CAUSE AND MODE OF INHERITANCE OF MALE STERILITY IN FOUR MUTANTS OF TOMATO (LYCOPERSICON ESCULENTUM MILL.)

DISSERTATION

Presented in Partial Fulfillment of the Requirements
for the Degree Doctor of Philosophy in the
Graduate School of The Ohio State
University

By

WARREN ROBERT HENDERSON, B. S., A. M.

*****

The Ohio State University
1959

Approved by

Adviser
Department of Horticulture
and Forestry

ACKNOWLEDGMENTS

The writer gratefully acknowledges the aid and advice of Dr. Freeman S. Howlett, Chairman of the Department of Horticulture, who served as advisor for this study. Sincere thanks are due Dr. Walter N. Brown of the Department of Horticulture for his able suggestions and guidance. He also wishes to thank Dr. E. Kenneth Alban and Dr. Fred O. Hartman of the Department of Horticulture; Dr. Bernard S. Meyer, Dr. Glenn W. Blaydes, and Dr. Richard H. Bohning of the Department of Botany for their helpful suggestions. The field and greenhouse space provided by the Department of Horticulture are gratefully acknowledged.

CONTENTS

CONTENTS (continued)

TABLES

TABLES (contd.)

TABLES (contd.)

TABLES (contd.)

ILLUSTRATIONS

ILLUSTRATIONS (contd.)

INTRODUCTION

In recent years there have been numerous reports of male sterility occurring in vegetable crops. Allard (1) reported finding a male-sterile mutant in the lima bean; Jasmin (21) in eggplant; Owen (30) in sugar beets; Welch and Grimball (47) in carrots; and Jones and Emsweller (22) in the onion. In addition male-sterile mutants have been studied in the cucurbits by Scott and Riner (44) working with winter squash; Shifriss (45) with summer squash; Gabelman (11), after Peterson, with Oriental cucumber; and Bohn and Whitaker (4) with muskmelon.

The first male-sterile mutant in the tomato was reported by Crane (9) in 1915. Nearly twenty-five years elapsed until the second male-sterile tomato mutant was reported (Lesley and Lesley, 27). However, since 1942 when Barrons and Lucas (2) first suggested the possible use of male sterility to reduce the cost of hybrid tomato seed, numerous and various types of mutants have been studied: Bishop (3), Hafen and Stevenson (15, 18), Larson and Paur (25), Rick (35, 37, 39, 40), and Rick and Robinson (43). Although 24 pollen sterile mutants and 12 morphological mutant types have been cited in the literature, Lesley and Lesley (29) in 1958 stated that only about 5 per cent of the tomato crop was grown from hybrid seed. The percentage of this hybrid seed produced by the use of male-sterile mutants was not given. The fact remains that a very low percentage of the tomato crop was hybrid, indicating that the ideal mutant is not yet available.

It is quite likely that increased use of hybrid tomato seed will be realized in the future, especially as improved male-sterile mutants become available and their use perfected. Notwithstanding these rather disappointing results with male-sterile mutants in the production of hybrid tomato seed, these studies have contributed to our knowledge of the tomato. For example, Rick (36, 38) and Soost and Rick (46) assessed the rate of natural cross-pollination in the tomato by use of male-sterile mutants. In order to induce mutation and possibly obtain new, desirable, male-sterile mutants, chemical treatment was tried by Rhem (33) and Hafen and Stevenson (14) and seed treatment with radioactive phosphorous (P_{32}) by Lesley and Lesley (29). Butler (8) showed that fertility may be restored when the mutant is transferred to a different genetic background, thus necessitating adequate genetic testing. Similarily, instability in certain environments, resulting in partial or complete restoration of fertility, was reported by Bishop (3), Rick and Robinson (43), and Bullard and Stevenson (6). These latter findings could lead to interesting studies outside the fields of plant breeding and genetics.

Inheritance studies by Rick and Robinson (43) with mutants producing abnormalities in floral structure indicated a multiple effect of certain genes on floral morphology and to a lesser extent on other organs. Genetically these multiple effects could be explained by extremely close linkage, a chromosome deletion, or a pleiotropic effect of the gene responsible. Since no crossover types were observed, the

former would be unlikely. These same studies added valuable information to the literature concerning the mechanism of self-pollination in the tomato as influenced by flower structure.

Finally, of course, the crossing operation with tomatoes has been simplified, and perhaps of greater importance, the amount of contamination from unwanted self-pollination has been reduced. In addition to these benefits to the breeder, additional mutants provide the geneticists with new genes for linkage studies. Although a large number of genes are available, new mutations are still being induced for the primary purpose of expanding the linkage map. According to the latest report of the Tomato Genetics Cooperative, (1958), 4 of the 12 chromosomes still possess no distinct established linkage groups.

Unfruitfulness in the tomato can most easily be recognized late in the growing season (Rick, 34). Such plants are generally two to two and one-half times the size of fruitful neighbors in both height and over-all size. They are also more resistant to early frost and generally do not suffer so drastically from drought, because of their extensive root system.

Four mutants possessing floral defects were found by this means and selected for this study. Each mutant was found in a different variety: mutant bn in Purdue F_2; mutant cl_3 in Red Jacket; and mutant sl(?) in the new variety, Ohio W-R Jubilee. A fourth mutant which appeared very similar to ps and consequently designated ps?, will also be discussed to a limited extent. It was found as a segregate in Big Early Hybrid.

As previously indicated, not only are male-sterile mutants potentially useful in hybrid seed production, but have proved their value in other studies. Therefore, information that can be obtained concerning these mutants might prove beneficial in breeding and genetic studies.

The objectives of this study were basically four in number: (1) To make a genetic study of each mutant: a) to determine the mode of inheritance; b) to establish or disprove a linkage relationship with c (potato leaf) and/or u (uniform unripe fruit color; and c) to conduct an allelism test among the four male-sterile mutants to test their identity. (2) To obtain a detailed description and an account of the phenotypic stability of each mutant as expressed in both field and greenhouse tests. (Objective #2 was of special importance with certain genes, in that multiple effects [or close linkage] were observed.) (3) To determine the cause of unfruitfulness, by studying the relationship between floral abnormality and unfruitfulness. (4) To evaluate each mutant from the standpoint of its use in hybrid seed production and/or its adaptability to other studies, as a result of a unique character possessed by the mutant.

REVIEW OF LITERATURE

A survey of tomato fields by Rick (35) disclosed that of 55,000 plants examined, unfruitfulness due to cytogenetic causes occurred in 0.1 per cent of the plants. Heteroploids were in the vast majority, constituting 80 per cent of the unfruitful plants. Of these heteroploids, approximately 90 per cent were triploids. Diploid mutants therefore constituted 20 per cent of the unfruitful plants, or 1-5000 (0.02%) of all plants. Three types of diploids were found: (1) completely sterile plants; (2) pollen sterile mutants; and (3) mutants with gross floral abnormalities. Since this study of Rick's consisted of three varieties, with the number of unfruitful plants distributed quite uniformly between the varieties (as were the various types of cytogenetic causes), Rick (35) was led to conclude that this probably represented the rate of unfruitfulness due to cytogenetic causes in most varieties of tomato.

The term male sterility has come to mean different things to different workers. This paper will follow rather closely that used by Gabelman (11). Male sterility according to his usage may mean any one of three types of inherited abnormality:

1. Pollen sterility. Mutants deviate almost exclusively by the absence or extreme rarity of functional pollen.

2. Staminal sterility. Stamens are usually extremely malformed or completely absent, and the effect not necessarily limited to the androecium.

3. Functional pollen sterility. A barrier present which prohibits pollination.

(a) Pollen dehiscence prevented

(b) Stigma exerted (added by this writer) recognizing the semi-sterile nature of this barrier.

Staminal and functional pollen sterility may at times by referred to by this writer as morphological sterility when a distinction between the two is not possible or necessary.

Male-Sterile Mutants in the Tomato

Utilizing the previous classification, male-sterile mutants in the tomato will be described briefly under the proper heading with the mode of inheritance indicated.

