# NUTRITIVE VALUE AND CULINARY QUALITY OF SOME BREEDING LINES OF CARROT, DAUCUS CAROTA, AS DETERMINED IN BIOASSAY BY THE MEADOW VOLE, MICROTUS PENNSYLVANICUS

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This is to certify that the

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NUTRITIVE VALUE AND CULINARY OUALITY OF SOME BREEDING LINES OF CARROT, DAUCUS CAROTA, AS DETERMINED IN BIOASSAY BY THE MEADOW VOLE, MICROTUS PENNSYLVANICUS

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Ronald Paton Lane

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C.E. Peterson
Major professor

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ABSTRACT

NUTRITIVE VALUE AND CULINARY QUALITY OF SOME BREEDING LINES OF CARROT, DAUCUS CAROTA, AS DETERMINED IN BIOASSAY BY THE MEADOW VOLE, MICROTUS PENNSYLVANICUS

By Ronald Paton Lane

A consistent preference by the meadow vole, Microtus pennsylvanicus, for the roots of certain inbred carrot lines and hybrids was observed in field plantings. This suggested the possibility of using the meadow vole to evaluate carrot breeding material for nutritive value and culinary quality on the basis of vole preference for the roots. In a preliminary field test, 50 carrot lines representing the full range of feeding damage were planted in a confined feeding experiment. Lines showing no damage and severe damage were selected for controlled feeding trials and further evaluation.

Crosses were made between the contrasting lines, and the $F_1$, $F_2$, and the backcross generations were evaluated to determine the inheritance of factors responsible for meadow vole preference. Analysis of the data suggested quantitative inheritance with significant interactions involved. The heritability estimate was low. Statistical analysis did not indicate the number of genes concerned.

Ronald Paton Lane

In laboratory feeding tests, all carrot diets were inferior to control diets. There was no relationship between vole preference and the nutritive value of the carrots as measured by the growth response of young voles. However, vole preference showed a significant positive correlation with the sucrose content of the roots while a significant negative correlation was found between preference and total reducing sugars. The specific growth response was not correlated with the sugar content of the roots. Neither growth response nor vole preference was correlated with crude fiber, protein, or total carbohydrates. No correlation was found between mean taste panel scores for overall rating of carrot samples and data on mean feeding index by the voles. The correlation between taste panel scores and the concentration of sugars in the root was also non-significant.

While the high correlations between sucrose content of the roots and preference by voles suggest that these indices could be used in screening carrot populations for sucrose content, the variability encountered may invalidate the use of such preference tests for selection purposes.

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By

Ronald Paton Lane

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# I. INTRODUCTION

A contemporary problem in vegetable breeding is the objective measurement of nutritive quality and flavor of genetic lines in early generations, preferably in large populations. The screening techniques should provide reproducible measurements of quality components so that genotypes with the maximum potential for desirable quality can be determined. There is a need for a total assessment of quality encompassing all of the characteristics which contribute to industry and consumer acceptance.

Field observations made at the Michigan State Experiment Station suggested a possibility of developing such a technique for carrot evaluation. A distinct preference by the meadow vole, *Microtus pennsylvanicus*, for selected inbred carrot lines and hybrids was observed in the field in 1962. In 1963, a confined feeding experiment confirmed the earlier observation and a number of lines and hybrids with common parentage were demonstrated to be consistently selected by the voles.

The purpose of this investigation was to determine the feasibility of using bioassays for evaluating nutritive value and culinary quality of carrot genetic lines. The objectives of this research were:

1. To evaluate the meadow vole as a bioassay for screening inbred carrot lines for quality components.
2. To establish and standardize techniques for selecting nutritive value in carrot lines.
3. To determine the inheritance of the factors responsible for vole preference in order to predict the quality of $F_1$ hybrids.

## II. REVIEW OF LITERATURE

Objective measurements of carrot quality are not widely reported. Early investigations were primarily concerned with the biochemical changes which the roots undergo in storage. Hasselbring (14) found that the sucrose content of carrots was highest at harvest and immediately began to decrease in storage. The decrease in sucrose was accompanied by a corresponding increase in reducing sugars. He further concluded that the flavor of carrots was determined largely by their natural content of sucrose. Denny, Thornton, and Schroeder (8) found that carrots lost sucrose and gained glucose during 16 days of storage at 5° C with high humidity. Werner (30) also found reducing sugar to increase in Red Cored Chantenay and Nantes carrot varieties during storage. He reported that there was a large amount of sucrose compared with reducing sugar in both xylem and phloem regions of the roots. This is consistent with the statement of Hasselbring concerning the content of sucrose in relation to flavor in carrots.

