The Dispersion of Pollen in Sugar Beet Seed Plots

DEWEY STEWART[1] AND SAM C. CAMPBELL[2]

Introduction

Cytoplasmic male sterility (6)[3] as a tool in sugar beet breeding facilitates the production of hybrid seed. If a completely male sterile equivalent of an inbred is used in the production of F_1 seed, a companion line with which it combines well should be used as the pollinator. Pollen dispersal within seed plots, always important in sugar beet breeding to maintain heterozygosity, becomes a necessity if seed set is to be obtained on the pollen-sterile plants. If only F_1 seed will be acceptable, then the land area planted to the pollinator will tend to reduce the acre yield of marketable seed and in other ways increase the cost of production (2). If this method of seed production should come into use it is therefore highly important to know the proper ratio and distribution of the pollinator with respect to a male-sterile parent which will assure pollination of all the pollen-sterile flowers without unduly adding to seed cost.

Anticipating the availability of completely male sterile equivalents of leaf spot-resistant inbreds for production of hybrid sugar beet seed, plantings were made in the fall of 1950 at the Plant Industry Station, Beltsville, Maryland, and near Medford, Oregon,[4] for the purpose of making a study of pollen dispersal from a pollinator.

Materials and Methods

A partially male sterile equivalent of U. S. 216, a second backcross designated as SP 47100-01, was used as the mother parent. When grown at the Plant Industry Station approximately 60 percent of the plants of this inbred produced flowers with shrunken white anthers devoid of pollen. Of the remaining plants, 15 to 25 percent produced flowers with plump yellow anthers which approached normal pollen production. However, plants *in* this latter category were not readily separated from intermediate types which comprised the remainder of the population.

Five hundred plants in each of the three plots in Oregon were classified as to anther type. The white anther class comprised 42.6 percent, 45.0 percent and 52.2 percent in Plots 1, 2 and 3 respectively. The yellow plump anther class for these plots given in the same order was 35.0 percent, 32.0 percent and 32.4 percent. These percentages may indicate variations between locations as to anther types or may merely be indicative of the difficulty experienced in establishing sharply defined classes. SP 47100-01 is the recessive genotype *rr*, for the factors determining color in the hypocotyl, buds and other parts of the plant.

A strain of U. S. 215, SP 461001-0, was used as the pollinator in this study. It is vigorous, produces pollen in abundance and its date of flowering is fairly close to that of SP 47100-01. SP 461001-0 is homozygous dominant with respect to the *R* gene.

[1] Senior Agronomist, Division of Sugar Plant Investigations, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Adrninistraton, U. S. Department of Agriculture.

[2] Manager, West Coast Beet Seed Co., Salem, Oregon.

[3] Numbers in parentheses refer to literature cited.

[4] The plots in Oregon were provided through the cooperation of the Farmers and Manufacturers Beet Sugar Association, Saginaw, Michigan.

The plots were approximately $^1/_8$ acre each with rows 40 to 50 feet in length. All rows were four feet apart except for Plot 3 in which the rows were two feet apart. The seeding rate was comparable to that used in commercial seed fields and the seedling stands were not thinned. In Plots 1 and 2 in Oregon and in Plot SF-4 at Plant Industry Station, the strip of the pollinator, SP 461001-0, was in the center of the field and on each side there were 12 rows of the partially male sterile strain, SP 47100-01. Oregon Plot 1 had a four-row strip of the pollinator; Plot 2 and SF-4 each had only a single row. Plot SG-12A at the Plant Industry Station had a four-row strip of the pollinator but it was not centrally located. In Oregon Plot 3, there were three four-row strips of the pollinator, one in the center and the other two as the outside four rows of the field. Between the central strip of the pollinator and the two outside ones there were 16 rows of SP 47100-01.

During the height of the blooming period approximately 20 white anther plants and 20 pollen-fertile plants of each row of SP 47100-01 were tagged for a separate harvest by anther types. The yield of seed for these 20-plant samples ranged from one to two pounds. After threshing and cleaning, each lot of seed was run repeatedly through a Boerner sampler to obtain a one-ounce sample for analysis. Each small sample was taken as the representative of its row and was used to obtain the results to be presented. Two hundred seedballs from each sample were planted in sterilized sand in a manner permitting the percent of germination of seedballs, the number of sprouts as well as the ratio of hypocotyl colors, pink to green in the seedling populations, to be determined. From each planting of 200 seedballs a population of approximately 380 seedlings was obtained.