Pollen Sterility

As a means of avoiding confusion at the outset, it might be wise to state that no evidence of cytoplasmic control of male sterility in the tomato has yet been reported or suspected. The first reported study of the inheritance of pollen sterility in the tomato, by Lesley and Lesley (27), indicated control by two recessive genes. Since then Rick (35, 37, 39, 40) has reported 20 non-allelic mutants (ms1-18, 23 and 24) and Hafen and Stevenson (15) four non-allelic mutants (ms19-22), all of which were controlled by a single recessive gene. These were by no means confined to one variety but were represented by 11 different varieties. Rick (35) indicated that it should be possible to obtain

pollen sterile mutants in any desired variety rather than resorting to the time consuming operation of backcrossing. All mutants studied by Rick (37) showed a smaller anther size (diameter x length). The anthers and corolla were usually paler in color than those of the fertile flowers. The difference in external appearance was sufficiently great in some mutants ($\underline{ms}_5$ or $\underline{ms}_{10}$) for identification by this means but would prove highly inaccurate if applied to others ($\underline{ms}_6$ or $\underline{ms}_9$). For positive identification of these near normal types, an examination of the anther content would accurately separate the fertile from the sterile flowers. It was hoped that the gene might produce other phenotypic effects prior to flowering that would be useful for early identification, but none were discernible by Rick in this study (37). An interesting and practical aspect of this paper was the precise time of action of each of the mutants. Breakdown usually occurred during meiosis but the actual time of breakdown was quite characteristic of the mutant, occurring generally from slightly prior to meiosis ($\underline{ms}_3$) to late microspore stage ($\underline{ms}_{13}$). It generally followed that the later the time of breakdown the more normal the external appearance of the anther. Gabelman (11) stated that the time of breakdown could prove important in the selection of a mutant for hybrid seed production. Since the later the time at which breakdown occurred, the greater would be the chance of viable pollen production resulting in unwanted self-pollination. A high rate of ovule abortion occasionally was associated with pollen sterility.

Staminal Sterility

Until the report of Bishop (3) in 1954, no stamenless mutant that had normal female fertility had been reported in the literature. This mutant was controlled by a single recessive gene and designated as sl. The mutant was described by Bishop as having only a vestigial stamen development which, under most conditions, produced no stamens or pollen. The vestigial anthers and/or filaments tended to adhere to the pistil which produced fruits that had rough radial lines at the point of attachment of the vestigial "stamens." Under greenhouse conditions during the winter, this mutant produced a few abnormally shaped stamens which contained viable pollen. The conditions that caused stamen development were not known. Such pollen could be used to advantage in the propagation of the mutant. Differing from many of the pollen sterile mutants, the stigma was readily accessible for pollination. It should be stressed that this mutant rarely produced stamens, so that contamination would be highly unlikely, especially if crossing were not done during the time of partial stamen formation. No other abnormality of the plant was noted beyond those cited above. Use of sl in the production of F_1 hybrid seed may have value according to Bishop (3).

Hafen and Stevenson (15) described a stamenless mutant with a sepal-like corolla, designated as cs. This mutant, in addition to being male-sterile, was also highly female-sterile. The stamen, corolla, and gynoecium abnormalities were always associated. A single recessive gene was found to account for the inheritance of cs. A second stamenless mutant (sl_2) was also described, in which only the

androecium was affected. A single recessive gene determined the mode of inheritance.

Hafen and Stevenson (18) have secured two additional mutants, $\underline{sl}_3$ and $\underline{sl}_4$. In the latest report of the Tomato Genetics Cooperative (1958), the phenotypes and allelic relationship of these two mutants ($\underline{sl}_3$, $\underline{sl}_4$), $\underline{sl}_2$, and Bishop's $\underline{sl}$ were compared. A fifth mutant called $\underline{sl}_5$ was also used. The reason for separation was the similarity between $\underline{sl}_5$ and $\underline{cs}$; in fact, the descriptions appeared identical, and will be assumed to be identical, although not so stated. Therefore, this report discussed $\underline{sl}$, $\underline{sl}_2$, $\underline{sl}_3$, $\underline{sl}_4$, and $\underline{sl}_5$. A brief description follows for the benefit of comparison under similar growing conditions; $\underline{sl}$ was stamenless, female-fertile, and produced yellow petals; $\underline{sl}_2$ was similar to $\underline{sl}$ but produced a small quantity of viable pollen; $\underline{sl}_3$ showed slightly greater anther and pollen development than $\underline{sl}_2$; $\underline{sl}_4$ was completely normal in the greenhouse during the winter (especially older plants); and anther production in $\underline{sl}_5$ was nearly nil, thus closely resembling $\underline{sl}$, but differed from $\underline{sl}$ by being highly female-sterile, and by possessing a sepal-like corolla.

As a result of allelism tests, the conclusion was drawn that these mutants were not distinct from one another but probably constituted an allelic series. Inconsistencies arose when, in place of sterile individuals expected if allelic, the plants were intermediate (incompletely fertile (IF). Selfing these IF plants, however, produced a 2:1:1 ratio (IF:S:S), indicating allelism. An IF phenotype resulted

when a plant was heterozygous for any two of the male-sterile factors. Therefore, rather than five distinct mutants all belonged to the same locus.

Rick and Robinson (43) found a stamenless mutant pi during their examination of mutants with gross floral defects. It would be interesting to know if this is distinct or allelic with the other mutants discussed above. No functional anthers were found, though rudimentary structures were present which adhered to the pistil as in sl. The pistil was malformed (bent, twisted) and the gynoecium was defective. (See later section for further vegetative pleiotropic effects.) Again one recessive gene would explain the inheritance of pi. This same paper described another mutant, vg. All floral parts excepting the calyx were modified to the extreme. When a stamen was present some adnation with the pistil occurred but to a lesser degree than in pi. A single gene (recessive) determined the genetic expression of this mutant. Therefore, all stamenless tomato mutants were inherited in a simple Mendelian manner.

Functional Pollen Sterility

a) Pollen dehiscence prevented

One of the first morphological mutants which looked promising for hybrid seed production was studied by Larson and Paur (25). The cause of sterility of this mutant (ps) appeared to result from a failure of the stromia to rupture. Fortunately identification of this mutant could be accomplished by a macroscopic examination of the flower as a

result of an association with a cleistogamous floral condition. The petals did not unfurl but remained locked together as the "seams of a tin can." Petals appeared to be fully developed and normal in size. Pollen was viable and thus ps could be propagated sexually. A single recessive gene accounted for the mode of inheritance of the mutant ps. Pollack (31) reported an exerted stigma in ps, controlled by more than one gene. This characteristic would be extremely useful, since emasculation was usually necessary with ps in order to expose the stigma for pollination.

Rick and Robinson (43) added another indehiscent mutant with normal ovule fertility which could be identified by the partially open petals at full bloom. Because of this cleistogamous condition, the symbol cl_2 was assigned. Indehiscence evidently was not perfect because occasionally a small proportion of selfed seed could be found.

b) Stigma exerted

Currence (10) in 1944 was the first to report a mutant of this type in which the mode of inheritance was determined. This mutant was dependent upon two recessive genes. Another mutant designated as ex was described by Rick and Robinson (33). Mutant ex differed from the mutant reported by Currence by an occasional flower with an inserted stigma, the style of which was no shorter than those which were exerted, but was twisted and bent. In addition, the ovule was also defective. A single recessive gene would explain the inheritance of the mutant ex.

Phenotypic Stability

To the breeder this is an extremely important subject, whether he be concerned chiefly with hybrid seed production or with genetic and breeding studies. The importance of penetrance and the effect of modifying genes upon phenotypic stability was stressed by Butler (8). The pollen sterile mutants $\underline{ms}_1$, $\underline{ms}_2$, and $\underline{ms}_5$ were all found in the variety San Marzano. Fertility was restored when incorporated in a different genetic background. Hafen and Stevenson (17) observed that fertility was partially restored in the mutants $\underline{ms}_{19}$, $\underline{ms}_{21}$, $\underline{ms}$ 22, and $\underline{sl}_2$ when carried along from year to year by backcrossing. Since the mutants $\underline{ms}_{19}$, $\underline{ms}_{21}$, $\underline{ms}_{22}$, and $\underline{sl}_2$ were originally highly sterile, these authors indicated that modifying genes were probably supplied by the male-fertile parent during backcrossing.

Environmental factors have also been suspected of contributing to the breakdown of the sterility mechanism in some mutants. Bullard and Stevenson (6), using $\underline{ps}$ to measure the rate of natural cross-pollination in Indiana, reported that a high percentage (50%) of the seed produced resulted from self-pollination. These same authors stated that $\underline{ms}_{16}$ was suspected of producing viable pollen under widely different environments. Both Bishop (3) and Hafen and Stevenson (14) found greater stamen production in certain stamenless mutants under winter conditions in the greenhouse.

Pleiotropism

The term pleiotropism was coined in 1938 by Grunberg (12). This term arose from studies with the rat (Mus norvegicus) in which he stated, "the number of genes controlling development is limited therefore it follows many, perhaps most, genes must not effect only one organ or character but several at a time. Their effect is manifold and the term pleiotropism has been coined to cover this diversity of actions of a single gene." The use of the term in this paper will follow this definition, but will not imply a physiological relationship between the several associated phenotypic characteristics.