According to Hawk and Bergein (15), the relative sweetness of some of the sugars commonly present in plants is as follows considering sucrose as 100:

| | |
|---|---|
| Maltose .................. | 32.5 |
| Glucose .................. | 74.3 |
| Sucrose .................. | 100.0 |
| Fructose.................. | 173.3 |

Since the sweetness of carrot roots may depend on the relative amount of the various sugars present, Platenuis (24) tested for their quantities by the specificity of sugars on the time required for osazone formation. His results showed that sucrose constituted almost all of the disaccharides, while glucose made up almost entirely the monosaccharide fraction. Fructose was assumed to be formed as an intermediate during sucrose hydrolysis and probably converted to other carbohydrates. He suggested that the lack of change in sugar content was caused by a balance between respiration and the hydrolysis of polysaccharides.

Rygg (26) determined fructose in carrot roots since up to that time most investigators had found reducing sugar to be composed largely or entirely of glucose. After testing specifically for fructose, he concluded that it was present in quantities closely approaching glucose. His findings appeared to be logical provided the hydrolysis of sucrose in carrots follows the generally accepted pattern of one molecule of glucose and one of fructose for each hydrolyzed molecule of sucrose.

In sweet corn, Winter, Nylund, and Legun (31) reported that the correlation coefficients between sugar content and taste panel scores for flavor were influenced more by variation in sugar content at relatively low sugar levels than at high. This indicated that with the sugar level of sweet corn sufficiently high, other variables were more important in determining palatability.

Various methods have been used to measure the tenderness in vegetables. One of the most common is the detection of crude fiber. Tenderness of carrot roots was considered by Platenius (24) to be proportional to the crude fiber content. In his experiments the percentage of crude fiber remained relatively consistent for a given variety harvested at various stages of maturity. Hasselbring (14) also found that crude fiber was not affected by maturity.

The age of the plant has been reported as an important factor in determining the amount of carotene in carrot roots. The investigations of Barnes (2), Brown (4), Hansen (13), Lantz (21), and Smith, Caldwell, and Burlinson (27) show that carotene increased consistently as the growing season progressed.

The dry matter percentages of carrot roots were reported by Werner (30) to fluctuate throughout the growing and storage season in a manner similar to the sucrose content. Brown (4), Riddle and MacGillivary (25), and Werner (30) found that the percentage of dry matter in the phloem was greater than in the xylem of the carrot root. Riddle and MacGillivary (25) also found that the top third of the root was higher in dry matter than the middle third by 13 per cent and the latter was about 4 per cent greater than the basal third.

Carlton (5) found greater variation in soluble solids, sugars, and dry matter between carrot roots within a variety than between variety means. Individual roots ranged from 13.3 to 7.7 per cent in dry matter while total sugar varied from 6.41 to 1.92 per cent and the soluble solids from 9.0 to 4.5 per cent on a fresh weight basis. He concluded that it should be possible to develop uniform varieties with levels of dry matter and sugar approximating the extremes recorded. Carlton and Peterson (6) reported that soluble solids were positively correlated with dry matter, total sugars, and non-reducing sugars, but with reducing sugars correlations were negative or non-significant. By selection and inbreeding, they were able to alter substantially the percentage of sugars and dry matter.

Lipton (22) showed that the dry matter content of carrot roots was affected by soil type, variety, and maturity. He found mean dry matter contents of 10.11 and 12.02 per cent for roots grown on muck and mineral soil respectively. Dry matter percentages ranged from 10.29 to 11.73 between three varieties and were 10.66 and 11.46 per cent for two harvest dates. He also found that dry matter content was useful in determining how sugars vary in relation to other constitutients. Barnes (2) and Platenius (23) reported on the effects of temperature, moisture, and age of the plant upon dry matter content of carrot roots. They found higher dry matter contents when the roots were grown under low soil moisture conditions. Dry matter also increased slightly with the age of the plant.

Bailey (1) has given a good description of the life habits of the meadow vole, Microtus pennsylvanicus. In their natural habitat they feed largely on grasses, sprouts, and roots; their feeding habits varying with the season of the year. In fields, gardens, and orchards, they feed on grains, most garden vegetables, and the bark of many trees, shrubs and vines. Often they destroy and waste far more vegetation than they eat. Commenting on the individuality of the meadow vole, Bailey stated, "Some are very fond of certain foods which others have not learned to like or will not eat." Although the meadow vole is recognized as playing an important role in the balance of nature, it is generally considered to be a menace to many economic crops.

