AN ABSTRACT OF THE THESIS OF

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Title: The Effect of Bud Self Pollination, Open Flower Self Pollination and Cross Pollination on the Field Characteristics of Broccoli Progenies

Abstract approved: Dr. J. R. Baggett

To determine the effect of bud pollination, compared to pollination of open flowers and crossing, on field characteristics of broccoli progenies, an experiment was conducted in 1974 and 1975 at Corvallis, Oregon. Single plants of 55 broccoli lines were pollinated, using four methods, i.e. bud pollination (BP), open flower pollination (OS), open flower pollination with ether treatment as an aid (OSE), and crossing with a common parent (CP).

BP resulted in a substantially greater number of seed set per cluster than OS or OSE and slightly less than CP. There was a significant overall effect of pollination methods on seed weight, but it was the result of greater seed weight resulting from CP compared to other treatments. Broccoli lines differed greatly in seed weight and number of seeds per cluster. The use of ether did not significantly increase number of seed per cluster or seed weight.

Thirty-nine lines were direct seeded in the field. Because of seed shortages and seedling losses, the experimental design of four replications in a randomized block was complete for only seven lines, and a total of 20 lines had at least one replication for each treatment. Separate analyses were made of the data for these seven lines and 20 lines respectively.

In the field-grown progenies, the quantitative characteristics of plant height, plant weight, and head diameter, were greater for CP, intermediate for OS-OSE, and lowest for BP. Head weight was similarly, but less conclusively affected. Number of days to head emergence was longer in progenies from BP than OS-OSE, and those from OS-OSE took longer than progenies from CP. Number of days to harvest maturity followed the same pattern in the analysis of 20 lines but was inconclusive in the analysis of seven lines. Results for floret size and rosetting were generally inconclusive. The experiment generally supported the hypothesis that BP results in progenies with less plant vigor than those pollinated after normal anthesis (OS). The use of ether as an aid in open flower pollination generally had no effect on the field characteristics of resulting progenies.

THE EFFECT OF BUD SELF POLLINATION, OPEN FLOWER SELF POLLINATION AND CROSS POLLINATION ON THE FIELD CHARACTERISTICS OF BROCCOLI PROGENIES

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INTRODUCTION

Though sprouting broccoli, Brassica oleracea L.(Italica group), has been grown in Europe since Roman times, it was not introduced to the United States until about half a century ago. Within the short five decades, however, it has gained greatly in popularity and now it can be seen in markets all over the country. It is reported that broccoli currently has an annual commercial value of $68 million in the United States. It is a vegetable with very high vitamin C content and is also high in vitamin A. As a cool season, annual crop, it needs an abundant and constant supply of moisture and nutrients for rapid growth and heavy yield. It grows best when the daily temperatures are between 60-70^{o} F. In recent years, it has also become one of the many economically important vegetable crops grown in the Willamette Valley of Oregon.

Broccoli belongs to the family Cruciferae and to the species Brassica oleracea. This species includes a number of horticultural types with diverse form: cabbage (Capitata group), cauliflower (Botrytis group), kale and collards (Acephala group), kohlrabi (Gongylodes group) and Brussels sprouts (Gemmifera group). While these forms are distinctive in plant form and use, they intercross readily.

In B. oleracea and other genera of the family Cruciferae, sporophytic self-incompatibility is a common phenomenon. Self-incompatibility refers to the situation where both the pollen and pistil are structurally and functionally normal, but self-fertilization fails because of a genetically controlled biochemical mechanism. The incompatible pollen usually fails to germinate, or if it germinates, the pollen tube fails to penetrate the stigma. Studies have shown that the incompatibility reaction is localized in the stigma. The actual mechanism is still not well understood. In sporophytic incompatibility, the reaction of the pollen, is determined by the genotype of the plant which produced it.

Self-incompatibility has been used commercially to prevent selfing of the female parent in F_1 hybrid seed production. However, in order to produce the F_1 seed, the inbred parent lines must be maintained by selfing. This requires some methods to break or circumvent self-incompatibility. The most common method is bud pollination, but other aids, such as stigma abrasion and chemical treatment (ether, CO_2) are also used. When selfing occurs, either naturally or by bud pollination, the line becomes weaker, later in maturity and possibly inferior in quality - the result of so-called inbreeding depression. Field observation has suggested that open flower pollination will produce plants which are bigger and superior in quality than those from bud pollination. Also it has been known that F_1 hybrids express heterosis so that hybrids perform better. This results because broccoli is a naturally cross pollinated crop. When cross polination occurs, the hybrids will usually be more vigorous, superior in quality, and earlier in maturity as compared to selfed lines.

In this study, bud self-pollination, bud cross pollination, and open flower self-pollination with and without ether treatment of stigma, were compared on 39 lines of broccoli with respect to (a) seed set and weight;

(b) field measurement of date of first head appearance, maturity date, plant weight, plant height, head size and weight, and head quality as degree of rosetting (uniformity of bud development) and floret size. The main hypothesis to be tested was that seeds from bud self-pollination would produce smaller and later developing plants than those from open flower selfs, either with or without the aid of ether, and that seeds from cross pollination would produce larger, more vigorous, and earlier developing plants than seeds from self-pollination.

REVIEW OF LITERATURE

General Review

Broccoli is a tall, annual plant which rapidly produces an inflorescense. It has long internodes and usually produces axillary branches freely. This now popular vegetable may have been known to the Greeks some 2,500 years ago. Buck (8) traced the origin and the taxonomy of broccoli and reported that the present day varieties have been developed principally from forms that have arisen in Italy in the past 2,000 years. The name, meaning "bucktooth", was probably a Latin colloquial term applied to any projecting shoots of members of the cabbage family. Sprouting broccoli was first grown on the east coast of the United States by Italian market gardeners and gained popularity after the seed was introduced from the Province of Calabria in Italy in the years 1926-27.