Numerous examples have already been cited in the tomato in which the sterility factor has been associated with other floral changes: Larson and Paur (25), Bishop (3), Hafen and Stevenson (15), Rick (37), and Rick and Robinson (43). Less frequent, however, is an association of sterility with an accompanying vegetative change.

Rick and Robinson (43) cited the only examples of such association in the tomato. The mutant pi produced an inflorescence very similar to lf. On the basis of plant habit, pi plants could usually be distinguished from a distance, exhibiting a denser and bushier appearance than fertile segregates. A change in leaf shape and plant habit was associated with cleistogamy in cl_2. Internode length was reduced; branching was more sparse; and the leaves rolled ventrally and usually were darker green than normal. These two mutants reported by Rick and Robinson were the only male-sterile mutants in the tomato accompanied by extensive modification to the vegetative plant parts (pleiotropism).

Although these associations have been referred to as pleiotropy, it should be indicated that this is not the only possible explanation. Closely linked genes inherited as a unit or a chromosome deletion including the genes affecting these characters could also account for this situation.

Lesley and Lesley (28) pointed out that, "pleiotropism has a practical significance to the plant breeder since it is probably desirable to know as much as possible about a mutant gene. A desirable character may be only one effect of the gene, other undesirable effects also produced by the same gene."

Hybrid Tomato Seed

Rick and Butler (42) stated that over forty different papers report yields of tomato hybrids exceeding that of the parents. Larson and Currence (24) also stated that the F_1 mean yield was 39 per cent above that of the parents and the F_2, 23 per cent higher.

Larson (23), using ms from the variety Earliana, reported no reduction in combining ability as a result of male sterility. Hafen and Stevenson (16), in an effort to check the effect on combining ability of male-sterile mutants, used ms_{19}, ms_{20}, ms_{21}, ms_{22}, and sl_2 from the varieties Rutgers and Garden state. This work supported the conclusions of Larson.

As early as 1912 Wellington (48) suggested utilizing hybrid vigor in the tomato. He stated that proper selection of parents was of prime importance, indicating he was aware of differences in

parental combining ability. High labor cost was also recognized by Wellington as a limiting factor in hybrid production. For this reason, perhaps, it was not until 1936 that interest was revived in the field by Hadfield and Calder (13), who stressed the importance once again of the proper selection of the parents, and discussed improvement in technique. The paper of Barrons and Lucas (2), however, was the stimulant that was needed. Recognizing as did others the limitations due to cost, they devised techniques in crossing by which cost could be reduced, at least to a limited extent. Their more important contribution to this field, however, was the suggestion that male-sterile mutants might be of service in further reducing the cost of hybrid seed.

What are those qualities of a male-sterile mutant which a breeder would seek? Rick (35) suggested using pollen sterile mutants. Since they can be found quite readily, he also suggested seeking such mutants in the variety to be used and thus avoid the time-consuming operation of transferring the mutant gene. Larson and Paur (25) differed from Rick, indicating a preference for a mutant of the stamenless or functional type which could be propagated by selfing, thus eliminating carrying it either in the heterozygous condition or by vegetative means. These same workers suggested that a highly desirable mutant be selected and then transferred to the variety to be used. Although additional time would be required, this was justified according to them if the mutant possessed favorable characteristics of a high order. A plan for transferring in four rather than five years was presented.

Since the elimination of emasculation was one of the primary reasons for using male-sterile mutants, the stamenless mutants possessed considerable merit (Bishop, 3). Types such as the pollen sterile mutants would require an exerted stigma to be of any great value unless the anther column were sufficiently distorted to allow ready access to the stigma; the same drawback would apply to the functional type which failed to dehisce (ps and cl_2). Seed production when using male-sterile mutants as the female parent must be high, thus many mutants could be eliminated in which the gynoecium was defective. The mutant should be phenotypically stable over a wide range of environmental conditions (Gabelman, 11). To avoid contamination, Currence (10) suggested the use of seedling markers such as cc (potato leaf) or gg (green stem) for identification of contaminants. A more desirable situation would be a pleiotropic male-sterile gene affecting a seedling character, such as the mutant cited by Allard (1) in the lima bean.

Rick (36, 38) and Soost and Rick (46) have investigated factors which might affect the rate of natural cross-pollination in the tomato. They concluded from these studies that the three main factors which affected the rate of natural cross-pollination were: planting design, locality (insect activity), and variety. From these studies much interest has been aroused concerning the production of F_1 hybrid seed by this method of pollination. Rick's (33) work showed promising results with the pollen sterile mutants in California. Using four pollen sterile and one stamenless mutant, Hafen and Stevenson (17) reported discouraging results. Similarly, Bullard and Stevenson (6)

with $\underline{ms}_{16}$ and $\underline{ps}$ also experienced poor seed yields and considerable self-pollination under Indiana conditions. Bullard and Stevenson did not discount the potential value of natural cross-pollination in the production of hybrid tomato seed. They suggested the use of a more reliable functional mutant under Indiana conditions and one that possessed an exerted stigma. The importance of stigma exertion as a determining factor affecting the rate of natural cross-pollination was also indicated by Lesley (26) in 1924.

Although differences in opinion existed among workers with respect to the method of pollination, and the most favorable type of mutant to be used, there was general agreement that a substantial reduction in the cost of hybrid tomato seed could be realized by utilizing male-sterile mutants. However, as indicated by Lesley and Lesley (29) in 1958, the search continues for improved male-sterile mutants and methods by which they can be obtained.

MATERIALS AND METHODS

Male-sterile mutants bn, cl_3, and ps? used in this study were found by Dr. W. N. Brown; bn and cl_3 at the Northwestern substation; and ps? at the vegetable crops substation at Marietta. The author also obtained sl(?) at the Northwestern substation, at which time a survey of the experimental plots was made to uncover new male-sterile mutants.

Cuttings were taken from these male-sterile plants, rooted, and grown at the Horticultural Greenhouses of The Ohio State University in Columbus, Ohio. The male-sterile mutants functioned as the female parent in the parental crosses made at the Ohio State University Horticultural Farm, Columbus, Ohio. F_1 plants were greenhouse grown during late winter and early spring. At this time backcrosses were made by utilizing these F_1 plants. The F_1 plants were also used in conducting an allelism test involving all four mutants. The seed was handled on an individual fruit basis. Rick (37) used the term "family" to designate those plants which came from the same fruit, which also will be followed in this paper. Generally, between 30-50 plants constituted a family in this study, excepting the backcross generation which was somewhat smaller.

Briefly the cultural practices were as follows: Greenhouse plants were raised in four-inch or six-inch pots, staked, tied, and trained to a single stem. A 15-30-15 fertilizer was applied periodically. The interval was dictated by plant size and growing conditions. All plants constituting the F_2, backcross, and allelism population were

field grown at the University Horticultural Farm at Columbus, excepting the F_2 and backcross progeny of sl(?). The latter were greenhouse grown in the spring of 1958 in order that inheritance data could be made available at this writing.

Field preparation followed the usual procedure. Five hundred pounds of a 10-10-10 fertilizer were broadcast per acre prior to plowing. Subsequent fertilizer applications consisted only of a 10-52-17 starter solution applied at the time of transplanting. No side dressing was applied. The soil type was a Miami Silt Loam. Neither staking nor pruning was practiced at either the 3'x5' 1956 spacing nor the 2'x5' 1957 planting distance. All progeny were transplanted to the field on August 2 in 1956, and July 10 in 1957. There were two exceptions with respect to the 1957 planting. The backcross population of bn and approximately half the allelism families were field set on August 28.

In addition to the large F_2 populations of bn, cl_3, and ps? grown in the field, a limited number were subjected to greenhouse conditions during the winter of 1957-58 and the spring of 1958. From these plants additional inheritance data could be secured. Also the phenotypic stability of the mutants could be better assessed and additional evidence could be acquired to lend support or question the pleiotropic effect of the male-sterile mutants.

F_2 fertile and sterile segregates of each mutant were utilized to assess their fruitfulness. Pedicels and fruits on each of four fertile and four sterile plants of the mutants bn and cl_3 were counted and two fertile and two sterile plants of ps?. These counts

were made the latter part of September, 1957. Only the first cluster was counted with s1(?), using 28 fertile and 30 sterile plants. These counts with s1(?) were made in the greenhouse during the middle of June, 1958. Further data with s1(?) should be obtained to determine fruitfulness under field conditions.

Hypothesizing that a single recessive gene conditioned each of the male-sterile mutants, a goodness of fit chi-square test of each mutant to a 3:1 F_2 ratio and a 1:1 backcross ratio was computed. The complete chi-square analysis measured the conformity of each family chi-square to the above ratios. In addition, by subtracting the pooled chi-square from the total of these individual family chi-square values, the degree of heterogeneity of each family chi-square from the pooled may be assessed. Such an analysis was not limited to the F_2 population as a unit. The F_2 population was subdivided and analyzed on the basis of the year tested, location, and the male-fertile parent used in the cross.