Results

The F_T from the mating, 47100-01 *rr* x 461001-0 *RR* can be identified in the seedling populations by pink hypocotyl color. It should be noted that pollen fertile plants of SP 47100-01 produced *r* pollen and the extent to which this pollen was effective in bringing about selfing as well as fertilizations in siblings will be indicated by the recessive phenotype, or green hypocotyl color. Since the F_1 in the reciprocal cross could not be identified the seed from the pollinator was of no interest.

White Anther and Pollen-Fertile Phases

In a stand of sugar beets producing a variety of anther types with respect to pollen sterility as indicated for SP 47100-01, the highest percentage of F_1 seedlings was expected in the progeny of the white anther plants after exposure to unrelated pollen. That is, any tendency for self-fertilization in flowers of the pollen-fertile plants would tend to reduce the percentage of hybridizations while in the white anther siblings only out-pollination could occur.

In Figure 1 the percentage of F_1 seedlings in the progeny of pollen-fertile and of white anther phases have been indicated for each row of SP 47100-01 in Plot SF-4. The respective anther types of the rows gave strikingly similar values for the percentage of hybridization at each four-foot interval from the pollinator. The mean percentages of F_1 seedlings for the west side of the pollinator were 18.9 percent and 17.1 percent for the

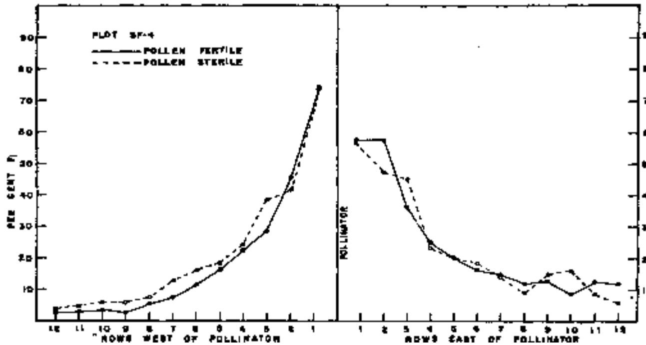


Figure 1.—The percentage of F_1 seedlings in the pollen-fertile and pollen-sterile phases of SP 47100-01 are shown for each row of Plot SF-4. The rows were four feet apart. There was a single row of the pollinator, SP 461001-0.

white anther and pollen-fertile phases respectively. On the east side the values given in the same order for anther types were 21.4 percent and 22.2 percent.

The tendency for the progenies of the white anther and pollen-fertile phases to give similar ratios of hybrids to non-hybrids was found also in the three Oregon plots. The mean F_1 value for all rows of Plot 1 was 24.5 percent for the white anther plants and 27.9 percent for the pollen-fertile phase. The other two plots gave a slightly higher percentage for the white anther plants than for the pollen-fertile ones. Results from 103 rows of S.P. 47100-01 in SF-4 and Oregon Plots 1, 2 and 3 which had been sampled in a similar manner were critically compared with respect to numbers of F_1 seedlings in progenies of the white anther and pollen-fertile phases. The percentages of hybrids in a balanced comparison of anther types were 38.34 percent and 37.80 percent respectively. The difference of 0.54 percent was not statistically significant as determined by Mest applied to differences in paired row samples. Since the pollen-fertile plants of S.P. 47100-01 did not differ significantly from the white anther phase with respect to the percentage F_a when exposed to a mixture of pollen from related and unrelated plants, the results of the two samples from each row have been combined for the remainder of the presentation.

