

one half of the total reducing sugars. The correlation coefficients are given in Table 2 for the 2 years. The 1966 results showed the correlation coefficient between mean feeding index and sucrose to be positive and significant at the .05% level while those for total reducing sugars and total sugars were not significant. Non-significant correlations were also found between specific growth and each of the concentrations; however, the correlation coefficients were very near significance at the .05% level for both total reducing sugars and sucrose. Data from 1967 revealed the mean feeding index to have a highly significant positive correlation with sucrose and a highly significant negative correlation with total reducing sugars. No significant correlation was found between mean feeding index and total sugars. Data from the 2 years were 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 were probably a better estimate of the true relationship since correlation coefficients were determined on a larger number of observations.

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 nonsignificant. 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 demonstrated that flavor and palatability were extremely difficult to elucidate. It is possible that the proportions of the various components of flavor were more important than the absolute amount of any one component.

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## Measurement and Classification of Genetic Variability in Horseradish<sup>1</sup>

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*Abstract.* The genetic variability of 20 cultivars of horseradish, *Armoracia rusticana* Gaertn., Mey., & Scherb., was measured and classified. These cultivars were chosen from a gene pool containing over 400 cultivars, and collectively they appeared to represent the extreme and intermediate forms of variability found in horseradish. Two methods of classification were compared. One classification was based on 2 highly diagnostic characters that showed the extreme and intermediate limits of the germplasm in the form of a scatter diagram. The other classification was based on 40 characters. For

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this classification methods of numerical taxonomy were used to show the germplasm complex by scatter diagrams and phenograms. The two classifications appeared to be equal in defining the extreme limits of the genetic variability. The main area of disagreement was found among the relative positions of the intermediate forms or genotypes. The use of scatter diagrams as an aid to the selection and conservation of genetic variability for future use is discussed.

#### INTRODUCTION

THE conservation of genetic variability is a continuous problem. To save all genotypes within a species is economically impossible. But in some crops, much of the potential variability can be retained by saving certain genotypes that are representative of the genetic complex. Loss of these genotypes may mean an irreplaceable loss of valuable germplasm. At a future time, other desirable combinations would be produced through hybridization of the selected genotypes.

Germplasm representative of the genetic complex can be identified only after the genetic variability is measured and the taxa in the complex are classified. Such a classification should be based upon characters that span a broad genetic background, otherwise, the classification will be too artificial to be of value for the identification of representative types.

Classification of germplasm may be grouped into two general types: (a) those based on a few carefully selected characters and (b) those based on many, randomly selected characters.

One method of classification based on a few characters, in some cases only 2, has been developed by Anderson (1) and is generally referred to as the method of scatter diagrams. This method is especially useful in detecting variation within a species caused by interspecific introgression or to define limits of variation within a genetic complex of a given species or species-hybrids. Anderson's method has received particular attention in the classification of corn races. Two early examples of such classification are those of Anderson and Cutler (2) and Wellhausen et al. (12).

The method of classification based on many characters, usually 40 or more, has been popularly called numerical taxonomy or phenetic taxonomy (11). The method implies that differences in genotypes can be measured from their overall similarity. If 2 genotypes are similar in overall appearance, then they are considered to be closely related. Conversely, if 2 genotypes are greatly different in overall appearance, then they are not considered to be closely related. Numerical taxonomy has been used mainly to study species and higher taxa; however, it has been shown to be useful in classifying certain genotypes within species as well (5, 7).

Although both methods, using few or many characters, are valuable in classifying taxa, only one direct comparison of the 2 methods is known to the authors. In this study, Edwards (5) found that the 2 methods produced similar groups of corn races.

The purpose of this paper is to compare classifications derived from the 2 methods using horseradish, *Armoracia rusticana* Gaertn, Mey, & Scherb., cultivars as a model and to determine if such classifications are useful in selecting diverse genotypes that are representative of the genetic variability within the group.