In captivity voles will eat a great variety of green vegetation including most vegetables. It has been calculated that each adult requires from 24 to 36 pounds of vegetation per year. In a cage of adult voles, Bailey found that 55 per cent of the weight of each animal was

eaten every 24 hours. These animals received considerably richer diets than would be available to them in nature. In another cage, an average of 107 per cent of the weight of each animal was consumed every 24 hours. This was on what he considered to be more nearly a normal ration and could be used for computing food consumption in nature. On this basis, one average 40 gram meadow vole would consume over 30 pounds of food per year.

The breeding season of meadow voles extends over most of the year except midwinter. It is considered to be one of the most prolific of the mammals. The animals mature very quickly with young females reaching breeding age in 25 to 30 days.

Various authors have estimated the size of the home ranges of the meadow vole. Hamilton (12) by the use of a live-trapping technique concluded that the range of the meadow vole was about 1/15 acre. While he did not distinguish between male and female ranges, he stated that the male wanders more widely than the female. A study by Blair (3) in Michigan indicated that the female vole had a home range of about one-fifth to one-fourth of an acre while that of the male vole was slightly less than one-third of an acre. Hayne (19) found a positive relationship between apparent size of home range and the distance used between traps and concluded that such determinations were of doubtful reliability.

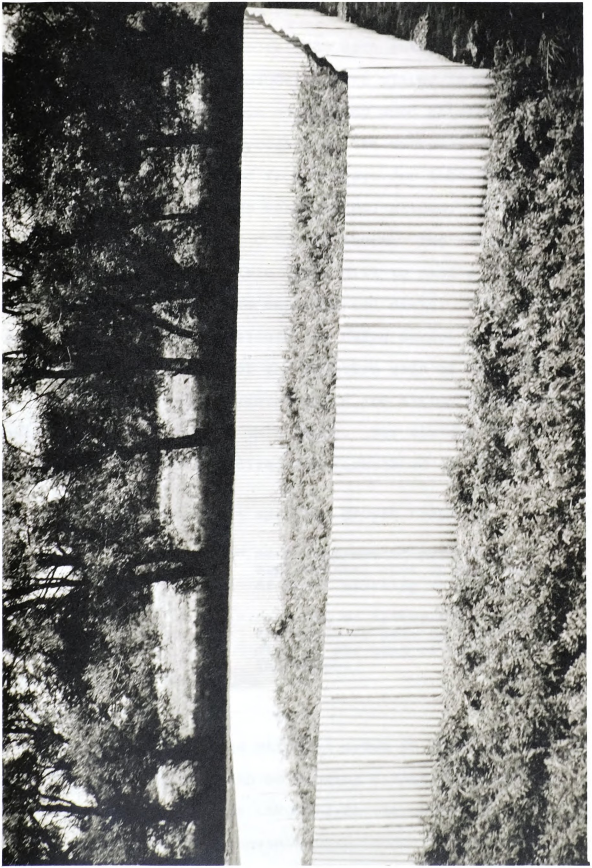
While mice, rats, guinea pigs, and rabbits are common laboratory animals, little use has been made of the meadow vole in feeding experiments. Elliott (9) reported on his colony, established in 1962 with wild voles, and described the initial problems of establishing satisfactory diets and getting the animals to reproduce under laboratory conditions. He developed a bioassay for individual alfalfa clones utilizing weanling meadow voles.

In subsequent work, Elliott (10) and Elliott and Olien (11) used the meadow vole to detect antimetabolites from individual alfalfa clones. They were able to separate by paper chromatographic and electrophoretic techniques a series of nitrogen base compounds some of which showed anti-metabolic activity to voles.

## III. METHODS AND MATERIALS

For a preliminary study, 50 lines of carrot from the breeding program at Michigan State University were planted May 14, 1965 at the Muck Experimental Farm in a randomized complete block with eight replications. The rows were spaced 18 inches apart with plots 18 inches long in which a stand of 10 plants was established by thinning. Guard rows of a standard variety were planted on all sides giving a 33 x 33 foot plot. On August 9, when the carrot roots were approximately one inch in diameter, 22 meadow voles of various ages and sizes were placed in the plot after a 40 x 40 foot galvanized metal pen was constructed around the borders (Figure 1). Baled straw was provided for bedding and a container of water was placed in the center of the plot. No supplemental diet was provided during this period. The carrots were harvested 28 days after the voles were placed in the plot. Feeding damage to individual roots was recorded using a numerical scale to classify damage with a value of one for no damage and five for severe damage. The amount of root exposed above the soil level was recorded to determine the effect of root accessibility since roots of some lines were below the soil while others had as much as two inches of the root exposed.