Regions and Pollen Dispersion

Plot SF-4 at the Plant Industry Station was similar to Plot 2 in Oregon both in size and number of rows. Each had a single row strip of the pollinator located in the center of the plot. The percentages of F_x seedlings for rows west and east of the pollinator in these plots have been indicated in Figure 2. The mean percentages of hybrid for Plot 2 in Oregon were 17.6 and 35.6 for the rows west and east respectively of the pollinator. For

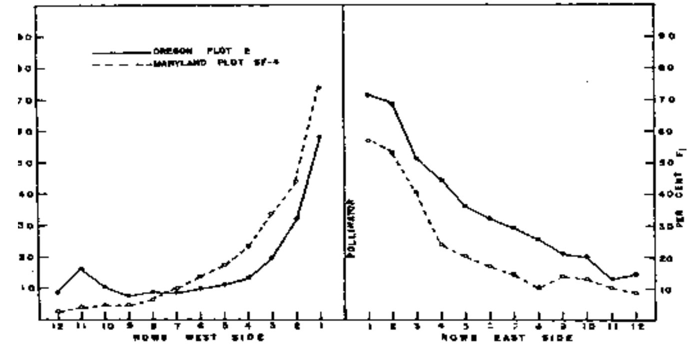


Figure 2.—Percentage T_1 seedings in row progenies of SP 47100-01 in Oregon Plot 2 and Maryland Plot SF-4. In each plot there was **a single row of the pollinator, SP 461001-0. The rows were four** feet apart.

Plot SF-4 in Maryland the values were 20.2 percent and 24.5 percent for the west and east sides respectively. The average lower percentage for the Maryland plot is attributed to a dike and trees on the west side which provided a windbreak, rather than to regional differences.

There was a tendency for the hybridizations to be higher on the east side of the pollinator than on the west in both plots but the tendency was most striking in Plot 2 where the mean percentage of F_t for the 12 rows on the east side was double that for the same number of rows on the west side. This tendency for a unilateral movement of pollen is an indication of the prevailing direction of air currents over the plots and is further evidence that insects are a minor factor in the pollination of sugar beets (7) .

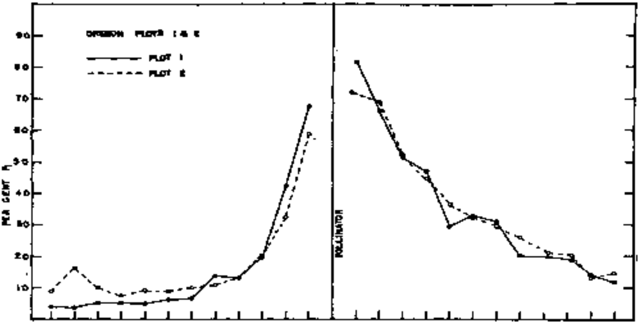
Width o£ Pollinator Strip

Plot 1 had a four-row strip of the pollinator and Plot 2 had only a single row strip, otherwise they were identical in design. The ratios of ~F_1 seedlings in the progenies of comparable rows in these plots may be used to indicate the relative efficiency in pollination of four-row and one-row strips. The percentages for the rows of Plots 1 and 2 have been shown (in Figure 3. It should be noted that the rows north of the pollinator in Plot 1 have been shown in comparison with those of the west side of Plot 2. These are the windward sides and presentation in this manner aids in making comparisons. The rows on the south and east sides of the plots are similarly contrasted.

Except for a slightly higher ratio of hybrid seedlings for the rows immediately adjacent to the four-row pollinator in Plot 1, the percentages were surprisingly similar in comparable rows of these two Oregon plots. The rows north of the four-rows of pollinator in Plot 1 and those west of the one-row pollinator in Plot 2 gave a mean F_x of 16.6 percent and

17.6 percent respectively. The rows on the south side and east side of the pollinator in these plots, given *in* the same order, were 35.1 percent and 35.6 percent. The mean percentage in all rows of SP 47100-01 in Plot 1 was 26.2 percent and in Plot 2 was 26.8 percent.