Horseradish was chosen because of the restricted nature placed on the germplasm within this species. The plant was considered to be highly sterile, and for centuries it has been propagated asexually by root cuttings rather than by seed. No doubt, an occasional new recombinant has appeared through sexual reproduction and has been preserved by man, but until the 20th century, there were no attempts, at least on record, to breed new cultivars (3). Because there has been relatively little breeding of new recombinants, it should be possible to define the limits of variation within horseradish from fewer genotypes than from a crop with a long history of breeding. This assumption is supported by comparing 2 sets of cultivars. One set consisted of 30 cultivars, of which 13 were seedlings of recent origin and the remainder were collected from areas in the United States. This set was grouped into 3, broad, overlapping types (8). The second set represented a recent collection of 76 lots from various parts of the world and about 300 new seedlings from various diverse cultivars. In comparing the 2 sets of cultivars, we did not find any cultivars in the second set that appeared to be more extreme in form than those of the first set. We assume

that these 2 sets of cultivars are fairly representative of the genetic variability found in horseradish being grown today.

#### MATERIALS AND METHODS

*Cultivars and field design.* The 20 cultivars (Table 1) selected for this

Table 1. Cultivars used in present study.

Illinois strain no.	Source and name
104a.....	Russia
125a.....	Wisconsin A.E.S. seedling X-1
135a.....	Wisconsin A.E.S. seedling Y-7
157a.....	Wisconsin A.E.S. seedling Y-8
167a.....	United States
196a.....	Canada, called Sassy
701a.....	England, called Big Top Western
722a.....	England, PI 304184
728a.....	Illinois, called Common
757a.....	Yugoslavia, PI 305393
760a.....	Poland, PI 305625
765a.....	Poland, PI 305630
774a.....	Hungary, PI 306123
777a.....	Hungary, PI 306207
781a.....	Denmark, PI 306237 (36 Danish type)
785a.....	Russia, PI 306335
786a.....	Sweden, PI 306701
844a.....	Vermont
845a.....	Iowa, called Swiss
849a.....	Czechoslovakia, PI 317042

study had been grown at the University of Illinois Horticultural Farm for at least 2 years. Some were vigorous and others showed severe symptoms of Turnip Mosaic 1, *Marmor brassicae*. But collectively they appeared to represent the extreme and intermediate forms of variability found in horseradish.

Roots, which had been subjected to cold treatment to induce flowering, were planted May 1, 1967. Each cultivar was represented by 10 plants consisting of 2 replications, with 5 plants per replication in a single row. Spacing was 78 inches between rows and 19 inches within the row. No fungicide or insecticide treatments were used throughout the growing season.

*Characters.* Data for flowering characters were taken during June. As a rule, some cultivars do not produce strong flower stalks and occasionally some do not flower at all; thus, 3 cultivars had some character states missing. To eliminate cultivars that did not flower or produce strong flower stalks might tend to eliminate genotypes important to the classification.

Most of the vegetative characters were recorded during the last week of August and the first week of September. Since the state of certain characters vary during the season, the date that these character states were recorded is given in Table 2.

Characters showing continuous variation were measured as follows, using radical leaf length as an example: 1 fully-developed, healthy leaf was se-

lected from each plant giving a total of 10 leaves per cultivar. The average length of 5 typical leaves of each sample was then calculated for later analysis. A typical leaf sample can be easily obtained because the older leaves or lower rosette of leaves are yellowing or dead, and the younger leaves or the upper rosette of the leaves are small.

An exception to this method of sampling was used for measuring the degree of leaf-crinkling of the radical leaves. Plaster of paris casts were made from old, intermediate, and young leaf-sections and a combined average rugose index of the 3 leaf ages were established as: 1 for smooth, 2 for slightly crinkled, 3 for crinkled.

For discrete characters, a state was determined after examining the 10 plants of each cultivar. These states are subjective ratings and a 3-state

Table 2. Characters and character states used in present study.