Introduction of the genes cc (potato leaf) and uu (uniform unripe fruit color) in crosses with bn, $c1_3$, and s1(?) permitted formulating and testing the hypothesis that each mutant assorted independently of c and independently of u. A goodness of fit test to a 9:3:3:1 F_2 ratio and to a 1:1:1:1: backcross ratio similarly was computed. A complete chi-square analysis was again made to measure the degree of heterogeneity of each family from the pooled data.

The following section will cover each mutant individually in that the materials or the methods used were rather specific for each mutant.

Mutant bn

Description

This mutant was first described by Henderson and Brown (19), at which time the symbol bn was proposed. This gene affected the plant in several ways, and was not limited to either the vegetative or the reproductive parts. Although all floral parts were modified to a degree, by far the greater change had occurred in the androecium to the extent of producing pistillate flowers (Figures 1 and 3). Figure 2, in which the corolla has been removed, reveals the range in stamen, number, and morphology most often encountered in flowers of bn plants. Some viable pollen in all probability could be obtained from stamens which were no more modified than those present in the two flowers at the bottom of Figure 2. This past spring (1958), flowers with three or four functional stamens occasionally were found. This latter situation was extremely rare in this mutant. Adnation of the stamen(s) to the pistil (Figure 2) produced radial scars on the fruit extending partially or completely from the stem end to the blossom end, as depicted in Figure 4. Mutant bn would thus be categorized as a stamenless male-sterile mutant according to the classification used in the Review of Literature.

Changes in the gynoecium are also revealed in Figures 1 and 2. The style was often bent, twisted, and grooved. A capitate stigma seldom was found in the mutant. Frequently the central area of the stigma was rough and depressed, the margins lobed or incised, which resulted in a reduced stigmatic surface. Fruit set was low and few seeds were produced when viable pollen was used from other fruitful plants. Thus the gynoecium was also defective. The abnormalities cited above, perhaps in part at least, contributed to this deficiency.

To a lesser extent, the corolla and calyx were affected by bn. The segments of both the corolla and calyx appeared somewhat more broad, and the sepals more blunt than in Bn. From a distance the flowers were less noticeable, partly as a result of the absence of the staminal column, and also as a result of differences in the relative size of the corolla and calyx.

Vegetative parts were by no means left unaltered when bn was present. The effect upon leaf form as represented in Figure 5 was typical of bn. There were fewer secondary leaflets than in the fertile plants. A ventral rolling of the leaf margin was found to occur more frequently and to a greater degree. The leaflets were obovate in size, the margins were nearly entire, and the apex was more blunt. Generally, a somewhat darker green color was evident. The overall appearance given to the leaf was a shorter, broader, and blunter leaf form than in fertile plants. From the latter feature the symbol bn was derived. A suitable cross was made to observe the phenotype of a

plant with both bn and c, and to determine segregation in the F_2. With a little experience four phenotypic classes could be distinguished. Plants homozygous for genes bn and c produced leaves similar to the specimen to the right in Figure 6. They were somewhat intermediate in form, usually lacked the basal lobes generally found in potato leaf, were more blunt, and thus more closely resembled bn plants.

Another most striking characteristic associated with bn was a distinct delay in flowering, attributed to a marked increase in the number of nodes preceding the first cluster. Although variation in node number did occur, the difference in node number between Bn and bn plants was of sufficient magnitude to utilize this feature as an additional criterion for classification. Subsequent node numbers between flower clusters of bn deviated in the same manner but were not as dependable.

A tendency toward the production of indeterminate flower clusters (Figure 7) commonly occurred, but the expression of this character has been variable (Figure 8). Usually the inflorescences in bn plants were found opposite the nodes as shown in Figures 7 and 8.

Methods

Male-fertile varieties used in the 1956 inheritance studies included Heinz Marketer, Marietta #1, Purdue F_2, Urbana, and WR-3. The variety Red Jacket was the sole male-fertile variety used in the 1957 field and 1957-58 greenhouse studies. The mutant bn served as the female parent in all crosses. The F_1 plants were observed

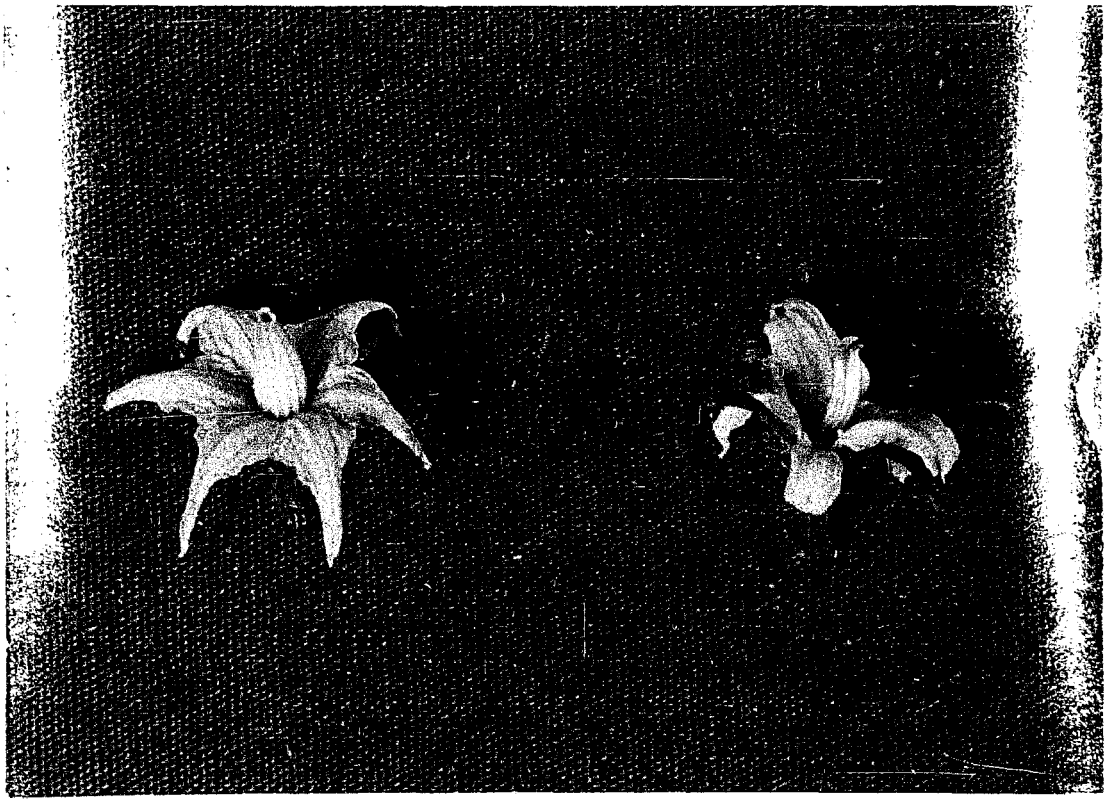


Figure 1. Flower from Bn plant on left and flower from mutant bn plant on right. X2½

Figure 2. Flower from Bn plant in center, flowers from mutant bn plant: upper left-- stamenless; upper right - a petaloid stamen adnate to the pistil; lower left - two stamens adnate to the pistil; lower right - a near normal stamen. All flowers shown with corolla removed. X1½