The incidence of pollen for a given distance from a point of release should be a function of the amount liberated. If one assumes that the ratios of the fertilizations produced by *R* and *r* pollen are directly related to their concentration in the air, then one would expect a higher percentage of hybridization from a four-row than from a single-row strip of the pollinator. The failure of Plot 1 with a four-row strip of the pollinator to show a general increase in effective *li* pollen over Plot 2 with a single row cannot be attributed to a sparse stand. Stand estimates were made and rows of the pollinator in Plot 1 were not noticeably deficient in plants. Furthermore, a comparison of seed yields for the pollinator in the two plots through the performance of the adjacent rows of SP 47100-01 indicated comparable stands. A partial explanation of the failure to get increased hybridization with the four-row strip of the pollinator should be



ROWS NORTH SIOE PLOT I; WEST SIDE PLOT 2 ROWS SOUTH SIDE PLOT i; EAST SIDE PLOT Z

Figure 3.—Percentage F_x seedlings in row progenies of SP 47100-01 in Oregon Plots 1 and 2. In Plot 1 there was a four-row strip of the pollinator SP 461001-0; in Plot 2 a **single row, otherwise the plots were identical in design.**

sought in the 12-row strips of the recipient. Rows progressively farther from the pollinator showed a marked reduction in the percentage of F_x seedlings. In a 12-row strip of a pollinator, it is evident that the innermost rows would contribute pollen mostly to companion rows and relatively little to a recipient variety in an adjoining area. However, one would expect the center rows of the four-row pollinator to increase the total hybridization a greater distance than one or two rows as indicated in Figure 3. If it could be established through additional tests that a single row of the pollinator is essentially as effective as a strip four rows wide or wider in bringing about fertilizations in adjoining area of a recipient variety, this information could lead to important savings in the cost of F_3 seed.

Dispersion of Pollen As a **Function of Distance**

The percentage germination, seedlings per seedball, and percentage !of F_x seedlings for rows of SP 47100-01 in Plot SF 4, and the combined values for Oregon Plots 1 and 2 are given in Table 1. In this summarization the values of the white anther and pollen-fertile phases have been combined for each row as well as the corresponding rows on each side of the pollinator.

Table 1.—Results Given as Means for Percentage Germination, for Seedlings Per Seedball, and Percentage Fi from Companion Rows of SP 47100-01 in Plot SF-4 Plant Industry Station, Beltsville, Maryland, and in Plots 1 and 2 Near Medford, Oregon.

	Plot SF-4			Plots 1 and 2		
Row[1]	Germination[2]	Seedlings per Seedball[3]	F_1 Seedlings[4]	Germination[2]	Seedlings per Seedball[3]	F_1 Seedlings[4]
	%	Seedlings	%	%	Seedings	%
1	90.3	1.97	65.5	90.3	1.94	69.5
2	89.1	1.88	48.3	91.1	1.91	52.3
3	89.3	1.93	37.0	91.1	1.89	36.3
4	91.5	1.90	23.7	91.8	1.95	30.3
5	87.1	1.80	19.0	89.7	1.91	23.0
6	91.6	1.88	15.7	90.9	1.88	21.3
7	95.1	1.84	12.2	93.7	1.94	19.2
8	89.9	1.79	8.4	93.8	2.00	15.1
9	89.1	1.89	8.8	92.2	1.92	14.0
10	89.0	1.82	8.5	93.4	1.90	13.7
11	87.0	1.77	6.9	89.9	1.87	12.1
12	89.0	1.83	5.8	90.1	1.80	10.0
Mean	89.7	1.86	21.7	91.5	1.91	26.4

[1] Each of the rows four feet from the centrally located strip of the pollinator is designated Row 1. Rows progressively farther from the pollinator are indicated by increasingly larger consecutive numbers.
[2] One seedling per seedball indicated germination. Values given as means of 800 seedballs, 400 from the pollen-fertile plants and 400 from the white anther phase of SP 47100-01.
[3] Values calculated from the total seedlings obtained from the seedballs germinating.
[4] Fi seedlings were identified by hypocotyl color.