Broccoli is classified as a botanical variety of the species *Brassica oleracea* L. Bailey (4) described the cultivated brassicas in 1930, separating *Brassica oleracea* into six subdivisions: *B. oleracea arvensis*, much like the wild plant, cultivated for its oil-yielding seeds and leaves for forage; *B. oleracea viridis*, the kales; B. *oleracea capitata*, the cabbages; *B. oleracea botrytis*, the cauliflowers and broccolis; *B. oleracea gonglydodes*, kohlrabi; *B. oleracea napobrassica*, rutabagas. More recent usage replaces the subspecies designation by groups i.e. Capitata group for cabbage, and places broccoli in the Italica group.

Yarnell (44) reviewed the cytogenetics of vegetable crops and discussed in detail the botanical relationships of the genus Brassica. The botanical groups of *B. oleracea*, supposed to have originated from wild leafy forms similar to cultivated kale and collards, cross readily and produce normally fertile progenies.

Self-Incompatibility

A typical characteristic of the Cruciferae family and of the *B. oleracea* species is self-compatibility (SI). Various workers (14, 28, 5, 2, 36, 21) have described incompatibility and suggested genetic schemes to account for its behavior in *B. oleracea*. Sampson (34) indicated that self and cross-incompatibility in green sprouting broccoli is controlled by multiple oppositional alleles at one locus. The pollen reaction is sporophytically determined. He also found that some self-incompatibile plants became self-compatibile with age, a change which seems to be conditioned by modifying genes.

It has long been known that a series of alleles at one locus determines whether pollen will be functional or non-functional. These alleles have been termed S_1, S_2, S_3,...S_n. Diploid stylar tissue will normally have any two alleles of the series, but never the same two. A single pollen grain will have one. When the sylar tissue has the same gene as the pollen grain, the developmoent of the pollen tube is inhibited.

In sporophytic incompatibility, all pollen grains from a particular plant behave similarly because the reaction of the pollen is determined by the plant producing it. Dominance is common in the pollen reaction and in the stigma reaction as well. Another characteristic of this system is that inhibition occurs in the stigma, consequently pollen tube growth is nearly or completely inhibited (29, 9, 12).

Thompson (41) in a study on marrow-stem kale found that self-incompatible plants may be homozygous either for an S allele low in the dominance series or for a recessive allele independent of the S allelic system. Female sterility may be due to genes in the same linkage group as the S alleles or independent of this system.

Kroh (17) in a study of the nature of the sporophytic incompatibility reaction, suggested that when transferring pollen from one stigma to another, the cutinase system of the pollen becomes irreversibly activated in combinations where pollen and stigma differ in the S alleles.

Sampson (35) in analyzing pollen tube inhibition on stigmas of open flowers, suggested a physiological mechanism by which a self-incompatible species of the Cruciferae can recognize pollen of the same species (or genus) and accept it, while rejecting pollen of foreign species. A stigma also rejects pollen when an S allele is mutually active. A macromolecular code with two pairs of sites, each with a complimentary structure, explains this behavior. Based on this hypothesis, he suggested that bud compatibility in self-incompatible crucifers results from the absence of barriers to pollen tube growth, rather than from the absence of the S allele controlled inhibition of the enzymes that normally overcome these barriers in compatibile crosses within the self-incompatible species.

It is known that after self-pollination in self-incompatible Cruciferae, pollen grains are unable to germinate, or even if germinated in a humid atmosphere, their pollen tubes are unable to grow into the stigma. In an electron microscopic study involving self-incompatible crucifers, Kanno and Kokichi (16) found that pollen tubes stopped their growth at the cellulose-pectin layer of the papilla cells and suggested that perhaps the specific protein within and/or around the cellulose-pectin layer exerts an effect on the pollen tubes.

Ockendon (26) found in a scanning electron microscope study of Brussels sprouts and kale that the stigmatic papillae are closely appressed in young stigmas, but are held apart in the mature stigma by the swollen bases of the papilla cells. Some self-pollen tubes show obvious signs of inhibition by being very short, or by coiling over the surface of the papilla without penetrating it.

Roggen (31) observed, however, the presence of a wax layer on the stigma papillae of Brassica in the scanning electron microscopital pictures. In young buds the stigma papillae are not completely covered with wax. It was shown that after a compatible pollination the pollen grains "stuck" to this wax layer, so that it was pierced and the pollen exine touched the cuticle sensu stricto, and the papillae collapsed. The wax layer seemed to be the incompatibility barrier. He also found pollen germination and penetration of pollen tubes into the papillae walls. After incompatible combinations, no "sticking" reaction and thus no germination took place and the papillae stayed turgescent. He discussed the importance of this initial pollen-stigma interaction, the 'recognition', in connection with the sporophytically determined self-incompatibility system.

Overcoming Self Incompatibility

It is often necessary to self pollinate self-incompatible plants of broccoli and other crucifers. For example inbreeding is often needed in breeding programs and it is necessary to maintain inbred lines used in F_1 hybrids produced commercially by means of the sporophytic incompatibility system. Thus the SI barrier has to be broken. Various methods have been used, of which the most popular one is bud pollination, that is the application of pollen to the stigmas of unopened flowers to maintain and increase inbred lines.

Kakizaki (14) found in experiments with cabbages that fertility of the normally incompatible plant may be increased to a considerable degree when flowers are pollinated in the late bud stage. In a follow up study, Kakizaki and Kasai (15) confirmed that the phenomenon was also found in two other species of Cruciferae, Chinese cabbage (B. Pekinensis Rupr.) and Japanese radish. Kakizaki explained that this phenomenon is due mainly to insufficient presence of the so-called 'inhibiting substance' which inhibits the pollen-tube growth in the stylar tissue owing to immaturity of the style, or it might be due to the fact that pollen tubes have a relative short distance to travel to bring about fertilization. Pearson (30) also described bud pollination in his experiments with plant breeding of the cabbage group.

