Refinements in the Technique of Isolating By Bags and Cages[1]

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The use of paper bags and cages has generally been adopted by most organizations conducting breeding work on sugar beets. This paper is designed to add to our available knowledge on the use of these isolators. The paper deals with the presence of foreign pollen in bagging work and its control; the use of paper bags for hybridization work ; the use of large paper bags as individual and group isolators, and the use of cloth as individual and group isolators.. The results are presented in this order.

Controlling Foreign Pollen in Bag Isolators

The use of small paper bags as isolators for breeding work with sugar beets is in general use. It has been reported that where paper bags are utilized for inbreeding occasional hybrids are observed. Seitz (9) reports that about one-third of the plants in his test were hybrids.[3] In our own inbred strains formed by this method, hybrids have appeared. This raised a question as to the purity of such strains.

An experiment was conducted to determine the amount of crossing taking place under our conditions by ntilizing a strain of sugar beets pure for green hypocotyl color and a strain of red beets pure for red root color, and spacing these plants alternately. Only the green hypocotyl mothers were bagged. As much as 17 percent red hypocotyl hybrids (Keller (7)) was obtained from the use of No. 6 brown kraft-paper bags in the progenies from the green hypocotyl mothers.

Suspecting the presence of foreign pollen on the plant at the time of bagging, a number of trials were made of collecting fresh pollen and placing it on the leaves and seedstalks of non-flowering plants in the greenhouse and confining these parts with No. 6 brown kraft-paper bags. Viability of the pollen was determined by its ability to germinate on 40-percent sucrose agar media as reported by Artschwager (3). It was found that the pollen remained viable under our conditions for an average of 7 days.

A series of individuals were bagged at the normal bagging time when the buds were swollen but before open flowers were present.

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[3]Figures in parentheses refer to Literature Cited.

Examinations were made daily to determine how long after bagging before the flowers opened and shed their pollen. It was found that the average plant began shedding pollen on the third day. Artschwager (3) reports that fertilization takes place approximately 1 day after anthesis. Seitz (9) reports 75 percent fertilization 12 hours after anthesis. Kharechko-Sawitzkaja (8) reports that the embryo sac remains viable for a period of 8 to 9 days after the flowers open.

According to these facts, foreign pollen present on the plant at the time of bagging would have a 4-day opportunity to produce undesirable hybrids. As a possible control a series of experiments were conducted comparing viability, where fresh pollen was placed on nonflowering plants and one set of plants sprayed with water before bagging and another set bagged without spraying. No viable pollen was found on the sprayed plants when examined at the end of 24 hours, whereas the pollen remained viable on the dry checks. The experiment was repeated 8 times with the same results each time.

Since aphids are a factor in bagging work the use of 2 commercial nicotine-sulfate spray compounds were compared with tap w^ater and a dry check. All 3 sprays controlled the foreign pollen and both chemical sprays controlled the aphid infestations. Field tests of these sprays showed no difference in the final seed obtained. These results are in contrast to those of Archimovitch (1) who reports 6.7 percent crossing when using the water-dip method and 5.4 percent crossing when using a tobacco-solution-dip method.

As a result of our experiments we have adopted the use of a spray consisting of 15 cc. of a commercial compound, containing 40 percent nicotine-sulfate, and 1 ounce granulated soap to 1 gallon of water. The spray is applied by an ordinary hand garden sprayer immediately preceding bagging of the individual plants. The results of adopting this technique have been very gratifying.

Hybridization by the Pair-Bagging Method

In sugar-beet breeding work it would be convenient to have a simple procedure whereby small quantities of seed could be obtained for numerous hybrid combinations. Grinjko (6) has suggested confining 2 branches in a parchment bag, placing the branches in a horizontal position with the branch of the father plant above that of the mother plant. We have utilized the procedure of confining single branches of 2 adjacent seed plants in No. 6 brown kraft-paper bags in an upright position. The seed from these bags was harvested separately by mother plant so that the parentage of the hybrids in the resulting progenies was known. Where the parent material contains contrasted characters or marker characters, the hybrids of the pro-

genies may be easily recognized and may be evaluated. Table 1 presents data comparing the production of seed by the inbreeding and pair-bagging methods.

Table 1.—Comparison of inbreds and hybrids formed by the bagging method.

	Inbreds	Hybrids
Number of No. 6 kraft bags	459G	954
Number of strains	1194	1908
Percentage of strains forming seed	70	90
Average weight per strain for the strains forming seed	0.51 gm.	1.03 grin.
Percentage of strains forming seed which produce progenies	05	71
Percentage of strains producing progenies	50	64

The data show that the production of hybrid seed in the bags is better than for inbred seed. The method is simple and its use makes it possible to study a large number of hybrid combinations. The possible utilization of the pair-bagging method for indexing the combining ability of strains has a great deal in its favor.

Large Paper Bags as Isolators

Preliminary tests conducted in the greenhouse during the winter 1939-40 on the use of large-sized brown kraft-paper bags of different shapes and weights as individual plant isolators indicated that size of bag was largely a matter of convenience, but that weight of paper in excess of 40-pound substance would not be satisfactory. Subsequent field tests utilizing 26 different types of bags verified these results inasmuch as no seed was obtained from any paper bags regardless of size, which were heavier than 40-pound substance.