From flower stalks:	
1.	Height of flower stalk
2.	Ratio of unbranched portion of flower stalk to total height
3.	Texture of flower stalk surface (1) smooth (2) striate (3) sulcate
4.	Angle between flowering braches and main axis
5.	Number of flowering branches per panicle.
6.	Length of longest flowering branch
7.	Ratio of sterile zone to total length of longest flowering branch
8.	Length of terminal branch of panicle
9.	Length of pedicel at midportion of upper lateral branches
10.	Width of petal
11.	Ratio of petal width to length
12.	Width of longest cauline leaf at midportion of flower stalk
13.	Ratio of width to length of longest cauline leaf at midportion of flower stalk
14.	Habit of cauline leaf (1) flat (2) curled
15.	Location of upper most cauline leaf (1) main axis (2) flowering branch
16.	Degree that lower cauline leaves are pinnately divided (1) none (2) few (3) most
From radical leaves:	
17.	Length of petiole (8/29/67)
18.	Length of leaf blade (8/29/67)
19.	Width of leaf blade (8/29/67)
20.	Ratio of leaf length to width (8/29/67)
21.	Ratio of upper to lower quarter widths of leaf blade (10/4/67)
22.	Basal angle of leaf blade as measured between midrib and basal margin of leaf blade (8/29/67)
23.	Percent change of basal angle of leaf blade between early season and late season (7/21/67 and 10/4/67).
24.	Rugose index of leaf blade: combined average for young, intermediate, and old leaves (1) smooth (2) slightly crinkled (3) crinkled
25.	Per cent hollow petioles (8/29/67)
26.	Reddish color on base of petiole (1) no (2) yes
27.	Black or gray spots on petiole (1) none (2) grey (3) black
28.	Shape of leaf apex (1) medium acute (2) broadly acute (3) rounded
29.	Shape of terminal portion of leaf apex (1) obtuse (2) acute (3) acuminate
30.	Type of leaf blade margin (1) crenulate (2) crenate (3) dentate
31.	Degree that midrib is arcuated (1) none to slight (2) moderate (3) much
32.	Degree that leaf blade is involuted (1) none to slight (2) moderate (3) much
33.	Degree that leaf is spiraled (1) none to slight (2) moderate (3) much
34.	Degree that leaves are pinnately divided (1) none (2) few (3) many
35.	Color of leaves (1) light (2) medium (3) dark green
36.	Vigor of leaves (1) weak (2) moderate (3) strong
37.	Growth habit of leaves (1) straggly (2) moderately upright (3) strongly upright
38.	Bacterial leaf spot symptoms (1) none to slight (2) moderate (3) severe
39.	Turnip mosaic leaf symptoms (1) none to slight (2) moderate (3) severe
40.	Cercospora-Ramularia leaf spot symptoms (1) none to slight (2) moderate

character, for example, was scored as 1, 2 or 3.

**Two character classification.** The data were first analysed by Anderson's method of scatter diagrams, which is essentially based on two characters. The two characters chosen were degree of leaf-crenating of the radical leaves, previously mentioned, and the basal angle of the radical leaves. This basal angle, taken from fully-expanded healthy leaves, was measured from the midrib of the leaf blade to the basal margin of the leaf blade. These 2 characters were selected because both appeared to be highly diagnostic for defining the extreme limits of the germplasm of horseradish.

These 2 characters were used to construct a scatter diagram. Each circle on the scatter diagram represents a cultivar and the position of a circle is determined from the data of the 2 characters, one used for the abscissa and the other for the ordinate. Rays or metroglyphs were added to the circles to show 5 other characters that are associated or correlated with the first 2 characters. The position and length of a ray indicates the character state for a particular character. After the rays have been added to the circles, the diagram is called a pictorialized scatter diagram (1).

**Forty character classification.** Data for 40 characters were analysed by methods of numerical taxonomy. Details of the calculations for these methods can be found elsewhere (11). A brief description is given below where we have departed from the general technique.

Each character for all cultivars was standardized to give a mean of zero and a variance of 1 (9). Standardization tends to remove unequal weighting of characters caused by the use of different units of measure for different characters.