Wiering (43) in a study on artificial pollination of cabbage plants suggested the most suitable stage for bud pollination is two or three days before opening. Martin (20) found that the age of male or female component of the flower had a small but statistically significant effect on seed set in an experiment involving green sprouting broccoli.

Bud pollination, though often used, is very time-consuming and demands skilled labor to produce a reasonable yield of seed (13). Other methods have thus been sought to break the SI barrier. Some of these include mutilation or removal of the stigmata (19); changing nutritional conditions (38); pollen transplantation (17); and high temperatures (10). Roggen et al (32) discovered that by using electrical treatment during self-pollination of open flowers in Brussels sprouts and Savoy cabbage, the SI barrier can also be broken. They also proved that they could use a steel-brush to mutilate the stigmata to acheive the same result of breaking SI in Brussels sprouts. Roggen et al (33) found that percentage of fruit set with electrical aided pollination is always higher than with bud pollination. On the other hand they found bud pollination produces generally higher seed set.

Nakanishi and Hinata (24) suggested that CO_2 was effective when supplied to a self-pollinated flower while hundreds of pollen grains were germinating on the stigma. They explained that since the effective time coincides with the attachment of pollen tubes to papilla cells, CO_2 produces a metabolic change in these cells during attachment.

Tatebe (39) was able to overcome SI in radish by treating the stigma with ether or KOH. It was suggested by Roggen (31) that this is done through the disturbance or removal of the wax layer on the stigms papillae.

Heterosis and Inbred Depression

Pearson (30) noted that hybrid vigor is evident in cabbage, and hybrid cabbage appears to produce certain advantages, such as larger and more uniform heads, earlier maturity and a better stand of plants, as compared with open pollinated varieties. It is also reported that for hybrid vigor to be most effectively utilized, lines or varieties with high combining ability must be identified (22).

In a follow up experiment, Pearson (30) developed a method of producing F_1 hybrid broccoli seed using honey bees working on plants protected by cheesecloth cages from visitation by outside insects.

Odland and Noll (28) in discussing the production of F_1 hybrid cabbage suggested that seed set (in the presence of self-incompatibility) is influenced slightly by temperature. In maintaining or increasing inbred self-incompatible lines best results may be obtained by holding the plants during the seed setting period in a relative cool temperature.

Anstey (1), in his study on SI in broccoli, has confirmed that SI may be used in the production of hybrid seed by growing self-incompatible, cross-compatible lines in close proximity.

Legg and Souther (18) identified heterosis in intervarietal crosses of broccoli by comparing the average of all F_1 hybrids with the average of all varieties for days of maturity, plant height, and compactness of the central head.

Borchers (7) discussed yield, uniformity of heading and season of maturity of broccoli inbreds, hybrids and varieties and found that broccoli inbreds may be as large as or larger-headed than the varieties from which they were derived. He noted that a majority of the hybrids produced larger heads than either of their parent inbreds. The average weight increase of the hybrids over their larger-headed parent was 36.3%, and the largest headed inbreds, in general produced the largest headed hybrids.

Nieuwhof (25) discussed the use of incompletely sib-incompatible inbred lines in breeding hybrid varieties of cole crops. He suggested that one advantage of the incompletely sib-incompatible lines is that their propagation is easier than that of completely sib-incompatible lines, especially when the S-factors are highly active.

Myers and Fisher (23) found inbreeding depression in their experiments with cabbage. They attempted to increase genetic uniformity by inbreeding but concluded that it is not advisable to inbreed beyond two generations because of continuing loss of vigor.

To increase inbred lines, Odland and Isenberg (27) discussed methods of asexual propagation in the production of F_1 hybrid cabbage seed. They experimented with three types of cuttings, the leaf, the leaf with axillary bud attached (heel cutting), and the leaf with a complete section of the stem attached, and found heel cutting gave the best results.

Martin (20) found in his experiment on factors affecting seed set in cross pollination of broccoli that the chief and most important source of variation in seed setting ability seems to be associated with specific parental combinations. He noted that even a very small amount of pollen placed on the stigma can fully fertilize the pod and thus attributed less of the variability in seed setting to pollen sources.

Seed Size Effects

In a study on seed size effect on germination, Thiele (40) found that heavier seeds of samples of carrot, lettuce, tomato, beet and cabbage have shown greater final percentage of germination than lighter ones.

Austin and Longden (3) in experiments with carrots, found that higher and more predictable field emergence and slightly higher yields of roots would be obtained by use of graded large seed. They proposed that this increased yield is likely to be caused by the increased "initial capital" in the larger seeds in the form of potential leaf or stored food, and by the better germination of the larger seed.

Tompkins (42) demonstrated that broccoli plants produced from large seeds had much higher early yields of center heads than plants from small seeds. In most cases, total center head production for the entire season was not influenced by seed size. Plants maturing early tended to have smaller center heads than plants maturing at midseason and later.

Halsey et al (11) discussed seed and plant size effects on cabbage cultivars and found that the principal responses perhaps are more readily accounted for by genetic rather than environmental factors.

MATERIALS AND METHODS

Plant Materials

All the plant used in this study were taken from an existing Oregon State University breeding program. They were chosen to have different genetic backgrounds. Some had club root resistance, some had both club root and downy mildew resistance, and some were general breeding lines with no particular disease resistance. Lines were designated by prefixes as follows:

Prefix	Type of Line	Degree Inbred
H	general, nonresistant	medium
HS	general, nonresistant	highly
B	club root resistant	medium
S	club root resistant	highly
CM	club root and downy mildew resistant	medium

The lines described as highly inbred were from about 6 or more generations of selfing and were being maintained by either selfing or sibbing, depending on their intended use. They were quite uniform. Medium inbred lines were from two to four generations of selfing, and were generally uniform in phenotype.