It has been assumed that the production of r pollen was uniform over the plantings of SP 47100-01. If the effectiveness of *R* pollen in the mixture is not changed with dilution, then the percentage of $¥_1$ seedlings in the row progenies can be taken as indicating its relative concentration over the rows which were located at four-foot intervals from the pollinator. The percentages of hybrid seedlings occurring on each side of the three plots were plotted on semi-logarithmic paper as a series of associations. The values for the south side of Plot 1 and the east side of Plot 2 gave a fair fit to a straight line for all rows but in the other plots the rows farthest from the pollinator showed wide deviations. This indicated for the three plots in general an exponential relationship for the rate of decrease of *R* pollen concentration and distance. In a generalized manner this relationship is given by the curvilinear regression line in Figure 4. The percentages for SF-4 and Oregon Plots 1 and 2 as given in Table 1 have been averaged for the calculations. The F_x percentages computed from the regression equation Log. Y = 1.83917 — .07715X and the averages of the observed values are significantly correlated, r being equal to 0.97. For each row interval of four feet from the strip of the pollinator the logarithm of the percentage $F_{\pm}$ which is interpreted as the relative concentration of jR pollen is reduced uniformly by 0.07715.

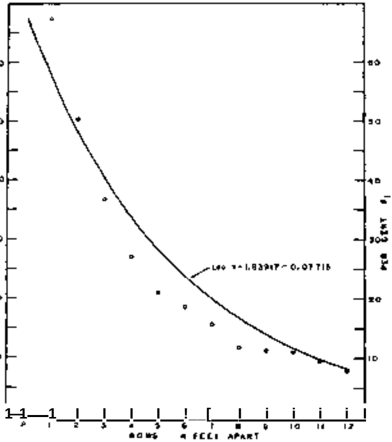


Figure 4.—Curvilinear regression line showing relation of row interval of four feet and percentage of T_1 seedlings. The average F_x percentages for SF-4 and Oregon Plots 1 and 2 as given in Table 1 were used in the calculations. The average values from the table are indicated by small circles.

If X is taken as 24 in the above regression equation the estimated percentage F_t at 96 feet from the pollinator would be 0.97 percent. It is interesting to note there was a fair confirmation of this estimation in Plot SG-12A on the Plant Industry Station. In this plot the row width and varieties were the same as before but the four-row strip of the pollinator was not centrally located. There were 50 rows of the recipient variety east of the pollinator. The first row, four feet from the pollinator, gave 69.8 percent F_x seedlings, which is comparable to values for similar locations in the other plots. The twenty-fourth row east of the pollinator gave only 0.6 percent hybrid seedlings, which conforms reasonably well with 0.97 percent computed from the regression equation. The ratio of hybrid to non-hybrid seedlings in the row progenies of Plot SG-12A gave further agreement with those obtained by the extrapolation of the regression line shown in Figure 4. For the portion of the field ranging from 100 to 200 feet east of the pollinator none of the seed samples gave more than 2.9 percent hybrids in the populations grown and the mean F_x for the 26 rows was 0.7 percent. Although not in accord with the regression equation but to an extent expected due to sampling error, ten of the 26 rows in the 100 to 200-foot sector of the field gave no F_x seedlings.

The percentage germination and the seedlings per seedball as given

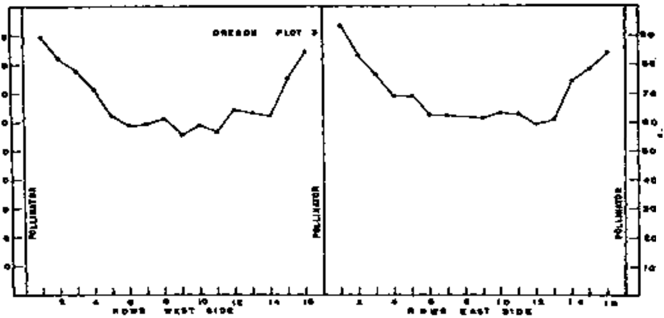


Figure 5.—The percentage of T_1 seedlings in row progenies of SP 47100-01 in Oregon Plot 3. There were alternating strips of the pollinator (four rows) and the recipient variety (16 rows). The rows were two feet apart.

in Table 1 do not show a significant change for the rows progressively farther from the pollinator, which is in accord with the report by Campbell (2). The quality of the seed harvested from rows farthest removed from the pollinator indicates that the pollen-fertile plants in SP 47100-01, judged to be 15 to 30 percent of the population, produced effective pollen in ample quantities to bring about the usual extent of fertilization obtained in commercial seed crops.