Two coefficients of similarity, Q-correlation coefficient and Sokal's distance coefficient, were employed to measure the overall similarity between each pair of cultivars. From the Q-correlation and distance matrices, the 2 cultivars showing the lowest magnitude of overall similarity were selected as representing the extreme limits of the germplasm under study. These 2 cultivars have the lowest values in the Q-correlation matrix and the highest values in the distance matrix.

The similarity values of the 2 cultivars when compared with the other 18 cultivars were used to construct scatter diagrams. Each circle on a scatter diagram represents one of the 18 cultivars

and a cultivar's position on the diagram is determined from its similarity values with the 2 extreme or reference cultivars.

Rays depicting the same 5 characters used in the 2 character scatter diagram were added to the circles. These characters are part of the 40 characters analysis and they were added as an aid in comparing the scatter diagrams.

From the data of the similarity matrices, phenograms were constructed to show clusters of cultivars. The phenograms are based on the unweighted pair-group method (UPGA) of clustering, using simple averages proposed by Sokal and Michenor (10). With distance coefficients, the magnitude of the coefficients represents the degree of dissimilarity rather than similarity; therefore, the sign of each coefficient was reversed before cluster analysis was performed. This was unnecessary in the case of Q-correlation coefficients, because the coefficients represent the degree of similarity. The rays portraying the same 5 characters mentioned above were added to the phenograms.

Computations for similarity coefficients and cluster analysis were performed on an IBM 360 75 computer using FORTRAN IV programs developed at the Agronomy Statistical Laboratory of the University of Illinois.

## RESULTS AND DISCUSSION

**Two character classification.** The pictorialized scatter diagram (Fig. 2) shows the relationship of the 20 cultivars as defined by 2 characters, degree of leaf-crenating (rugose index) and the leaf base angle. The rays on the

<b>CHARACTER 21</b>	<b>CHARACTER 32</b>
↳ 1.13 TO 1.32	↳ NONE TO SLIGHT
↳ .94 TO 1.12	↳ MODERATE
○ .79 TO .93	○ MUCH
<b>CHARACTER 13</b>	<b>CHARACTER 31</b>
↳ .17 TO .23	↳ NONE TO SLIGHT
↳ .24 TO .26	↳ MODERATE
○ .27 TO .46	○ MUCH
<b>CHARACTER 20</b>	
↳ 4.6 TO 6.1	
↳ 3.8 TO 4.5	
○ 2.9 TO 3.7	

Fig. 1. Key to ray or metroglyph coding of 5 characters used in Fig. 2 to 6.

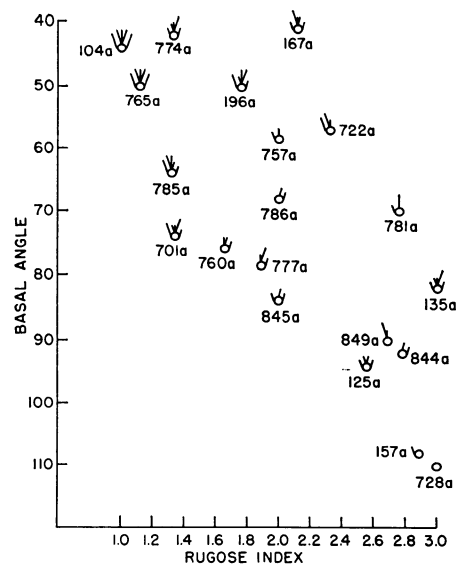


Fig. 2. Pictorial scatter diagram showing relationship of 20 cultivars based on degree of leaf crinkling (character 24) and basal angle of leaf blade (character 22). Each circle represents a cultivar. The rays on the circle show how the 5 characters from Fig. 1 are associated with crinkling and basal angle.

circles, as coded in Fig. 1, indicate how 5 other characters are associated as a complex with the first 2 characters in Fig. 2. From an inspection of the elliptical-shaped diagram, it is easy to see the variation pattern of the germplasm. The cultivars located in the lower right-hand corner are generally referred to by commercial growers as the common type and the cultivars in the upper left-hand corner as the bohemian type. The cultivars in the center of the ellipse are intermediate in form.