Figure 1. Galvanized metal pen used to confine meadow voles to the carrot breeding plot for feeding preference tests.



Random samples of roots from each entry for use in feeding studies or seed production were placed in cold storage in polyethylene bags containing wood shavings.

Roots from lines representing the extremes in field feeding damage were removed from storage for laboratory feeding tests. The samples were washed, sliced, and dried in an electric oven at 70° C for 24 hours. The material was ground in an Intermediate Wiley Mill to pass a 20 mesh sieve and incorporated into experimental diets. A bioassay previously developed by Elliott (9) using meadow voles for laboratory determination of the relative gain of pairs of weanling voles fed experimental diets and litter mates fed control diets was used in this study. His six-day growth-response test was standardized and formulated as follows:

$$G_{sp} = \frac{G_x - G_c}{G_c}$$ where: $G_{sp}$ = specific growth response,

$G_x$ = mean response of pair on an experimental diet in per cent of their starting weight, and

$G_c$ = response of litter mate randomly chosen or pair on the control diet in per cent of their starting weight.

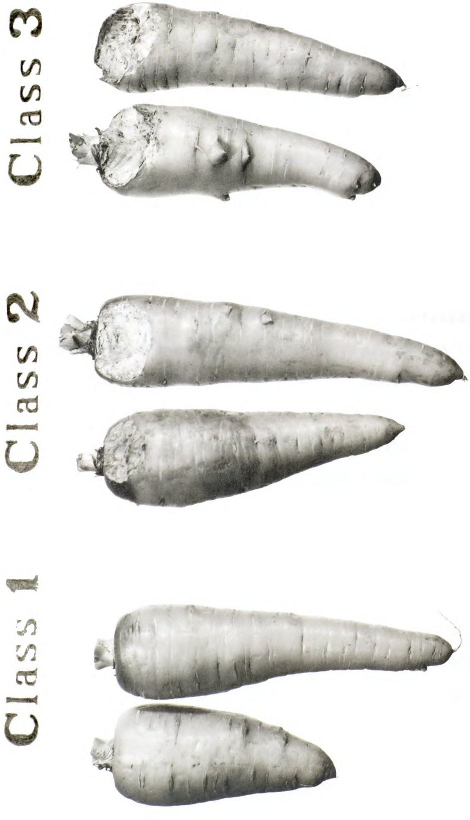
Genetic Study:

From the replicated material grown in the field in 1965, two inbred lines with no feeding damage were crossed with each of three inbreds involved in the parentage of the two $F_1$ hybrids that showed severe feeding damage the previous year. The five inbred lines used in the study were W 33 and MSU 378 representing the non-damaged lines; MSU 670, MSU 1558, and MSU 8549 representing the inbred parents of the severely damaged $F_1$ hybrids, 1558 x 670 and 1558 x 8549. The populations derived from these crosses included the two parental inbreds, the $F_1$, $F_2$, and backcrosses to parental lines.

The initial crosses were made in the greenhouse during the winter of 1965-66. Cloth cages were used to enclose the umbels and houseflies were introduced into the cages to effect pollination. During the summer of 1966, the $F_1$'s and parents were screened in the field using 28 days exposure to a population of 22 voles in the field pen. Damage was rated on a scale of numerical values of one, two, and three for undamaged, moderately damaged, and severely damaged roots as illustrated in Figure 2.

Seed of the $F_2$ progenies and the reciprocal backcrosses was produced in the greenhouse during the winter of 1966-67 and were planted along with the parent lines for field evaluation with confined voles as previously described. Due to a large $F_2$ population and the limited capacity of the vole pen, the number of replications was reduced to four. Each $F_2$ population occupied 15 feet of row while all other populations were planted in plots of approximately 7.5 feet. With the spacing used, this provided an $F_2$ population of approximately 100 plants in each of the four replications. The number of

Figure 2. Rating scale used to evaluate individual carrot roots for feeding damage by meadow voles.


Class 3
Class 2
Class 1

roots of the other progenies approximated 50 plants per replication.

The model formulated by Hayman and Mather (18) and applied by Hayman (16) to data of the kind obtained in this research was used to elucidate the mean rating of the various generations. The model is as follows: $\bar{Y} = m + a_1d + a_2h + a_3i + a_4j + a_5l$, in which $\bar{Y}$ is the mean of a particular generation and the a's are coefficients. The parameters are defined as follows: m = the mean, d = pooled additive effect, h = pooled dominance effect, i = pooled interactions between additive effects, j = pooled interactions between additive and dominance effects, and l = pooled interactions between dominance effects.