Alternating Strips of Pollinator and Recipient

Alternating strips of the pollinator and SP 47100-01 were used in Oregon Plot 3. The strips of the pollinator were four rows wide and those of the recipient 16, giving an arrangement of 4-16-4-16-4 for the plot. The results are presented in graph form in Figure 5.

It should be noted that the rows in Plot 3 are two feet apart instead of four as in the plots discussed previously. The influence of distance on percentage hybridization is strikingly demonstrated in Figure 5. Row 1 of the west strip of SP 47100-01, and row 1 of the east strip, occurring on the leeward side and two feet from a strip of the pollinator, gave for F_a seedlings 89.9 percent and 93.1 percent, respectively. Row 16 of the west and of the east strip, which might be considered as occupying the windward side of the pollinator, gave 84.1 percent and 84.3 percent respectively. These values show increased hybridization in the two-foot rows over the four-foot width used in SF-4 and Plots 1 and 2. The percentage values for the windward and leeward side of the pollinator indicate a strong unilateral distribution of pollen in the plot from west to east, which is attributed to wind transport.

It is evident that a 16-row strip (32 feet) of SP 47100-01 is too wide to give a high F_x percentage in the seed crop. The percentages tend to level off at approximately 60 percent for the eight or nine centrally located rows but this is a marked reduction from the high percentages given for the rows adjacent to the pollinator. Judging from the values for the second row from the pollinator, it is estimated that 80 percent or more F_1 seed would have been obtained from a four-row strip of SP 47100-01. Likewise, the values for the rows adjacent to the pollinator indicate that alternate single row planting might have given 90 percent F_1 seed for the recipient in this plot.

Discussion

No attempt has been made to determine the amount of *R* pollen produced by the pollinator or the amount of r pollen produced by SP 47100-01, but it is assumed that the latter type was produced uniformly over the strips of this variety. The percentage of F_1 seedlings in the row progenies should give the relative ratio of the two pollen types for the various parts of the field. That is, a reduction in the percentage of hybrid seedlings in the progenies of row progressively farther from the pollinator is taken to indicate a relative dilution of *R* pollen in the pollen mixture which reaches a given plant.

The data which have been presented indicate clearly that most of the pollen liberated by the pollinator and settling in the plots came to rest only a short distance from the point of release. This is illustrated by the regression line in Figure 4. If the equation Log. Y = 1.83917 — 0.07715X is given in the exponential form then the estimated percent F_1 = $(69.05)(.8372)^x$. Thus, according to this equation the concentration of effective *R* pollen over a row as an average was 83.72 percent of that over the adjacent row nearer to the pollinator, or a reduction of 16.28 percent for the four-foot interval.

It should be recognized that the settling out of the initial quantity of *R* pollen in the plots is not the major factor in bringing about a reduction in its concentration except possibly for clumps of pollen which would tend to settle rapidly on the rows nearest the pollinator. Basically, the reduction in pollen concentration with distance is due to its dispersion brought about by turbulence and gustiness of the air in a manner similar to the spread of smoke over and across a landscape from a campfire. That the rate of dispersion as well as the distribution pattern of pollen from a point of release is greatly influenced by the vagaries of the wind has been demonstrated by Colwell (3).

Although the hybridizations occurring in Plot SG-12A showed a general concordance with percentage of F_1 seedlings computed from the regression equation giving average values for three other plots, the real significance of the equation is to show the logarithmic rate of reduction with units, of distance rather than to establish specific values. It is reasonable to assume that the dispersion of *R* pollen would be expressed by a reduction *in* hybridization and a concomitant increase in fertilizations by *r* pollen. The nonlinear relationship found in this study for pollen dilution with distance from a pollinator is *in* accord with the reports in literature concerning the dispersion of pollen, fungous spores and other particles polluting the air. (5).

The exponential equation focuses attention on distance as the major factor in maintaining high concentration of pollen over a recipient variety in the production of hybrid seed. In Plot *3*, SP 47100-01 gave over 90 percent hybrid seed in a row two feet from the pollinator, which indicates the extent hybridization may occur. The minimum distance between plants of two lines to be hybridized could be obtained by the use of a seed mixture as planting stock. This method has been used in the commercial production of U. S. 215 x 216 (4) and other combinations of leaf spot-resistant inbreds. However, this scheme of producing hybrid seed requires a strong tendency for self sterility as well as relatively high productivity in the parental lines, otherwise the non-hybrid phase even though small may markedly reduce the yielding capacity of the variety. Hybrid varieties of sugar beets containing only F, seed can be produced if completely male-sterile equivalents of an inbred line are used as the seed bearer and supplied with a pollinator.