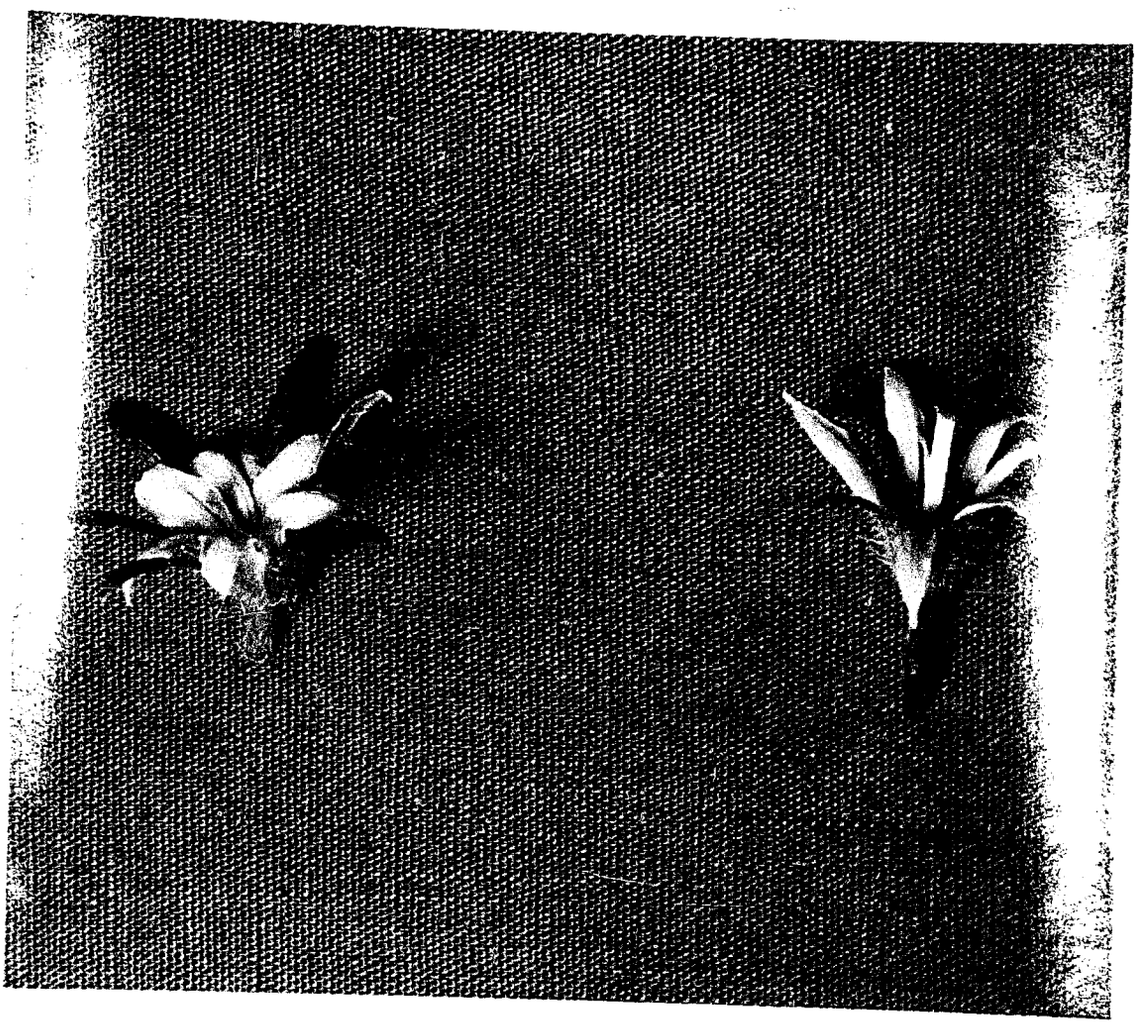


Figure 3. Flower from mutant bn plant on left and flower from mutant sl(?) plant on right. X2½

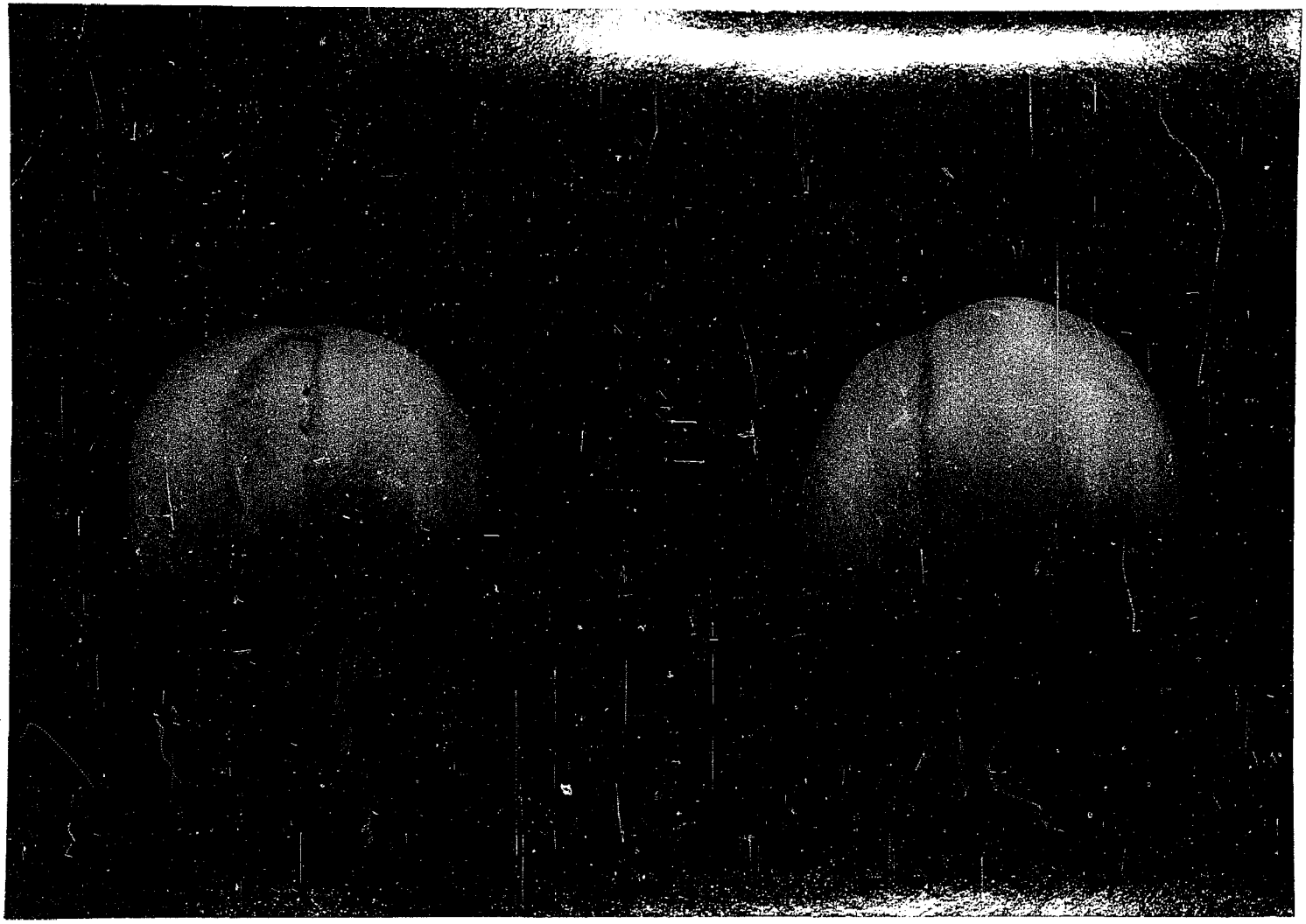


Figure 4. Scarred fruits from mutant bn plant. Remnants of petaloid stamen on the fruit at left. X1

Figure 5. Leaf from cut leaf plant (Bn-C-) on left and leaf from blunt leaf plant (bnbnC-) on right. X½