The coefficients in the above model are given for the mean of each generation included in this study as follows:

| $\bar{Y}$ | $a_1$ | $a_2$ | $a_3$ | $a_4$ | $a_5$ |
|---|---|---|---|---|---|
| $P_1$ | 1 | -1/2 | 1 | -1 | 1/4 |
| $P_2$ | -1 | -1/2 | 1 | 1 | 1/4 |
| $F_1$ | 0 | 1/2 | 0 | 0 | 1/4 |
| $F_2$ | 0 | 0 | 0 | 0 | 0 |
| $B_1$ | 1/2 | 0 | 1/4 | 0 | 0 |
| $B_2$ | -1/2 | 0 | 1/4 | 0 | 0 |

The model was fitted by least squares and tests of significance of genetic effects were made by the F test (28) from an analysis of variance of generation means.

An estimate of heritability was obtained from the ratio formed by dividing the additive genetic effect by the total genetic effect. Thus

$$\text{Heritability} = \frac{\sigma_a^2}{\sigma_G^2}$$

Laboratory Studies:

Stored material from the 1966 field trial was used in additional feeding studies following the procedure described for 1965. Other laboratory studies included a proximate feed analysis for ash, crude fiber, ether extract, moisture, protein, and nitrogen-free extract determined in root samples according to the methods of the Association of Official Agricultural Chemists (20). The samples used for these determinations were taken at the time of preparation for the feeding study. Duplicate samples were analyzed independently.

The concentration of sugars in each sample was determined by the method of Ting (29) following extraction using the procedure described by Clegg (7).

In order to study the biochemical aspects of the vole preference phenomenon, carrot tissue from both eaten and non-eaten roots which had been frozen at the time of harvest was extracted in cold water, condensed by freeze-drying, and the extracts applied reciprocally to previously preferred and non-preferred roots of a different pedigree. The treated roots and untreated checks of preferred and non-preferred roots were exposed overnight in cages containing three voles in a preference test, replicated six times. The voles were supplied with a complete diet and water in addition to the carrot roots. This test was designed to determine if selection under laboratory conditions paralleled field preference as well as to provide information on the dominant or recessive nature of the preference factor.

Except for the preference test using voles, all laboratory studies were repeated in 1967 using essentially the same procedures. In addition, the quality of each entry was rated by taste panels which consisted of ten individuals who served repeatedly and evaluated the roots for flavor and acceptability under controlled conditions. Each panel member scored two randomly selected discs of each variety for desirability of flavor and for general acceptability. The scale used for scoring all factors ranged from 1 (poor) to 8 (excellent). Certain other characteristics were measured to ascertain desirability factors of the breeding lines. These included color, crispness, and sweetness.

## IV. RESULTS AND DISCUSSION

### Preliminary Study

Results from the preliminary study showed that the mean feeding index ranged from 1.00 to 4.75 on the scale used for evaluation of the 50 carrot lines grown in 1965. The five lines representing the extremes in preference are presented in Table 1. The coefficients of variation expressed for these lines were not typical of the much higher ones found in the remainder of the entries where coefficients of variation greater than 75 per cent were obtained in some instances.

An analysis of variance for the data obtained on the amount of root exposed above the soil level is shown in Table 2. There was a highly significant difference between the various entries for the amount of root exposed but this factor was not correlated with the preference by the voles. Factors other than a readily accessible food source must determine the feeding preference of the animals. In many instances the voles dug below the surface of the soil to feed on certain highly preferred carrot lines.

Results of a preliminary feeding test conducted on some of the carrot root samples are summarized in Table 3.

Table 1. Carrot lines representing extremes in meadow vole preference from 1965 field trial.

| Entry Number | Pedigree | Feeding Index 1 | 2 | 3 | 4 | 5 | Mean | S.E. | C.V. |
|---|---|---|---|---|---|---|---|---|---|
| 6510 | 1108 S | 31 | | | | | 1.00 | 0 | 0 |
| 6515 | 1558 x 670 | | | 1 | 16 | 51 | 4.75 | 0.130 | 7.77% |
| 6522 | 378 | 15 | | | | | 1.00 | 0 | 0 |
| 6538 | 1558 x 8549 | | | 4 | 13 | 48 | 4.70 | 0.186 | 11.17% |
| 6549 | W 33 | 32 | | | | | 1.00 | 0 | 0 |

Table 2. Components of variance for the amount of root exposure for 50 carrot breeding lines.

| Analysis of Variance | | | |
|---|---|---|---|
| Source of Variation | Degrees of Freedom | Mean Square | F Value |
| Total | 399 | | |
| Replications | 7 | | |
| Entries | 49 | 0.580 | 15.26** |
| Error | 343 | 0.038 | |