The concentration of pollen required in the air surrounding male sterile plants to give fertilization in all the flowers is not known. It should be mentioned that Owen (6) has reported high seed yields of a hybrid from alternating strips, four rows of the pollinator and 16 rows of 100 percent male-sterile plants obtained by clones. In the light of the results which have been presented, one would expect the egg nucleus in the sugar beet to remain receptive for a fairly long period, and with low pollen concentrations the chance of fertilization would become a function of the period of receptivity. This emphasizes the need for complete male sterility of all plants in the equivalent inbred used as the seed bearer, since an occasional fertile plant would produce proportionately more fertilizations under conditions of low total pollen concentration. For the same reason the problem of volunteers from previous seed crops looms large if cytoplasmic male-sterile plants are used in the production of commercial F_x seed.

The exponential relationship of units of distance and the rate of reduction in F_1 production in a recipient variety by a pollinator has a bearing on the polycross method of breeding sugar beets. If the plants to be interpollinated occupy a large area, or if they are planted in rows of considerable length, the pollen mixture over the various hills of the plot would be strikingly unlike qualitatively. Therefore, a progeny test could not evaluate the relative combining ability of the plants comprising the polycross group, but more precisely it would indicate the combining ability of the seed bearer and a few nearby plants as the major source of pollen. A pollinator with a genetic marker as in this study if otherwise acceptable would be an aid in determining the relative breeding value of individuals in a group, since a specific hybrid combination would be identifiable.

This study of pollen dispersion within sugar beet seed plots does not indicate the extent to which pollen from the pollinator may be transported beyond the boundary of the field. However, from the literature, which has been reviewed by Archimowitch (1) , it is known that sugar beet pollen may be wafted to great heights and transported over long distances.

Summary

The dispersion of pollen from a pollinator in sugar beet seed plots has been studied by means of identifiable hybrids in row progenies of a recipient

variety. In plots of similar size and design a four-row strip of the pollinator did not give appreciably higher percentages of hybridization than a single row. A slightly lower percentage of hybridization in a plot at the Plant Industry Station, Beltsville, Maryland, than in one near Medford, Oregon, with equal plantings of the pollinator was attributed to a local condition and not to a regional influence. There was a marked unilateral distribution of pollen within the plots from the pollinator, which was attributed to wind transport.

The percentage of hybrid seedlings *in* the row progenies of the recipient was reduced at a logarithmic rate with distance from the pollinator. The curvilinear regression based on averages of three plots indicated that the computed percentage of F_1 seedlings in a row progeny would be 83.72 percent of the adjacent row, four feet nearer to the pollinator.

The bearing of the results of this study on commercial hybrid seed production and on methods of sugar beet breeding has been discussed.

Literature Cited

(1) ARCHIMOWITCH, A.
1949. Control of pollination in sugar beet. Bot. Rev. 15: 613-628.

(2) CAMPBELL, S. C.
1950. Methods of utilizing the male sterile factor in sugar-beet seed production. Proc. Amer. Soc. Sugar Beet Tech. 187-190.

(3) COLWELL, R. N.
1951. The use of radioactive isotopes in determining spore distribution patterns. Amer. Journ. Bot. 38: 511-523.

(4) COONS, G. H.
1949. The sugar beet: product of science. Scientific Monthly 6: 148-164.

(5) GREGORY, P. H.
1945. The dispersion of air-borne spores. Trans. Brit. Mycol. Soc. 28 26-72.

(6) OWEN, F. V.
1945. Cytoplasmically inherited male-sterility in sugar beet. Journ. Agr. Res. 75: 423-440.

(7) STEWART, D.
1946. Insects as a minor factor *in* cross pollination of sugar beets. Proc. Amer. Soc. Sugar Beet Tech. 1946: 256-258.