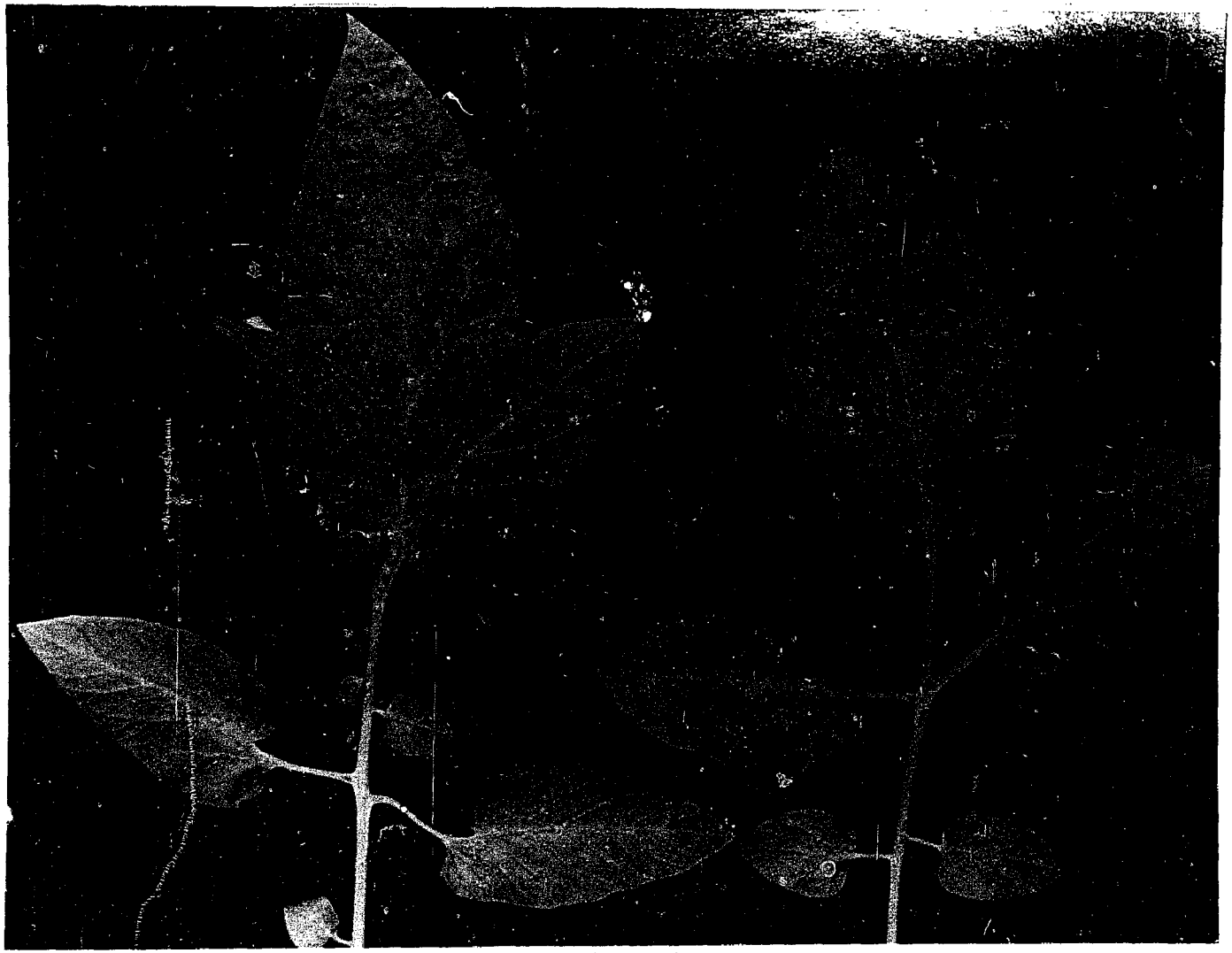


Figure 6. Leaf from potato leaf plant (Bn-cc) on left and leaf from blunt-potato leaf plant (bnbncc) on right. X½

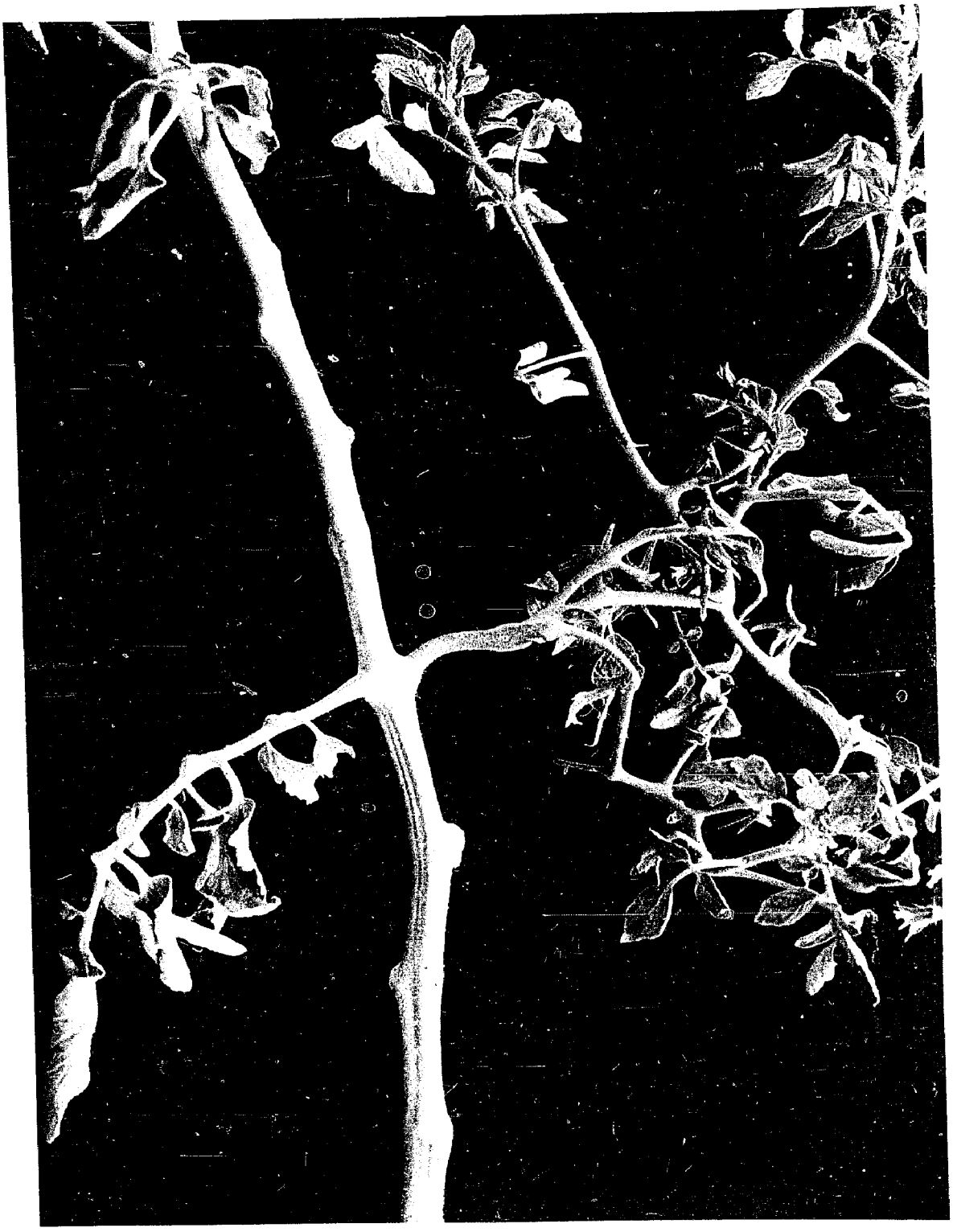


Figure 7. Indeterminate inflorescence from mutant bn plant. X1/3

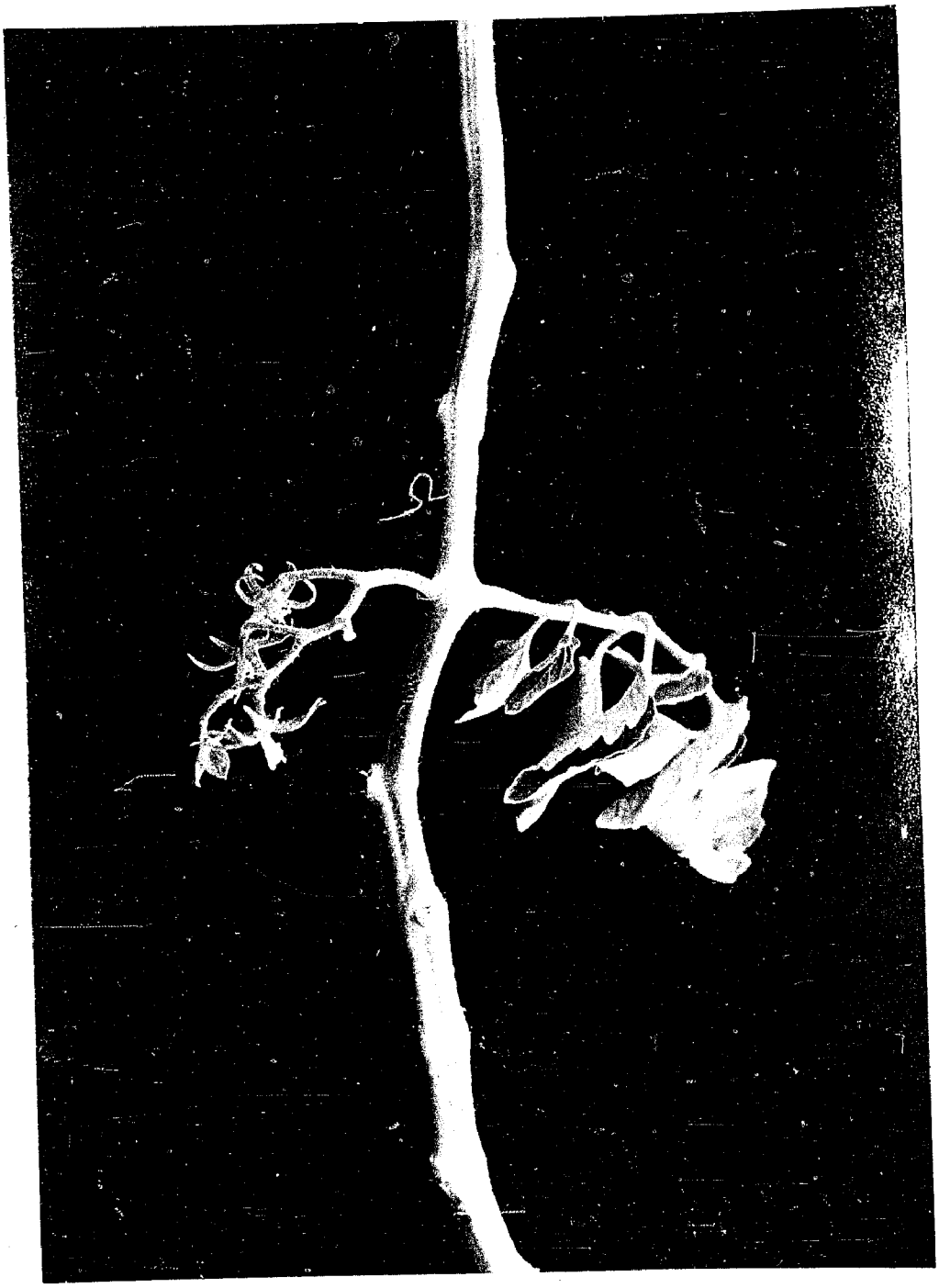


Figure 8. Determinate inflorescence from mutant bn plant. X1/3

periodically for mutant characteristics in the event the gene was dominant or cytoplasmically inherited.

