830	39	54	47
9	317	14.1	6.6	10.4	27.4	22.9	25.2	41.6	29.5	35.6	56	49	52	664	814	740	920	810	860	50	66	58
10	319	3.1	2.0	2.6	5.7	3.4	4.5	8.8	5.4	7.1	15	17	16	463	510	487	840	740	790	47	61	54
11	322	4.9	1.0	2.9	19.8	13.3	16.6	24.6	14.3	19.5	30	30	30	1092	856	975	1060	990	1020	52	61	56
12	320	17.3	11.1	14.2	11.1	9.0	10.0	28.4	20.0	24.2	42	31	36	808	937	873	900	880	890	57	63	60
13	323	13.1	9.6	11.4	9.5	4.9	7.2	22.6	14.5	18.6	35	31	33	672	504	588	850	870	860	48	55	51
14	424	2.3	0.5	1.4	7.5	5.0	6.2	9.8	5.5	7.6	29	15	22	531	397	464	910	860	880	45	52	48
15	518	4.6	0.5	2.6	16.4	17.8	17.1	20.9	18.3	19.7	37	34	36	628	645	637	820	920	870	54	58	56
16	425-1	2.6	3.5	3.0	9.1	13.0	11.0	11.7	16.5	14.1	21	31	26	619	955	788	840	980	910	58	71	65
17	521	20.9	15.0	18.0	25.7	30.5	28.1	46.6	45.5	46.1	47	75	61	476	695	586	830	880	860	52	68	60
18	523	23.1	13.7	18.4	23.1	21.4	22.3	46.2	35.1	40.7	60	51	56	1137	706	922	1150	1170	1160	54	59	56
19	417	6.9	2.7	4.8	16.1	17.6	16.9	22.9	20.3	21.7	29	33	31	901	895	899	1180	1080	1130	51	63	57
20	524	9.2	3.1	6.2	18.1	13.0	15.6	27.3	16.1	21.7	42	30	36	642	510	576	960	790	880	54	51	52
Mean		9.0	5.5	7.2	14.3	12.5	13.4	23.3	18.0	20.6	31	31	31	671	673	672	960	900	930	49	56	52
CV (%)				25			18			18			17			19			7			8

APPENDIX TABLE 4.
Correlation Coefficients of Individual Plant Measurements for Chemical and Morphological Characters in Male Hops, 1958 and 1959. 1/

		Alpha-acid			Beta-acid			Total hop-acid			Resin gland number			Flower weight			Flowering date			Gland diameter		
		1958	1959	Ave.	1958	1959	Ave.	1958	1959	Ave.	1958	1959	Ave.	1958	1959	Ave.	1958	1959	Ave.	1958	1959	Ave.
Beta-acid	1958	.610																				
	1959		.441																			
	Ave.			.543																		
Total hop-acid	1958	.881			.912																	
	1959		.754			.922																
	Ave.			.836			.915															
Resin gland number	1958	.737			.733			.818														
	1959		.640			.844			.894													
	Ave.			.746			.823			.897												
Flower weight	1958	.163			.274			.248			-.025											
	1959		.186			.390			.366			.151										
	Ave.			.164			.302			.276			.036									
Flowering date	1958	.277			.326			.337			.510			-.187								
	1959		.113			.187			.186			.364			-.135							
	Ave.			.179			.224			.233			.437			-.212						
Gland diameter	1958	.290			.457			.468			.380			.468			.365					
	1959		.121			.371			.323			.242			.391			.392				
	Ave.			.228			.457			.408			.308			.542			.349			
Gland volume	1958	.306			.460			.433			.381			.478			.370			.982		
	1959		.093			.325			.278			.206			.386			.422			.982	
	Ave.			.213			.427			.381			.291			.533			.372			.982

1/ r of .255 required for significance at the five per cent level. r of .331 required for significance at the one per cent level.

APPENDIX TABLE 5.
Correlation Coefficients of Line Means for Chemical and Morphological Characters in Male Hops, 1958 and 1959. 1/

		Alpha-acid			Beta-acid			Total hop-acid			Resin gland number			Flower weight			Flowering date			Gland diameter		
		1958	1959	Ave.	1958	1959	Ave.	1958	1959	Ave.	1958	1959	Ave.	1958	1959	Ave.	1958	1959	Ave.	1958	1959	Ave.
Beta-acid	1958	.599																				
	1959		.452																			
	Ave.			.543																		
Total hop-acid	1958	.878			.910																	
	1959		.756			.925																
	Ave.			.835			.916															
Resin gland number	1958	.777			.749			.852														
	1959		.680			.877			.932													
	Ave.			.769			.837			.917												
Flower weight	1958	.132			.260			.224			-.052											
	1959		.205			.369			.357			.155										
	Ave.			.160			.289			.266			.022									
Flowering date	1958	.269			.341			.344			.549			-.241								
	1959		.090			.200			.184			.384			-.150							
	Ave.			.160			.232			.229			.448			-.246						
Gland diameter	1958	.297			.503			.456			.440			.517			.386					
	1959		.149			.469			.407			.296			.499			.449				
	Ave.			.227			.489			.429			.324			.575			.363			
Gland volume	1958	.308			.503			.461			.445			.518			.393			.989		
	1959		.113			.407			.346			.247			.479			.479			.990	
	Ave.			.215			.456			.402			.311			.561			.394			.989

1/ r of .444 required for significance at the five per cent level. r of .561 required for significance at the one per cent level.

APPENDIX TABLE 6.

Intra-plant and Intra-line Correlation Coefficients for Chemical and Morphological Characters in Male Hops, 1958 vs. 1959.

	Intra-plant	Intra-line
Alpha-acid	.861**	.920**
Beta-acid	.808**	.883**
Total hop-acid	.650**	.905**
Resin gland number	.630**	.732**
Flower weight	.638**	.720**
Flowering date	.595**	.725**
Resin gland diameter	.363**	.563**
Resin gland volume	.385**	.559*