General Methods

Plants used as seed sources were obtained from cuttings of broccoli grown in the summer of 1974. After rooting, the plants were maintained in pots in a greenhouse free from pollinating insects in the autumn of 1974 through spring of 1975. Pollination was done during the winter of 1974 through the spring of 1975. Altogether 55 lines were pollinated. For each line a single plant served as a seed parent. No replication was done. Each plant was pollinated using four pollination methods: 1. bud self-pollination (BP), consisting of pollinating stigmas of flowers in the bud stage, two to three days before opening, with pollen from the same plant; 2. open flower pollination (OS), in which flowers on the day of their opening were self-pollinated with pollen from mature flowers of the same plant; 3. open flower pollination with ether treatment (OSE), in which flowers, on the day of their opening were treated with ether applied to the stigma with a straw and then pollinated with pollen from the same plant; 4. bud cross-pollination (CP), which was similar to bud self-pollination except that pollen was taken from one standard male parent, a uniform inbred line HS 118. Each of the treatments consisted of ten buds or ten flowers.

The seeds from these plants were harvested in the late spring of 1975. Number of seeds/cluster and seed weight were determined at that time. They were subsequestly seeded in place in the field 30 cm apart in rows which were 90 cm apart. Ten seeds were planted in each plot. Rows were previously hand fertilized with 600 kg/ha of 8 N-10.3 P-6.6 K with no additional fertilizer applications. Water was applied by sprinkler every ten days or as needed to maintain normal growth. Insect controls were applied to protect against such pests as flea beetles, aphis, and root maggots. However, some loss or damage of seedlings from flea beetles did occur.

Experimental Design

A randomized block was used with a maximum of four replications for each line and treatment. The number of replication was actually variable because in some cases there were not enough seeds and only two or three replications were possible. Due to loss of seedlings, treatments were also missing for many lines. The result was that data for only 20 lines were complete enough for analyses, as explained below under Analyses of Data. In each replication the four pollination treatments of each line were grouped together, but the order of the treatments within lines and the location of the lines in the block were at random.

Obtaining Data

Greenhouse data were obtained for number of seed set per cluster and weight per 100 seeds. Field data were obtained for each plant for date of first head appearance, head (inflorescence) maturity date, plant height, plant weight, head diameter, head weight, floret (bud) size and degree of rosetting. Maturity date was that judged to be most practical, considering both yield and quality, in a commercial situation. Plant height and head diameter were measured in centimeters. Head weight , in grams, was obtained from heads trimmed to 12.5 cm length from buds to base. Floret size was scored from 10 to 50 with 10 being smallest and 50 largest. Degree of rosetting, which is an uneven appearance of the head caused by gradients from the young center buds to the older outer buds of a section of the inflorescence, was scored from 10 to 50 with 10 for slight rosetting and 50 for extreme rosetting. Individual plants were identified to facilitate note taking at different stages of development.

Anaylses of Data

All data were transferred to punch cards and computer analyzed. Greenhouse data on seeds per cluster and seed size were subjected to the analyses of variance using line x treatment interaction as error term.

Because of many missing plots due to lack of seed supply and loss of seedlings, two separate analyses of field data were undertaken and are separately presented in the results. Seven lines for which complete data were available (four replications of four treatments) were included in a conventional analysis of variance, and then an analysis of covariance to determine if there was a stand effect. Where the F value for stand effect was significant at 5% probability, covariance analyses and adjusted means are presented in Results.

Based on the absence of significant replication effects in the analysis of seven lines, described above, additional analyses were done as follows. Analysis of covariance, was applied to any line x treatment combination, having data for at least three replications; 81 line x treatment combinations were included. Covariance adjusted means were used for further analysis except where the stand effect was nonsignificant (in plant height, bud size score, and rosetting score). An Unweighted Means Analysis (37) was then used on 20 lines with all treatments present and at least one replication for each treatment. This method initially used individual observations (replications) to obtain a per plot error mean square. Then a harmonic mean was calculated to derive a per cell (20 x 4) error mean square. Treatment means from this analysis included the plots acctually present; no missing data was created.

Treatment means for seeds per cluster, seed weight, and the eight variables measured in the field were compared by t test. Based on an initial fixed hypothesis, the following formulae were used to calculate T.

For BP versus the mean of OS and OSE and CP verses the mean of OS and OSE:

$$t = \frac{\frac{\overline{X}\,OS + \overline{X}\,OSE}{2} - \overline{X}\,BP\ (or\ CP)}{\sqrt{s^2\left(\frac{1}{2N} + \frac{1}{N}\right)}}$$

For OS verses OSE:

$$t = \frac{\overline{X}\,OS - \overline{X}\,OSE\ \ (or\ \overline{X}\,OSE - \overline{X}\,OS)}{\sqrt{s^2\left(\frac{2}{N}\right)}}$$

For BP verses CP:

$$t = \frac{\overline{X}\,CP - \overline{X}\,BP}{\sqrt{s^2\left(\frac{2}{N}\right)}}$$

In addition, LSD values were calculated for the express purpose of showing an approximate significance level on the graphs (Figures 1, 2, 3), using the formula:

$$LSD = t\sqrt{s^2\left(\frac{2}{N}\right)}$$

RESULTS AND DISCUSSION

Greenhouse

The effect of line on number of seeds set per cluster was significant at the 1% level (Table 1). Line means ranged from 5.5 seeds/cluster to 107.25 seeds/cluster. This was to be expected and did not relate to the objectives of the experiments. The effect of treatment on seed set was highly significant (F=40.8) (Figure 1, Table 1). Bud pollination produced substantially more seed than either kind of open flower pollination (OS or OSE). OS and OSE did not differ statistically, but OS did produce more seeds than OSE. CP resulted in more seeds than all other treatments. These results are generally consistent with expectations, based on literature reports, although the ether treatment did not improve seed production over OS as it was expected to do.

The effect of line on seed weight was significant at the 1% level (Table 1). Line means ranged from 323 mg to 651 mg/100 seeds. Again, a difference between lines for seed weight was expected because cultivar differences for seed size are common and obvious. The effect of pollination method on seed weight was significant at the 5% level, but not at 1% (Figure 1, Table 1). Cross pollination produced larger seeds than other treatments, probably because of the effects of hybrid vigor on the daughter embryos. The other possible comparisons were non-significant.