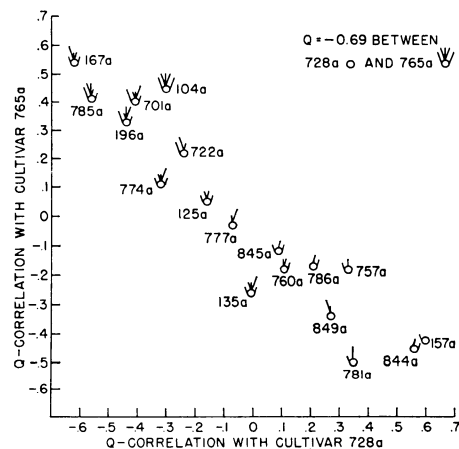


Fig. 3. Q-correlation scatter diagram based on 40 characters showing relationship of 18 cultivars with 2 reference cultivars, 728a and 765a. Each circle represents a cultivar. The rays on the circle show the relationship of 5 of the 40 characters. For key to ray coding see Fig. 1.

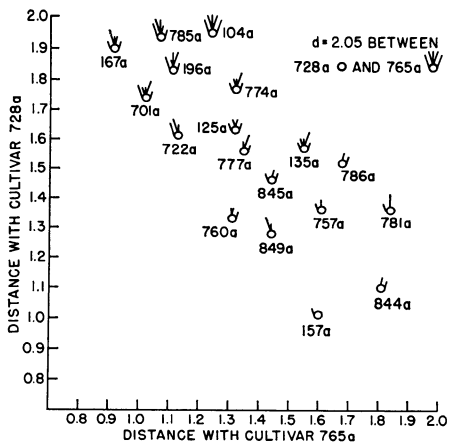


Fig. 4. Distance scatter diagram. For explanation see Fig. 3.

**Forty character classification.** An inspection of the Q-correlation and distance matrices (not shown) revealed that cultivars 728a and 765a showed the lowest magnitude of overall similarity in both matrices.

The 2 elliptical-shaped diagrams, one based on Q-correlation values (Fig. 3) and the other based on distance values (Fig. 4), show the relationships of the other 18 cultivars with the 2 extreme cultivars, 728a and 765a. The rays on the circles show how 5 of

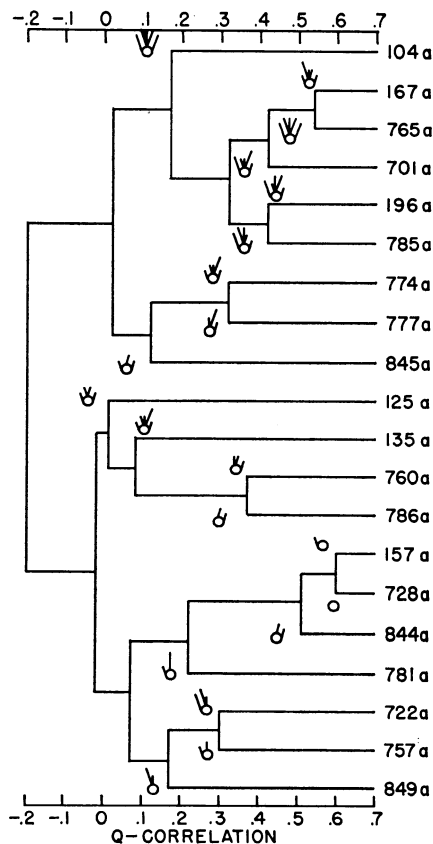


Fig. 5. Q-correlation phenogram obtained by unweighted pair-group method (UPGA) of clustering. Circles with rays show character states of the 5 characters from Fig. 1.

the 40 characters are associated as a complex. These 5 characters are the same as those shown in Fig. 1 and 2.

A comparison of Fig. 3 and 4 indicates that the various cultivars fall in about the same diagonal order. It can be concluded that both Q-correlation and distance coefficients in this instance are of approximate equal value in delineating the relationship of the 18 cultivars with the 2 extreme reference cultivars.