Before the inflorescence had become visable on male-fertile segregates, the F_2 backcross progeny were scored for leaf shape. Without referring to previous notes on leaf shape, flowers were examined and the plants classed as either fertile or sterile on the basis of stamen number and morphology. A periodic examination of all plants for each character was made during the growing season for evidence of a significant variation in expression. Inheritance studies included genes c and u. To obtain segregation data with the latter, sterile plants were artificially pollinated.

In an effort to evaluate more accurately the stability of this mutant with respect to stamen production, and possibly relate any marked variation to a specific set of environmental factors, the number of stamens present was counted. Such data were acquired in 1956, at different dates in 1957, and in the greenhouse during the spring of 1958. A stamen would be recorded if on the basis of external appearance it was judged capable of bearing at least some viable pollen.

The node number to the first cluster and between the first and second clusters was counted in both fertile and sterile plants of the F_2 population. The number of days required to reach anthesis was recorded (based upon the first flower to reach full bloom in the first cluster).

In an effort to evaluate the stability of these characteristics (node number and days to reach anthesis) and the relative effect upon

fertile and sterile plants, data were recorded according to the following cultural practices and treatments (under greenhouse conditions). One family was transplanted twice, once to flats and then to four-inch pots (seeded January 31, 1958). A second family was sown directly ("direct seeded") to four-inch pots (February 22, 1958). The first family of plants was divided into four groups. One of these four served as a check, the remaining three subjected to one, two, and three applications of gibberellin in a 100 ppm aqueous solution per treatment. The entire plant was sprayed on the following dates: February 20, March 5, and March 14. The first treatment was timed according to leaf and cotyledon size (when the cotyledon had fully expanded, and the first true leaves had reached one-half inch in size). The second treatment was applied when the first true leaves were two to three inches in length. The purpose of including these gibberellin treatments resulted from the report of Bukovac et al (5) in which was cited an increased node number and days to anthesis in the tomato, as a consequence of gibberellin treatment. The time of application was guided by the same report. Plants of the size described above for February 20 were indicated to be most sensitive to gibberellin. Subsequent applications were reported to be effective, but to a lesser degree. Also, gibberellin was found to affect leaf shape, by producing a potato leaf in varieties with the gene for cut leaf. Thus the relative effectiveness of gibberellin on node number and leaf shape of fertile and sterile plants was tested.

Mutant $\underline{cl}_3$

Description

Other than the unfruitful condition of this mutant the most obvious deviation from the normal was the apparent permanent juvenile state of the flowers. The corolla, which rarely opened in normal fashion, was responsible for the deviation. The $\underline{cl}_3$ flower type shown in the left-hand specimen in Figure 9 represents the type which has most commonly been found in this study. The petal tips parted but remained paired forming three groups, in contrast to the usual six reflexed petals found at anthesis in Cl_3 flowers. The features of this mutant flower closely resembled a fertile flower just prior to anthesis. Petal color remained a pale yellow-white, margins curled inward (Figure 10), and consequently were more narrow than in Cl_3 flowers. During late fall and under greenhouse conditions in mid-winter, the petals deepened in color and were occasionally found to be in a horizontal position to the floral axis. Generally the range in expression during the remainder of the year would be represented by one of the mutant flowers in Figure 10. Referring then to the cleistogamous condition of the flower, the symbol $\underline{cl}$ is proposed for this mutant. Since two such mutants have been reported by Rick and Robinson (43), one controlled by a dominant gene Cl_1, the other a recessive $\underline{cl}_2$, the subscript next in order, $\underline{cl}_3$ is added.

Further deviations in floral structure of $\underline{cl}_3$ are apparent from Figures 9 and 10. The position of the stigma in relation to the

staminal column indicated possible difficulties in self-pollination resulting from stigma exertion. Evidence that this may be an effective barrier to self-pollination will be presented in the results. Therefore, if this be true, mutant $\underline{cl}_3$ would be categorized as a functional pollen sterile mutant (exerted stigma).

There appears to be no concomitant change in the size or color of the anthers. In fact, anther color was used by the author to determine the time of anthesis in this cleistogamous flowered mutant. The pollen was fertile and the mutant has served as the male parent in hybridization.

Sepals usually failed to reach a reflexed or perpendicular position, but rather, remained in a position parallel to the floral axis. The relative length of the sepals and the petals were changed as compared with fertile flowers. Mutants had approximately equal length sepals and petals, whereas in fertile flowers the corolla generally exceeded the length of the calyx. This difference is principally attributed to a reduction in corolla length.

The fruit of this mutant possessed a prominent protuberance at the stylar end which resembled the phenotype produced by the bk gene. Often associated with this protuberance was the retention of the style, long after abscission had occurred in fruits of Cl_3 (Figure 11).

Difficulties in setting fruit and in producing seed have been encountered wich $\underline{cl}_3$. The cause of this defect is not known.

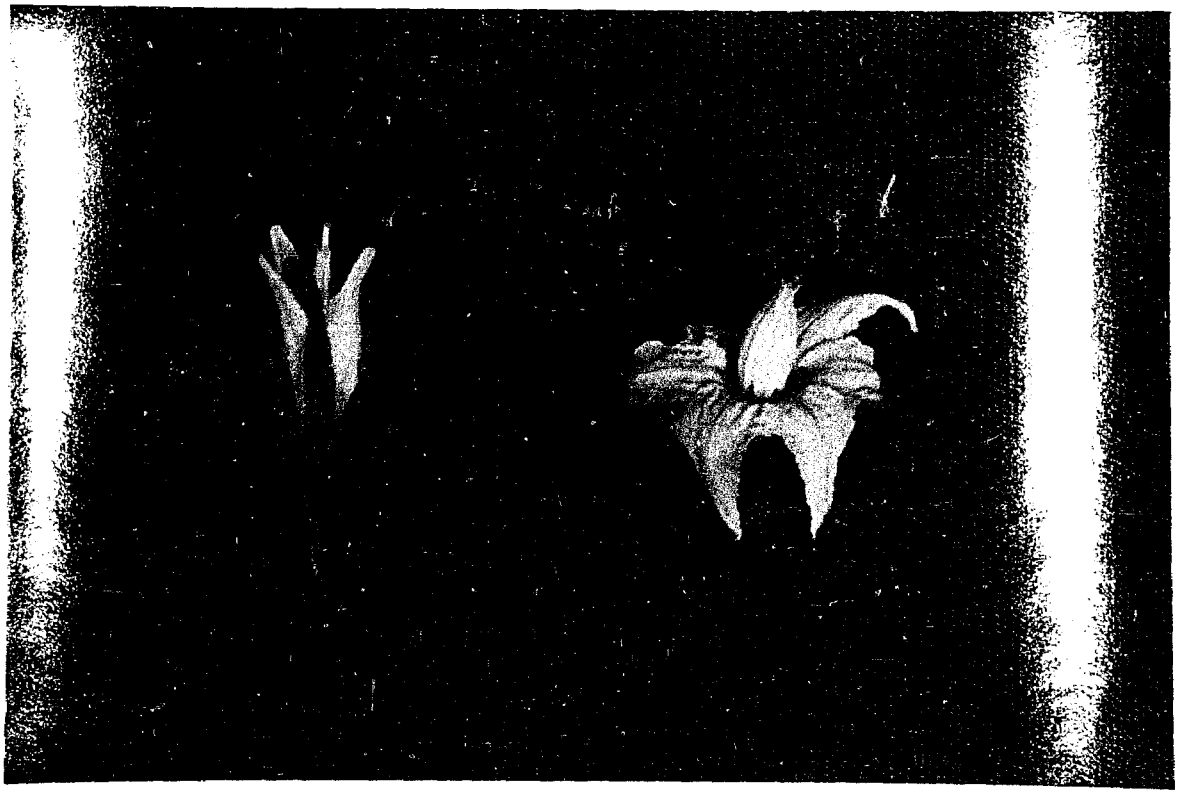


Figure 9. Flower from mutant $\underline{cl}_3$ plant on left and flower from Cl_3 plant on right. X2½