There was no apparent correlation between seed number per cluster and seed weight (r = .1401).

Table 1. Effect of greenhouse pollination methods, in 39 broccoli lines, on number of seeds produced per cluster and weight in milligrams/100 seeds.

Variable	Treatment means[1]					t values for treatment comparisons[2]				Line effect
	1	2	3	4	F	1 vs 2,3	2 vs 3	2,3 vs 4	1 vs 4	F
Seed Number	64.077	30.897	24.308	73.128	40.8**	7.893**	1.235NS	9.852**	1.696*	4.9**
Seed Weight	462.89	461.79	467.87	497.77	2.7**	0.153NS	0.411NS	2.609**	2.361*	7.2**

1. Treatments: 1-bud pollination (BP); 2-open flower self (OS); 3-open flower self ether aided (OSE); 4-crossed (CP)

2. $2,3 = \frac{\bar{X}2 + \bar{X}3}{2}$

NS = non-significant at 5% probability level

* = significant at 5% probability level

** = significant at 1% probability level

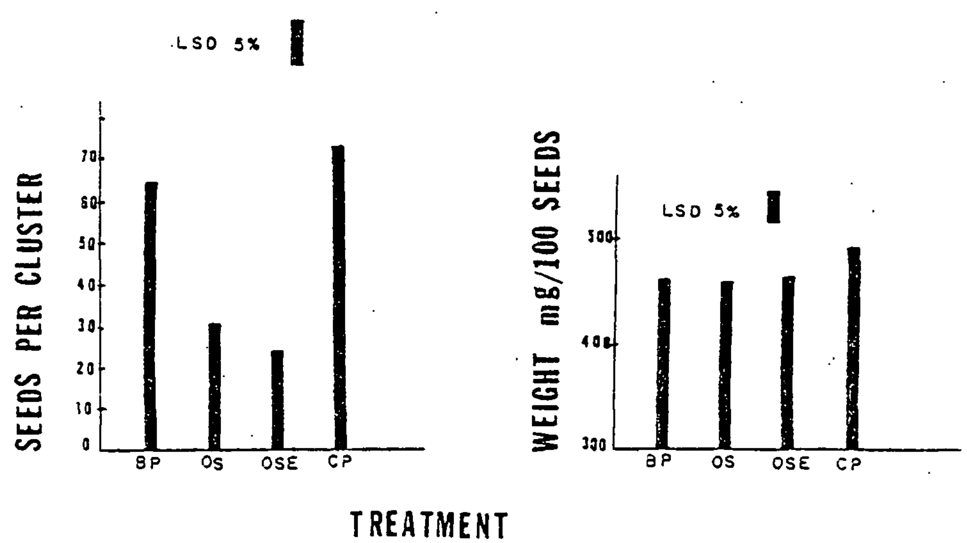

Figure 1. Effect of greenhouse pollination methods, in 39 broccoli lines, on number of seeds produced per cluster and weight in milligrams per 100 seeds. (BP-bud pollination, OS-open flower self, OSE-open flower self ether aided, CP-crossed)

Field Results

There was no replication effect, as indicated by the analysis of seven lines with complete data, for any variable. The line effect was significant at the 1% level for all variables in both the 7-line and 20-line analysis. This is to be expected because of the wide genetic base of the broccoli materials used in the study.

In all characteristics except bud size score and rosetting score, the relationship between BP, OS-OSE, and CP was generally as hypothesized with BP resulting in lower quantitative values (higher in the case of dates), OS-OSE [(OS + OSE)÷2] intermediate, and CP giving the highest values. This trend was present even though the differences were not significant in some cases. This is readily apparent in Figures 2 and 3, and Tables 2 and 3. In the case of rosetting, there was no logical relationship with treatments and in fact no significant treatment F value was obtained. In the case of floret size, treatment F values were significant in both the 7-line and 20-line analyses, but in the 20-line analysis BP gave greater floret size than the average of OS and OSE.

OS and OSE did not differ in their effect on field characteristics, except in two cases noted below for plant weight and head diameter in the 20-line analysis.

A number of significant line x treatment interactions occured, the result of individual lines which did not fit the patterns predominating. There were more of these inconsistencies in the 20-line analysis than in the 7-line analysis. They were perhaps due to plant damage or soil and cultural variation, or, in the case of the CP plots, to genetic effects of the second parent used in all crosses, HS 118. In any case, expected differences in most lines were apparently large enough to offest such

Table 2. Pollination treatment effects on field performance of seven broccoli lines with complete data available.

	Treatment means[2]					t values for treatment comparisons[3]				Line x treatment
Variable	1	2	3	4	F	1 vs 2.3	2 vs 3	2,3 vs 4	1 vs 4	F value
Plant height (cm)	423.6	430.3	444.8	460.1	4.6**	1.506NS	1.353NS	2.436**	3.413**	0.9063NS
Plant weight (g)	969.8	983.9	1021.3	1205.1	10.5**	0.795NS	0.787NS	4.914**	4.944**	1.6888NS
Head emergence (days)	65.4	63.5	63.4	61.8	11.8**	3.635**	0.059NS	3.227**	5.943**	1.3706NS
Harvest date[1] (days)	80.25	79.16	79.09	79.10	1.6NS	2.10*	0.0697NS	0.0194NS	1.835*	2.72**
Head diameter[1] (cm)	164.8	174.2	173.7	188.3	6.8**	2.17*	0.183NS	3.41**	4.84**	2.99**
Head weight (g)	190.2	203.1	206.7	229.6	3.2*	1.313NS	0.285NS	2.203*	3.044**	2.74**
Floret size (score)	22.0	23.0	23.7	25.7	4.0*	1.439NS	0.615NS	2.447**	3.365**	1.0321NS
Rosetting (score)	22.7	23.6	23.8	21.1	2.1NS	0.893NS	0.178NS	2.506**	1.397NS	2.1771*