**Phenograms.** Although phenograms are more efficient in showing clusters of taxa that are defined mainly by discrete or non-overlapping character states, such as are generally found in species and higher groups, they are of some value in cases of overlapping character states as are commonly found in horseradish. Fig. 5 and 6 show phenograms based on Q-correlation and distance coefficients, respectively.

In general, the phenogram based on the Q-correlation coefficients is slightly more representative of the scatter diagrams than the phenogram based on distance coefficients. The rays depicting the same 5 characters as found in the scatter diagrams are shown on the phenograms where a cultivar first joins a cluster and these indicate that the Q-correlation phenogram is more

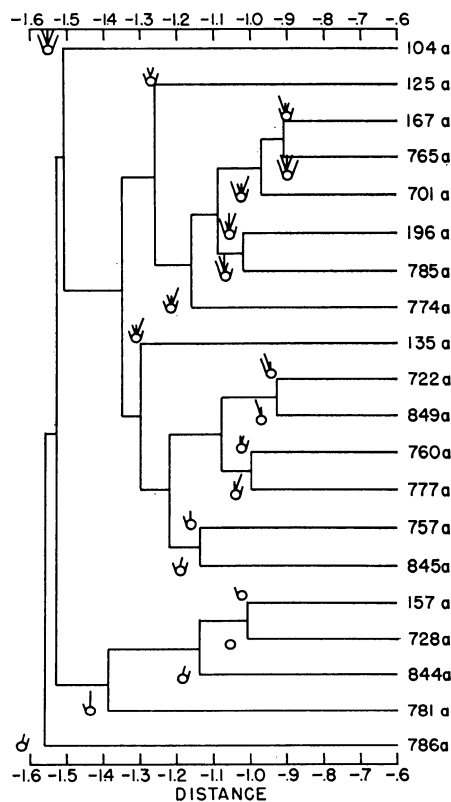


Fig. 6. Distance phenogram. For explanation see Fig. 5.

indicative of the results obtained by Andersonian analyses.

**Comparison of the methods.** It is generally recognized that classifications based on many characters usually produce a more natural classification than classifications based on only a few characters (4). Within a restricted taxa as horseradish, however, where the germplasm complex shows an association of certain characters due to linkage (1) or other causes of correlation (6), a 2 to few character classification appears to be equal to a many character classification in defining the extreme limits of the germplasm. The main area of disagreement is found among the intermediate forms, such as cultivar 125a.

The chief advantage of the 2 to few character classification is that less time is consumed in obtaining the data than in a many character classification. These characters, however, must be carefully chosen to produce the desired results, while in the many character classification the characters are generally chosen rather randomly from all parts of the plant.

In classifications devised for selecting genetic variability, it would probably be best to first study a many character classification based on a reasonable sample of diverse genotypes, and then, try to obtain similar results using the 2 to few character method on a large number of genotypes.

**Gene bank selections.** In regard to retaining germplasm for future use, cultivars of diverse type should be saved. If only 2 cultivars can be saved, then 2 extremely different types, which are interfertile, should be retained. Intermediate types can theoretically be produced by crossing the two extreme types. This assumption can be partially supported from data of an unpublished study in which the ratio of the leaf blade length to width of 52 seedlings was compared with their parents, cultivars 196a and 728a. Cultivar 196a, a bohemian type, had a ratio of 3.2 and cultivar 728a, a common type, had a ratio of 1.9. Forty-three of the 52 seedlings had ratio values that were intermediate to the parents and the values ranged from 2.2 to 3.1. The other 9 seedlings had ratio values that were slightly greater than cultivar 196a.