**Indicates significance at the .01 level.

Table 3. Relative gain of meadow voles fed on standard diet and on diets including carrot lines selected for degree of field preference.

| Entry Number | Pedigree | Feeding Damage | Mean Gain[1/] Control | Mean Gain[1/] Experimental | $G_{sp}$[2/] |
|---|---|---|---|---|---|
| 6522 | 378 | None | 44.0 | 36.0 | -0.18 |
| 6538 | 1558 x 8549 | Severe | 42.0 | 22.0 | -0.48 |
| 6510 | 1108 S | None | 30.3 | 9.5 | -0.76 |
| 6515 | 1558 x 670 | Severe | 40.0 | -10.0 | -1.30 |
| LSD .05 | | | | | 0.16 |
| .01 | | | | | 0.29 |

[1/]Per cent of starting weight.

[2/]$G_{sp} = \frac{G_x - G_c}{G_c}$ where: $G_{sp}$ = specific growth response.

$G_x$ = mean response of pair on an experimental diet, and

$G_c$ = response of pair of litter mates on control diet.

Significantly different specific growth responses were found among the inbred lines and hybrids tested. All experimental carrot diets were inferior to the control diet, their relative value being expressed as negative specific growth responses. The lack of any relationship between vole preference as indicated by feeding damage to carrot roots in the field and growth responses of young voles under laboratory conditions is of special interest. Carrot roots that were preferred in the field seemed to be lower in nutritive value in laboratory feeding tests than some that were not preferred. While the number of observations may be too small for valid conclusions, there is evidence that characteristics other than nutritional value influenced the vole's selection pattern.

## Genetic Study of Vole Preference

The analysis of variance for the data on feeding damage in the segregating generations is shown in Table 4. This analysis allowed separation of the variance components contained within the $F_2$ and backcross generation means as well as those of the genetic parameters. No significant additive effect was detected by this analysis; likewise, the dominance effect was non-significant. Interactions between the two factors was highly significant. Highly significant differences were also found among the backcross means but not among the $F_2$ means.

Table 4. Components of variance for feeding damage to carrot roots by meadow voles in confined field exposure test--1966-67.

| Analysis of Variance | | | |
|---|---|---|---|
| Source of Variation | Degrees of Freedom | Mean Square | F Value |
| Years | 1 | 0.9116 | |
| Reps within years | 10 | 0.2339 | |
| Genetic parameters | 5 | 1.6847 | 8.23** |
| Additive | 1 | 0.1152 | 0.57 |
| Dominance | 1 | 0.7024 | 3.45 |
| Interactions | 3 | 2.2663 | 11.14** |
| Within $F_2$ | 3 | 0.4286 | 2.11 |
| Within backcrosses | 6 | 0.6596 | 3.34** |
| Error | 118 | 0.2035 | |

**Indicates significance at the .01 level.

The various generations included were expected to reveal different inheritance patterns based on segregation ratios. The fact that the backcross generation means showed greater variation than the $F_2$ means suggests the presence of heterozygosity within some of the inbred parents for the factors involved. Variation obtained from the heterozygous parents would be manifested in the segregating populations and would be expressed as interactions in the analysis of variance.

Analyses of individual generations are shown in Table 5. Incomplete data were obtained for the W 33 x 8549 and 378 x 8549 crosses due to loss from a flooded condition of the plot early in the 1967 growing season; therefore, these generations were omitted from the analysis. Results from the latter analysis suggest that the significant variation within the backcross generations found in the genetic analysis may have resulted from inbred MSU 1558 being heterozygous for characters influencing vole preference. In both populations where this inbred was a parent, the backcrosses to it showed greater variations than the other generations analyzed.

The genetic model employed in this study did not take into account possible linkages among loci affecting the factors for vole preference. Linkages confound the interactions effect (17); therefore, the variation in linkage terms will contribute to the residual variance

Table 5. Means, standard errors, and coefficients of variation feeding damage to different generations of carrot roots by meadow voles in a confined field exposure test--1967.