1. Used adjusted means derived from analyses of covarience
2. Treatments: 1-bud pollination (BP); 2-open flower self (OS); 3-open flower self ether aided (OSE) 4-crossed (CP)
3. $2,3 = \frac{\overline{X}2 + \overline{X}3}{2}$

NS = non-significant at 5% probability level
* = significant at 5% probability level
** = significant at 1% probability level

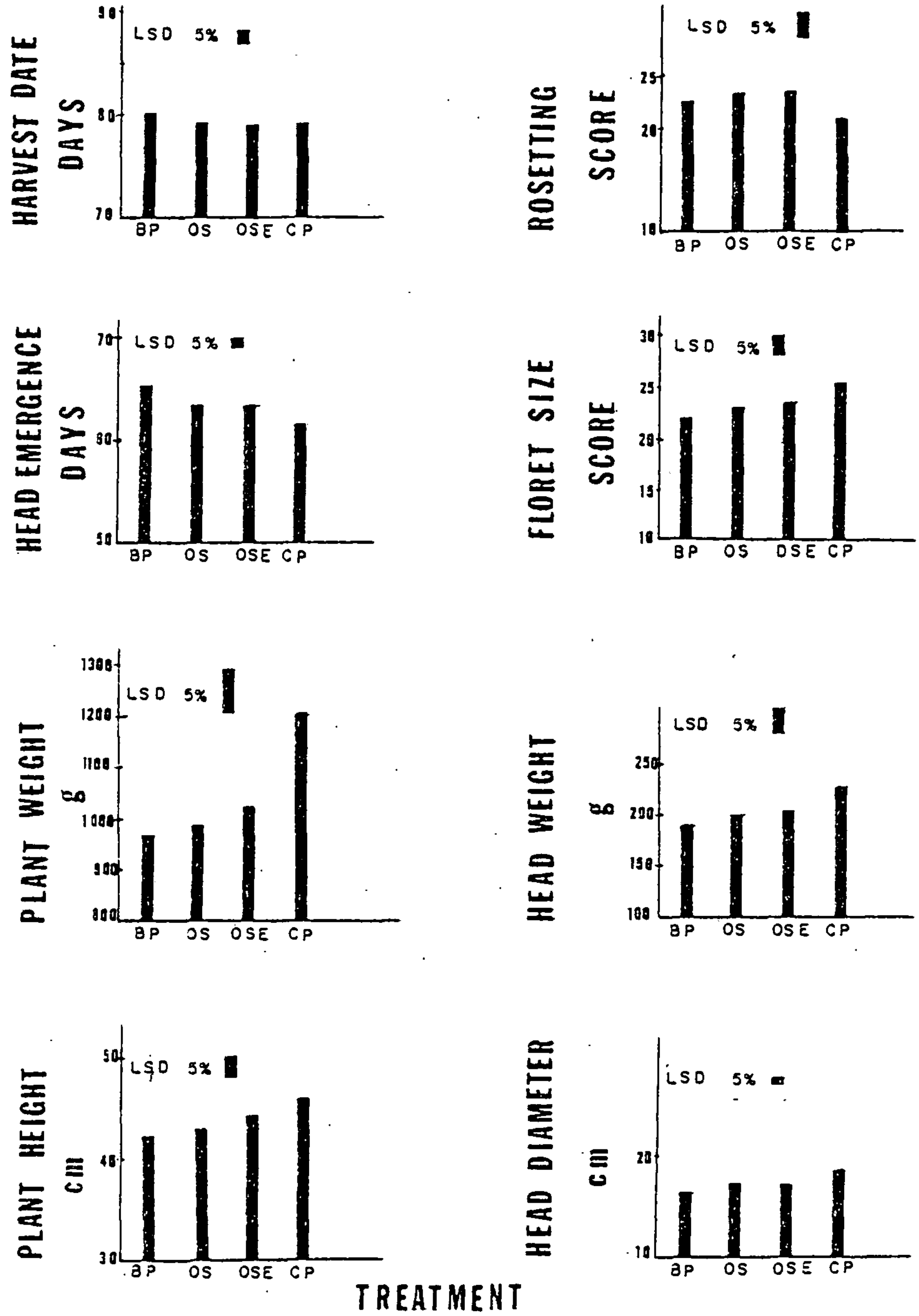

Figure 2. Pollination treatment effects on field performance of seven broccoli lines with complete data available. (BP-bud pollination, OS-open flower self, OSE-open flower self ether aided, CP-crossed)

Table 3. Pollination treatment effects on field performance of 20 broccoli lines, analyzed by unweighted means method.[1]

Variable	Treatment means[2]					t values for treatment comparisons[3]				Line x treatment
	1	2	3	4	F	1 vs 2,3	2 vs 3	2,3 vs 4	1 vs 4	F value
Plant height (cm)	392.0	397.0	398.2	437.4	23.1**	1.037NS	0.190NS	7.428**	7.338**	1.47**
Plant weight (g)	1176	1301	1204	1317	5.3**	2.054*	2.246*	2.054*	3.272**	1.74**
Head emergence (days)	67.72	66.35	66.42	63.11	27.4*	2.888**	0.132NS	7.144**	8.689**	1.34NS
Harvest (days)	83.09	82.39	81.77	80.01	11.9**	2.17*	1.146NS	4.417**	5.707**	1.80**
Head diameter (cm)	179.01	189.41	183.90	189.80	3.5**	2.292*	2.021*	0.941NS	3.960**	1.94**
Head weight (g)	221.24	243.94	240.93	248.84	2.3NS	2.178*	0.268NS	0.658NS	2.456**	2.00**
Floret size (score)	24.34	23.73	24.54	25.73	2.7*	0.329NS	1.141NS	38.936**	1.939*	1.32NS
Rosetting (score)	22.74	23.03	22.10	21.29	1.7NS	0.241NS	1.101NS	1.753*	1.727*	1.76**