Where the economic values of the various genotypes are fairly well known and where several cultivars can be saved, the scatter diagrams can serve as a useful guide in choosing a representative sample of diverse germ-

# The Effect of Flower Age, Time of Day and Variety on Pollen Germination of Onion, *Allium cepa* L.<sup>1</sup>

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**Abstract.** Onion pollen of 2 different parents, B12115C and B2215C, was collected at 9:00 AM and 2:30 PM from flowers at approximately 1, 3, and 6 days after anthesis. This was germinated in hanging drops of a sucrose-gelatin medium. The percentage of pollen germination from B12115C was significantly higher than the percentage from B2215C. There was no significant difference in germination between pollen collected and germinated in the morning versus pollen collected and germinated in the afternoon. The results also show a linear relationship between pollen age and pollen germination. The ability to ger-

minate declined rapidly after the first day and approached zero by the sixth day after anthesis. Hand pollination and style studies indicate that onion pollen tubes are capable of growing the entire length of the style, a distance of approximately 4 mm, in 24 hr.

## INTRODUCTION

IN the commercial production of hybrid onion seed, the pollen parent, C-line, is interplanted with the male-sterile seed parent, A-line. Because the seed parent produces no pollen, emasculation is not necessary and the production of hybrid onion seed is economically feasible. However, in the past few years low seed yields in the onion seed industry, particularly among hybrids, has become a major problem. Factors which may affect the seed set of onions include the comparative blooming dates of both parents, the ratio of pollen rows to seed rows, bee activity, weather con-

ditions during bloom, and the duration of receptivity of the stigma (9). In addition, the pollen longevity is an important factor because pollen viability is a prerequisite to fertilization and seed set.

Several factors may affect the viability of the fresh pollen. The age of pollen affects the percentage of germination and the seed-set ability of certain pollens. Maximum germination of various fruit and vegetable pollens occurs on the day of flower anthesis or anther dehiscence (5, 6). The germination percentages of pollen taken from *Solanum* and *Capsicum* flowers 1 or 2 days before anthesis and pollen from closed or abscised flowers was very low (5, 10). Dempsey (2) observed that maximum fruit set in pepper was obtained with pollen collected on the day of anthesis. Pollinations with pollen collected 1 day before or after anthesis resulted in reduced fruit set and seed production, and pollinations with pollen collected 2 days prior to anthesis or 3 days after anthesis were not successful.

The time of day of anther dehiscence and pollen collection also may affect seed-set ability of pollen. Adlerz (1) reported that the percentage of fruit set in watermelon was higher following pollination between 8 to 9 AM than between 9 to 10 AM, 7 to 8 AM, or 6 to 7 AM. Morning pollination with stored sorghum pollen produced more seed than afternoon pollination (11). Eršov and Vorob'eva (4) found that pollinations in onion, both within and between varieties, at 10 AM or 6 PM resulted in a higher seed set than pollinations at 2 PM.

The work reported here was designed to outline the influences of various factors on the germination of onion pollen.

## MATERIALS AND METHODS

Preliminary studies using various levels of sucrose, boron, and calcium were conducted to find the individual medium requirements of onion pollen. The resultant selected medium was a modification of one proposed by Kwack (7) consisting of 10% sucrose, 100 ppm H<sub>3</sub>BO<sub>3</sub>, 300 ppm Ca(NO<sub>3</sub>)<sub>2</sub>·14H<sub>2</sub>O, 200 ppm MgSO<sub>4</sub>·7H<sub>2</sub>O, and 100 ppm KNO<sub>3</sub>. This medium was modified by adding 1% gelatin to increase the viscosity.

The pollen and the medium were thoroughly mixed by stirring with a glass rod, and a drop of this mixture was placed in a petri dish which had a grating etched into the bottom (Fig. 1). Thirty-six separate hanging drops were placed in each petri dish which

plasm. For example, cultivars 157a, 196a, 701a, 728a, and 845a are grown commercially in the United States. Cultivars 196a and 701a represent one extreme type, and cultivars 157a and 728a represent the other extreme type. Cultivar 845a is intermediate in form. If 3 cultivars are to be saved, then one would want to select 196a or 701a, 157a or 728a, and 845a, thus retaining much of the genetic variability of this group. In actual practice, a larger number of cultivars would no doubt be retained for a gene pool and the scatter diagrams could be used to select a diverse group of genotypes.

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