| Entry Number | Pedigree | Generation | Mean Feeding Index | S.E. | C.V. |
|---|---|---|---|---|---|
| | | | | | Percent |
| 6708 | W 33 x 670 | $F_2$ | 1.50 | 0.155 | 20.73 |
| 6709 | (W 33 x 670) x W 33 | BC | 1.06 | 0.041 | 7.74 |
| 6710 | (W 33 x 670) x 670 | BC | 1.06 | 0.058 | 10.85 |
| 6701 | W 33 x 1558 | $F_2$ | 1.82 | 0.065 | 7.09 |
| 6702 | (W 33 x 1558) x W 33 | BC | 1.80 | 0.071 | 7.83 |
| 6703 | (W 33 x 1558) x 1558 | BC | 1.90 | 0.372 | 39.11 |
| 6704 | 670 x 378 | $F_2$ | 1.04 | 0.028 | 5.48 |
| 6705 | (670 x 378) x 670 | BC | 1.08 | 0.028 | 5.28 |
| 6706 | (670 x 378) x 378 | BC | 1.00 | 0 | 0 |
| 6711 | 378 x 1558 | $F_2$ | 1.57 | 0.082 | 10.38 |
| 6712 | (378 x 1558) x 378 | BC | 1.26 | 0.150 | 17.34 |
| 6713 | (378 x 1558) x 1558 | BC | 1.73 | 0.200 | 31.75 |
| 6714 | W 33 | | 1.00 | 0 | 0 |
| 6715 | MSU 378 | | 1.01 | 0.014 | 2.77 |
| 6716 | MSU 670 | | 1.23 | 0.097 | 5.85 |
| 6717 | MSU 1558 | | 1.57 | 0.129 | 16.36 |

due to deviation from regression. The significant interactions found here suggest that linkages may be a factor.

The estimate of heritability for the population studied was 6.9 per cent. Only the $F_2$ and the backcross generations were used in the estimation of heritability. This estimate was probably subjected to bias by genotype x environmental interactions.

## Laboratory Studies

Correlation coefficients were calculated as presented in Table 6 to evaluate the relationship between 1966 data obtained from a proximate feed analysis, the feeding index, and the specific growth response. None of the correlation coefficients are significant at the .05 per cent level; however, some trends may be noted. Two of the responses are of particular interest. The negative response between mean feeding index and crude fiber suggests the possibility that voles tend to select against high crude fiber content in the field. The mean feeding index and the specific growth response appear to be inversely related. This is supporting evidence for the results obtained in the preliminary study where vole preference was not related to nutritive value of the roots as measured by feeding trials.

Results of the 1967 study are presented in Table 7. The correlation coefficients are not significant at the

Table 6. Correlations of growth response and feeding preference by meadow voles with proximate feed analysis of carrot inbreds and hybrids from 1966 data.

| | Ash | Crude Fiber | Ether Extract | Crude Protein | N-free Extract | Total CHO | $G_{sp}$ |
|---|---|---|---|---|---|---|---|
| Mean Feeding Index[1/] | -.014 | -.310 | .080 | -.056 | .000 | .040 | -.285 |
| Specific Growth Response($G_{sp}$)[2/] | .280 | .213 | .000 | .126 | -.003 | -.192 | |

"r" $_{.05}$ at 14 DF = .497

1/ Means of Eight Replications

2/ $G_{sp} = \frac{G_x - G_c}{G_c}$ where: $G_{sp}$ = specific growth response,

$G_x$ = mean weight gain of pair on experimental diet, and

$G_c$ = mean weight gain of litter mates on control diet.

Table 7. Correlations of growth response and feeding preference by meadow voles with proximate feed analysis of carrot inbreds and hybrids from 1967 data.

| | Ash | Crude Fiber | Ether Extract | Crude Protein | N-free Extract | Total CHO | $G_{sp}$ |
|---|---|---|---|---|---|---|---|
| Mean Feeding Index[1/] | .275 | -.170 | -.030 | .211 | -.193 | -.262 | -.158 |
| Specific Growth Response($G_{sp}$) | .200 | -.237 | .266 | .133 | -.014 | -.148 | |

"r" $_{.05}$ at 34 DF = .32

[1/] Means of Four Replications

.05 per cent level. A comparison of the data obtained for the two years reveals some discrepancies but these are not serious considering that data from neither year were significant.

The data obtained from analysis of carrot root samples for sugar content were tabulated and correlated with the feeding index and specific growth. Total reducing sugar ranged from 10.0 to 33.9 milligrams per gram of dry weight. Sucrose ranged from 1.1 to 35.0 and total sugars from 29.4 to 45.8 mg/g dry weight. In agreement with the work of Rygg (26), fructose was generally found to constitute a little less than one-half of the total reducing sugars. The correlation coefficients are given in Table 8 and Table 9, respectively, for the two years. The 1966 results show the correlation coefficient between mean feeding index and sucrose to be positive and significant at the .05 per cent level while those for total reducing sugars and total sugars were not significant. Nonsignificant correlations were also found between specific growth and each of the sugar concentrations; however, the correlation coefficients were very near significance at the .05 per cent level for both total reducing sugars and sucrose.