1. Snedecor (37), observations adjusted for stand effect by analyses of covarience, except for plant height, floret size, and rosette score.
2. Treatments: 1-bud pollination (BP); 2-open flower self (OS); 3-open flower self ether aided (OSE); 4-crossed (CP)
3. $2,3 = \frac{\overline{X}2 + \overline{X}3}{2}$

NS = non-significant at 5% probability level
* = significant at 5% probability level
** = significant at 1% probability level

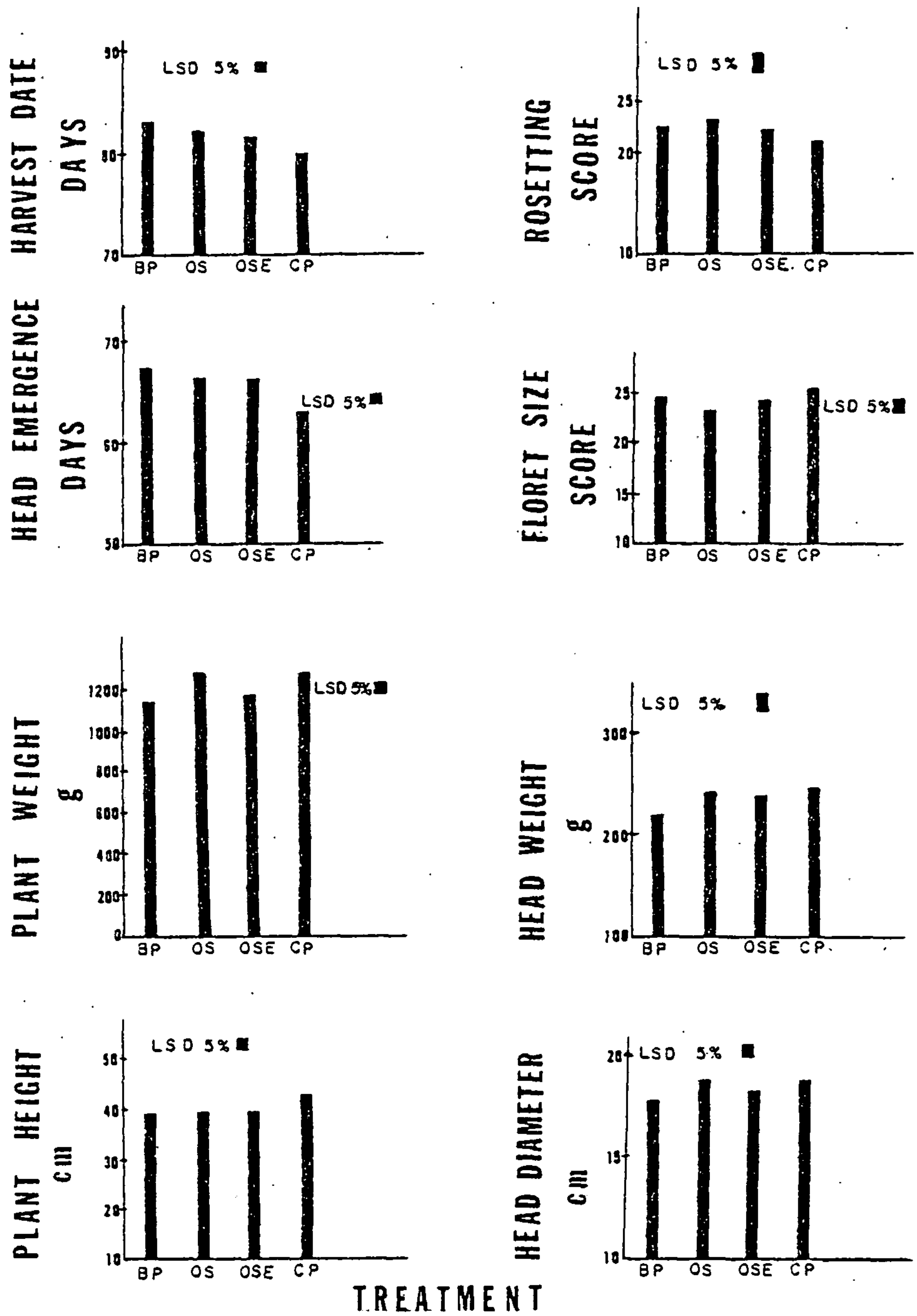

Figure 3. Pollination treatment effects on field performance of 20 broccoli lines, analyzed by unweighted means methods. (BP-bud pollination, OS-open flower self, OSE-open flower self ether aided, CP-crossed.)

variations, with the result that treatment F Values were mostly significant and the expected effect of vigor, as related to treatment, was found.

Based on the 5% significance level, the following additional details of the relationships were found.

Plant height - The treatment F value was significant for both the 7-line and 20-line analyses. The height values of both BP and OS-OSE were lower than those of crosses, but the height for BP and OS-OSE treatments did not differ.

Plant weight - The treatment F value was significant for both the 7-line and 20-line analyses. The weight for the crosses was higher than for both BP and OS-OSE. BP gave lower plant weight than OS-OSE for both analyses, but the difference was not significant to the seven lines.

Head emergence - The treatment F values were significant in both analyses. Increased vigor, in ascending order from BP to OS-OSE and CP, hastened plant development. BP resulted in later head emergence than OS-OSE and heads for OS and OSE emerged later than those crosses. There was no difference between OS and OSE.

Harvest date - The treatment F value was significant in the 20-line analysis but not in the 7-line analysis. In the 20-line analysis, BP resulted in later harvest date than OS-OSE, and OS-OSE were later than crosses. There was no difference between OS and OSE.

Head diameter - The treatment F value was significant in both the 7 and 20-line analyses. With both 7 and 20 lines, BP gave smaller heads than OS and OSE, and also lower than crosses. In the 20-line analysis, OS gave larger heads than OSE, one of only two cases where the effects of OS and OSE differed significantly. Head diameter for OS-OSE was less than for crosses in the 7-line analysis, but not in the 20-line analysis.