In 1940 a number of 30-pound substance No. 3 brown kraft-paper banana bags measuring 14 x 11½ x 47 inches were obtained for use as small group isolators. During the summer, 143 groups, of from 2 to 4 mother roots, planted in the same hole, were set out and as soon as seedstalks appeared the groups were covered with the large paper bags. Seed was obtained from 55 (38 percent of the isolators). The average yield of those forming seed was only 2.62 grams. Many bags were damaged by wind and had to be replaced during the bagging period.

In 1941 an experiment was conducted utilizing the 30-pound substance banana bags as individual plant isolators. One hundred thirty-three plants were isolated by these bags. Seed was obtained from 33 (25 percent) of the bags. The average yield of seed per bag was 1.56 grams.

Another experiment was carried out in 1941 on 324 groups of from 2 to 5 mother roots using bags made up with waterproof glue of 40-pound substance brown kraft paper, measuring 16 x 18 x 48 inches. Seed was obtained from only 22 (7 percent) of these iso-

lators. The average yield of those forming seed was only 0.925 gram. Wind was also a factor in survival of bags in both of the 1941 experiments. The results of these experiments are compared with No. 6 brown kraft-paper bags and cloth bags in table 2.

Table 2.—Comparison of large and small paper bags and cloth bags.

	Percentage isolates setting seed	Average weight of seed
4—No. 6 kraft grocery bags per plant for inbreds	76	0.51 gm.
1—14 x 11 1/2 a x 47 inches, 30-pound kraft bag per plant for inbreds	25	1.56 gm.
1—18 x 36 80 square bleached muslin per plant for inbreds	67	5.80 gm.
1—14 x l1 1/2 X 47 inches, 30-pound kraft bag for small groups	38	2.62 gm.
1—16 x 18 x 48 inches. 40-pound kraft bag for small groups	7	0.92 gm.

More seed was obtained from 1 large 30-pound kraft bag than from 4 small ones when used for inbreds, but fewer plants set seed under the large bags. A 30-pound kraft was belter for small groups than the 40-pound kraft, however, the amount of seed obtained was very small with either bag. The performance of the cloth bags for inbreds in comparison with the paper bags was encouraging.

Cloth. Cages

Periodically, in the literature on sugar-beet breeding, there have appeared references to experiments on the use of cloth cages as isolators for individual roots and for groups of roots, Archimovitch. (2), Brewbaker (4), Down and Lavis (3), Kharechko-Sawitzkaja (8), and Seitz (9). The results of some of this work have been put into practical use in breeding programs.

The following experiments give additional information on cloth cages. In all experiments a strain of beets pure for green hypocotyl and several strains of the common garden beet and the german garden beet, pure for red root color, were used. In the initial experiment in 1937, roots of the reds and greens were planted in a checkerboard manner and ail plants were covered individually and entirely by 100-pound inside white sugar-bag liners before any free pollen was present. All. of these bags survived a severe hailstorm without damage. The progenies of the green mothers showed a variation in crossing of from 0 to 27 percent. The average for all cages was 5.30 percent red hybrids.

In 1938 the experiment was expanded to include other types of cloth and changed inasmuch as only the greens were covered with the cloth cages, leaving the reds in the open. All cages of muslin were made up in a size of approximately 18 by 36 inches as single-plant isolators and the remainder were bags of the lOO-pound size. Table 3 presents the results of this experiment.

Table 3.—The results of the 1938 cloth-cage experiment.

Reference No.	Type of cloth (10 cages each)	Threads (inches)	Average percentage of hybrids	Average weight seed per cage (in grams)
1	1 thickness inside white liner	40x40	23	6.09
2	2 thicknesses, inside white liner	40x40	18	1.85
3	Salt bag	44x44	21	4.50
4	"Missouri" unbleached muslin	48x50	3	2.60
5	Outside white sugar bag	5Ox50	4	.86
6	"Glenwood" bleached muslin	60x64	27	1.64
7	"Hope" bleached muslin	72x80	8	4.30
8	"Nameless" unbleached muslin	80x80	16	4.43
9	"80 Square" bleached muslin	80x90	6	1.06

From these results it would appear that cloth numbers 4, 5, 7, and 9 gave the best protection against crossing.

An experiment was arranged in 1939 to obtain additional information, but due to the loss of many plants by rot the data are not considered reliable.

In 1940 a small experiment was successfully carried out including the 4 most promising cages of the 1938 experiment. Table 4 presents the results of this experiment.

Table 4.- The results of the 1940 cloth-cage experiment.

Reference No.	Type of cloth (5 cages)	Threads (inches)	Average percentage hybrids	Average weight seed per cage (in grams)
4	"Missouri" unbleached muslin	48x50	17	1.4
5	Outside white sugar bag	50x50	33	1.4
7	"Hope" bleached muslin	72x80	14	1.3
9	"80 square" bleached muslin	80x90	7	6.0

From these data it is obvious that these types of cloth cages used in this manner do not afford ample protection against foreign pollen. However, re-examination of the 1937 and 1938 data shows for the rather porous inside white-liner cages that where the red beets remained in the open, 23 percent crossing was obtained but when all plants within the isolation were confined, only 5 percent crossing was obtained. This indicates that the 1940 type of cages might have been successful if all plants had been enclosed.