Data from 1967 reveals the mean feeding index to have a highly significant positive correlation with sucrose and a highly significant negative correlation with

Table 8. Correlation of growth response and feeding preference with sugar concentrations of carrot inbreds and hybrids from 1966 data.

| | Total Reducing Sugars | Sucrose | Total Sugars |
|---|---|---|---|
| Mean Feeding Index[1/] | -.349 | .499* | .322 |
| Specific Growth Response ($G_{sp}$) | .491 | .454 | .160 |

* Indicates significance at the .05 level.

[1/] Means of Eight Replications.

Table 9. Correlation of growth response, feeding preference, and taste panel scores with sugar concentrations of carrot inbreds and hybrids from 1967 data.

| | Total Reducing Sugars | Sucrose | Total Sugars |
|---|---|---|---|
| Mean Feeding Index[1/] | -.577** | .632** | .032 |
| Specific Growth Response ($G_{sp}$) | .222 | .110 | .169 |
| Mean Taste Panel Score[2/] | -.016 | .011 | -.018 |

** Indicates significance at the .01 level.

[1/] Means of Four Replications.

[2/] Mean score of ten-member panel.

total reducing sugars. No significant correlation was found between mean feeding index and total sugars. Data from the two years are generally similar, the only difference being the non-significant negative correlation noted between feeding index and total reducing sugars in 1966 and the highly significant negative correlation found in 1967. The 1967 data are probably a better estimate of the true relationship since correlation coefficients were determined on a larger number of observations.

In the laboratory preference test all carrot samples exposed to the voles were indiscriminately eaten. While no information was gained on the biochemical aspects of selection, results from the study demonstrated that selection under laboratory conditions does not parallel field selection. Study of the biochemical aspects was not pursued since in the absence of a discriminatory bioassay, it would be impossible to identify the active fraction. This is not to imply; however, that the biochemical make-up of the roots is an unimportant factor in vole preference for certain carrot lines and hybrids.

No correlation was found between average taste panel scores of carrot samples and mean feeding index by the voles. The correlation between taste panel scores and the concentration of sugars in the sample was also non-significant. On the other hand, highly significant differences for average taste panel scores were found between the

breeding lines sampled and among the panel members making the evaluation. The variation encountered between taste panel members demonstrates that flavor and palatability are extremely difficult to elucidate. It is possible that the proportions of the various components of flavor are more important than the absolute amount of any one component.

There are many sources of variation in the vole selection technique for evaluating breeding material in carrots. An attempt was made to assess the various factors which might account for the variability in the results observed. Of primary interest to the plant breeder is the genetic variation. Variation was found among breeding lines and within a given line. It should also be recognized that two biological systems are involved in this type of evaluation. There is no information to indicate the degree of variability among the experimental animals in their preference for specific flavor compounds or sugars. On the contrary, some of the variation observed may be attributed to genetic differences among experimental animals. This may account for some of the experimental error in this study. Environmental variation cannot be ignored. As mentioned in connection with the genetic study, the field plot was flooded for several days early in the 1967 growing season. This occurred just when the primary roots were beginning to elongate. The general effect was death of the distal portion of the root with subsequent branching

or distortion. There is no way of assessing the extent to which this might have affected the carrot plants. Since genetic material was involved, it is possible that the effect was not of the same magnitude in each instance. In fact, the more vigorous plants were observed to be affected to a greater extent probably due to deeper penetration at the time of flooding.

# V. SUMMARY

Observation of a distinct feeding preference by the meadow vole for certain carrot breeding lines in the field suggested the possibility of using them in a bioassay to evaluate breeding material for potential quality and nutritive value. Fifty carrot breeding lines representing the full range of feeding damage were screened for preference in a confined feeding experiment and several lines representing the extremes were selected for more critical study.

Appropriate crosses were made between contrasting lines, and segregating generations were evaluated in an attempt to determine the inheritance of the factors responsible for selection. The data indicate quantitative inheritance for the factors concerned with preference by the vole. Linkages appear to be important in the inheritance pattern.

Data from laboratory feeding tests indicated no relationship between vole preference and nutritive value as measured by growth response of young voles. Selection by the vole was significantly correlated with sucrose content and negatively correlated with reducing sugars. There were no significant correlations between feeding

damage and crude fiber, protein, or total carbohydrates. Growth response was not correlated with any of the above factors. No correlation was found between average taste panel scores for overall rating of carrot samples and data on mean feeding index by the voles. The correlation between taste panel scores and the concentration of sugars in the sample was also non-significant.

Controlled exposure of carrot breeding material to populations of the meadow vole in the field did not prove to be a reliable bioassay for either nutritive value or culinary quality.

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