Head weight - The treatment F value was significant in the 7-line analysis, BP gave lower weight than CP but did not differ significantly from OS-OSE. When 20 lines were analyzed BP gave lower weight than either OS-OSE or crosses. OS and OSE did not differ in either analysis, and differed (gave lower weight) from CP only in the 7-line analysis.

Floret Size - The treatment F value was significant in both the 7 and 20-line analyses. BP and OS-OSE gave smaller floret size scores than crosses, but the effects of OS and OSE were not different, nor did BP differ from OS-OSE. Because floret size is closely related to maturity estimation it is possible that scores assigned were not biologically meaningful; i.e. it would be possible for slight misjudgment in maturity to result in scores which were too high or too low.

Rosetting - The treatment F values were non-significant in both the 7 and 20-line analyses. Although the rosetting score was lower in the crosses, as might be expected from observation of inbred lines compared to F_1 hybrids, the non-significant F values and lack a trend suggest there were essentially no treatment effects for this character.

GENERAL DISCUSSION

This study was designed to confirm or contradict experimentally a field observation that bud pollination (BP) resulted in progeny plants less vigorous and later in head development than plants of the same line produced from open flower selfing. Open flower produced seed involved in those observations was not from controlled pollinations, and it was necessary to differentiate open flower selfs (OS) from accidnetal greenhouse crosses by observing plant type and uniformity. This could usually be done, and crosses were recognizable by a very sizable increase in plant and head size and often earliness. The present study provided the controlled pollinations necessary to make more accurate comparisons and determine if BP truly does result in less vigorous plants than OS. The explanation for reduced vigor of plants derived from BP seed may be in the use of immature, small flower buds as compared to fully sized and mature flowers in the case of OS. The seeds resulting from BP might have less size or weight, but the data obtained did not indicate this was true (although CP seeds were larger than those from the other three treatments). There could have also been other differences, not measurable, such as reduced content of stored food reserves.

The use of a large number of more or less unrelated broccoli lines was prompted by the observation that the hypothesized BP effect appeared to be present in some lines and not in others. The approach was thus to involve a wide range of genotypes.

Generally, the hypotheis is that BP produced less vigorous plants, than OS, and that CP produced more vigorous plants than either of the types of selfing was confirmed, though the differences were often small and sometimes statistically non-significant. Floret size and rosetting appeared to fit a trend less than the characteristics more directly related to vigor.

One of the reasons that treatment differences were often small, was the occurrence of a certain number of individual lines with clearly contradictory behavior, i.e. CP derived plants were not larger, or earlier, or produced larger heads than BP or OS plants. While there were also cases where BP plants unexpectedly expressed more vigor than OS or OSE plants, the most conspicuous discrepancies were in CP plants with less than expected vigor. Lines in which this occurred, offset the rather distinct differences which occured with most lines, reduced the treatment difference, and resulted statistically in line x treatment reactions. Some of these contradictory results could have been due to plant damage and random environmental variations such as irrigation patterns. However, it is possible that some of the experimental lines used did not combine well with the common parent used in all crosses, HS 118, and the resulting F_1 hybrids were actually less vigorous than the respective inbred line. While F_1 hybrids are usually more vigorous than either parent, this may not always be true. Since HS 118 is not a very large and vigorous line, it may have reduced the size of some of its hybrids, when they were compared to the second parent. A reduction in head diameter would not be surprising, since HS 118, when used in experimental combinations with certain club resistent inbreds, produces F_1 hybrids with taller plants but smaller heads than the second parent. In the planning of the study, the possibility of specific combining ability effects were overlooked because of the general expectation for heterosis in the crosses.

Of the plant characters measured, plant height, plant weight, and head emergence are possibly most suited for objective measurement. Harvest date, head diameter, and head weight are subjective in that it is necessary to make a judgment of typical or acceptable commercial maturity. These three characters are closely interrelated. Since the lines differ basically in floret size, and also differ in shape and tendency to open rapidly, determination of comparable harvest stages is difficult. In both the 7-line and 20-line analyses, the line x treatment interaction was greater for harvest date than for head emergence date, probably because of greater error in maturity determinations.

The use of a large number of broccoli lines in this study was a disadvantage because it did not permit the concentration of effort needed to obtain sufficient seeds per line. A smaller number of lines with seeds enough to obtain a good stand and a complete experimental design would have yielded more information. The statistical analysis of the seven lines with complete data was more direct and simple and possibly more reliable than the larger analysis with 13 additional lines with missing plots. Stand variations introduced error, and it is likely the analyses of covariance did not fully correct them.

SUMMARY AND CONCLUSIONS

1. BP resulted in a greater number of seed set per cluster than OS-OSE, and slightly less than CP.

2. CP produced larger seeds than BP, OS or OSE, but the latter three treatments did not differ.

3. Broccoli lines differed greatly in both size and number of seeds produced.

4. The use of ether as an aid in open flower pollination generally had no effect on seed production or the field characteristics of resulting progenies.

5. In the field grown progenies, the quantitative characteristics, plant height, plant weight, and head diameter, were greater for CP, intermediate for OS-OSE, and lowest for BP. Head weight was similarly, but less conclusively affected. Thus, BP produced plants of lower vigor than those from OS-OSE or CP.

6. Number of days to head emergence was longer in progenies from BP than from OS-OSE, and those from OS-OSE took longer than progenies from CP; this was also an expression of relative vigor. Number of days to harvest maturity followed the same pattern in the analysis of 20 lines but was inconclusive in the analysis of 7 lines. Days to maturity is less accurately determined and more subjectively influenced than days to head emergence.

7. Results for floret size and rosetting were generally inconclusive. These characteristics are probably not sufficiently related to plant vigor to be useful in a study of this nature.

8. The experiment generally supported the hypothesis that BP results in progenies with less plant vigor than those from flowers pollinated after normal anthesis (OS).

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