Cloth number 9 was selected on the basis of the 1940 results for use in 1941 for an experiment where all plants were covered by a new type of cage. The cages had a wooden frame 30 inches wide, 12 feet long, and 4 feet high with an additional cross-section support in the

center. The base was made of 1-inch by 6-inch and the remainder 1-inch by 2-inch pine material. The "80 Square" bleached muslin was sewed together to form a cover for this frame with a stocking-like aperture affixed to one end and tied with a string for later visual inspections. This size cage accommodated 50 stecklings or 24 mother roots in two 20-inch rows. One 20-inch row was left blank between the cage rows and 3 feet were left blank between cages within the rows. The frames were assembled by screws and staked down. The cloth was placed over the frames and nailed in place by lathes around the base. Water was applied by subirrigation from furrows adjacent to the cages. In 1941, 101 such cages were utilized. One cage was devoted to reds and another to greens and located in the path of the prevailing winds to check the amount of crossing obtained. The remainder of the cages were devoted to breeding material. The seed from the cage containing the green hypoeotyl roots was all planted in the greenhouse to determine the amount of crossing obtained. There were 1,890 plants produced, 1 of which was a red hybrid, showing a trace of crossing amounting to 0.05 percent. This indicates that these cages used in this manner are not perfect but do afford very good protection for small groups and small hybrid isolations.

The cages were placed early in the season, and aphids developed in most of them. It is believed that this greatly reduced the amount of seed obtained. Table 5 compares the yield of seed from these cages with similar material in space-isolated plantings.

Table 5.—The results of the 1941 cloth-cage experiment.

	Caged	Space isolated
Percentage of planted stecklings setting seed	40	38
Average number setting seed per cage	19	
Variation in yield per individual steckling	Trace—28 grams	Trace—112 grams
Average yield per individual sleckling	.5 gram	33 grams
Variation in yield per cage	Trace—255 grams	
Average yield per cage	90 grams	
Average germination	36 percent	28 percent

The yield of seed from these cages was disappointing but the fact that they reduce to a minimum the probability of accidental hybridization warrants their further consideration. The use of stecklings in these cages may answer a need in breeding programs for a means of obtaining small amounts of seed of advance generations, backcrosses, and hybrids without increasing the number of space isolations. From this viewpoint these cloth cages utilized in this manner appear promising. We are hopeful that the control of aphids through the use of fumigants, the selection of a better location, better use of manure and commercial fertilizers, and better control of the material to insure uniform bolting may greatly increase the yield of seed.

Summary

Foreign pollen present on seed beets at the time of bagging, which may give rise to undesirable hybrids, and infestation of bags by aphids may both be effectively controlled simultaneously by a nieotine-sulfate-and-soap-solution spray applied by an ordinary garden sprayer immediately before bagging.

The pair-bagging method of confining branches of adjacent plants in a single bag may be useful in indexing the combining ability of sugar-beet strains.

The use of large-sized paper bags for inbreeding and small group isolating does not appear promising in view of the relatively high mortality of the isolates.

The use of "80 Square" bleached muslin as an isolating cloth where all plants are confined within bags or cages appears promising.

Literature Cited

1. Archimoviteh, A. Z. On the Methodology of Isolating and Hybridizing Sugar Beets. Pflanzenzuechtung. Vol. XV: Part 4, p. 357. 1930.
2. Archimovitch, A. Z. A New Type of Isolator for Groups of Sugar Beet Stecklings. Jour. Bot. Inst. Acad. Sci Ukraine, USSR No. 26-27: 257-270. 1938.
3. Artschwager, E. and R. C. Starrett. The Time Factor in Fertilization and Embryo Development in the Sugar Beet. Jour. Agr. Res. 47: 823-843. 1933.
4. Brewbaker, H. E. Self-fertilization in Sugar Beets as Influenced by Type of Isolator and Other Factors. Jour. Agr. Res. 48: 323-337. 1934,
5. Down, E. E. and C. A. La vis. Studies on Methods for Control of Pollination in Sugar Beets. Jour. Amer. Soc. Agron. 22: 1-9. 1930.
6. Grinjko. On the Method of Crossing the Sugar Beet. Bulletin of the Breeding Station Iwanowka, Charkov Government, Ukraine NR, 4, S. 47-63, 1930.
7. Keller, W. Inheritance of Some Major Color Types in Beets. Jour. Agri. Res. 52: 27-38. 1936.
8. Khareehko-Sawitzkaja, B. Flowering, Fertilization and Different Types of Sterility in *Beta vulgaris* L. Translation furnished by U. S. D. A. of a reprint with no reference to where originally published.
9. Seitz, F. W. The Fertilization of the Sugar Beet. Zuckerraebenbau. 19: 85-93, 111-115. 1937.