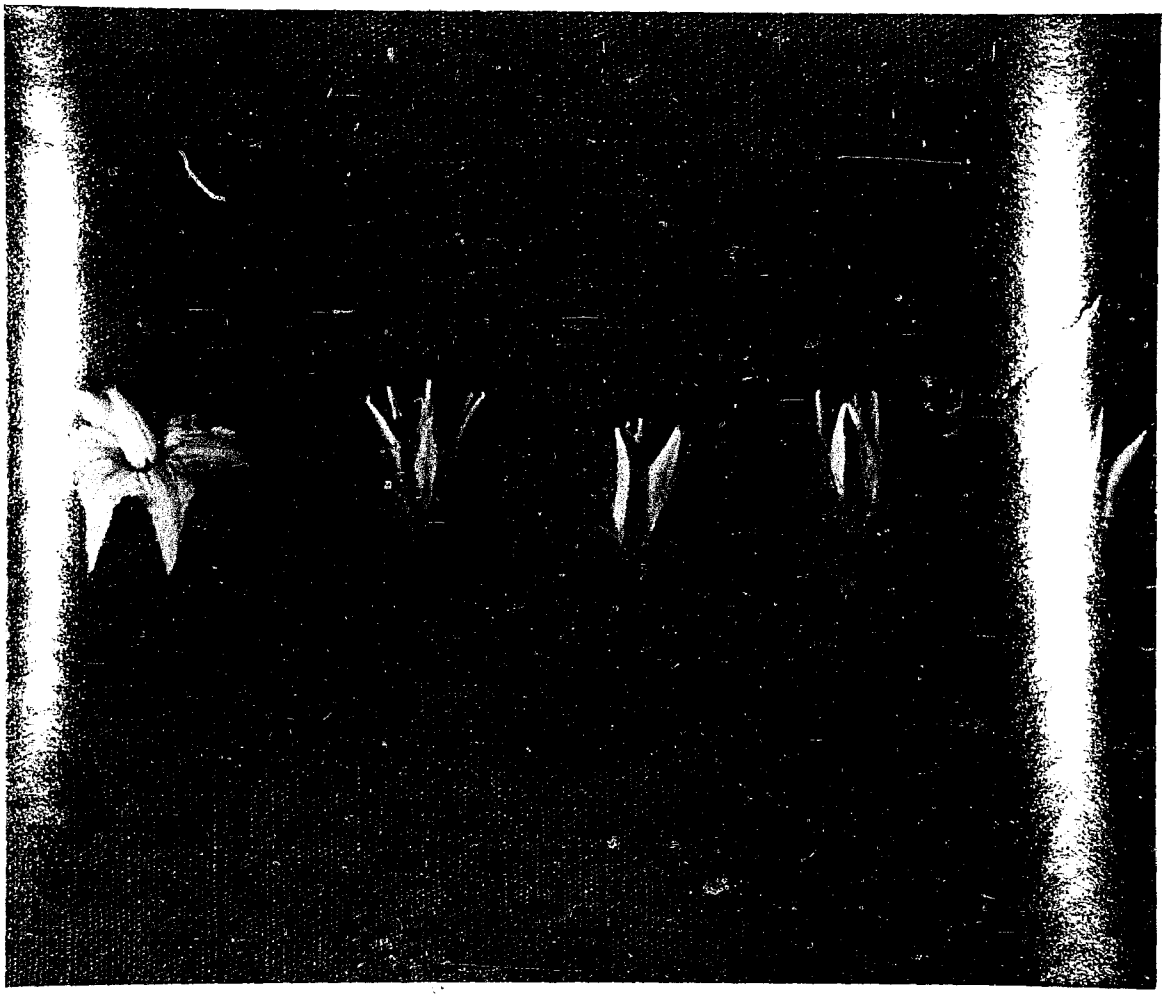


Figure 10. Flowers from mutant $\underline{cl}_3$ plant with a flower from Cl_3 plant at extreme left. X1-1/3

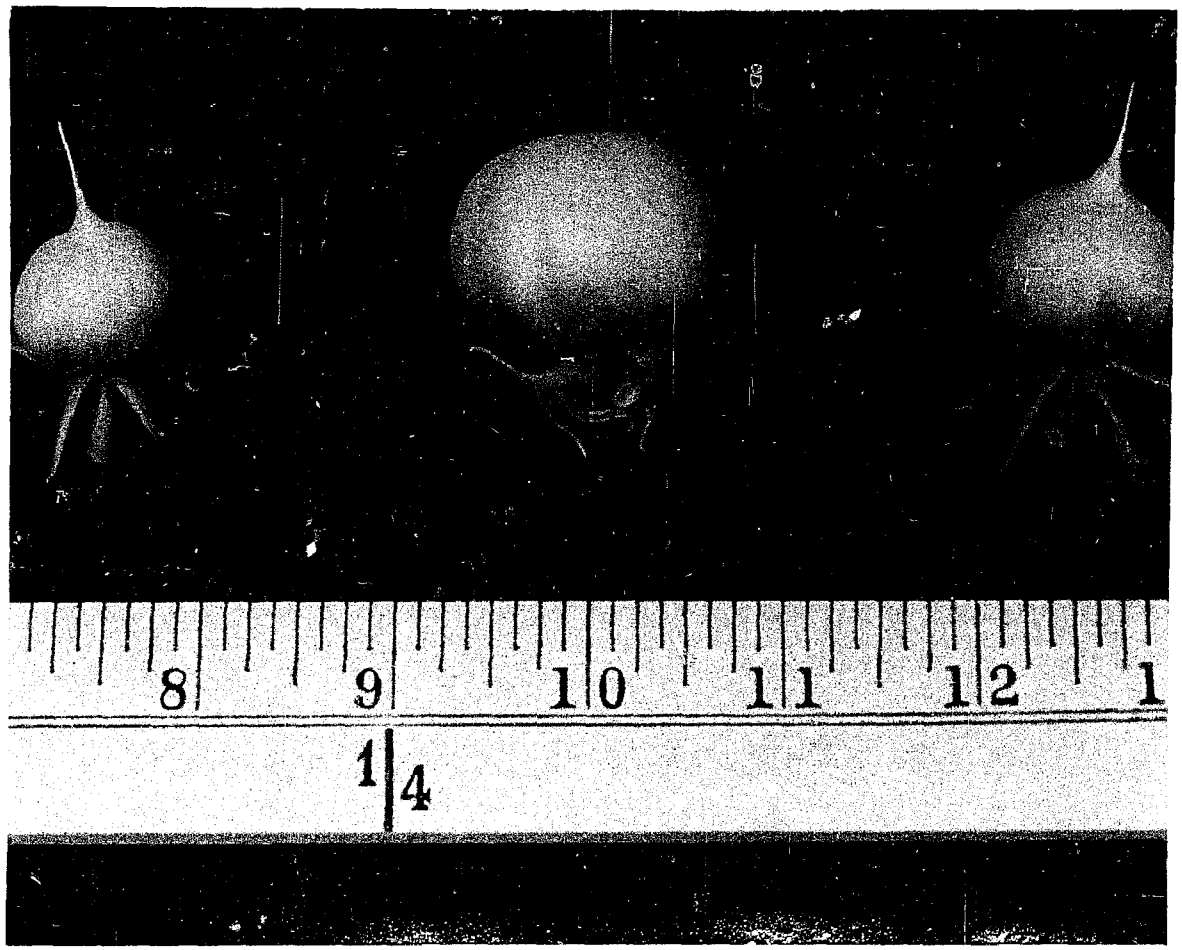


Figure 11. Fruit from Cl_3 plant in center, fruits from $\underline{cl}_3$ plant on left and right. X1

Methods

Inheritance data were obtained during the summer of 1957 and in the greenhouse during 1957 and 1958. The variety Red Jacket, in which $\underline{cl}_3$ was found, also carried the genes $\underline{c}$ and $\underline{u}$. Therefore a variety containing the dominant alleles was selected (Rutgers) which functioned as the male parent. A reciprocal cross was also made. The F_1 plants, as in all the genetic studies, were observed periodically for evidence of mutant characteristics and for $\underline{c}$ and $\underline{u}$.

Previous observation of vegetatively propagated plants over a period of a year, and plants grown from natural and controlled selfing had revealed that the cleistogamous condition of the flower might well serve as a criterion in classifying for sterility. This was most fortunate, for classification of the progeny was expedited. It should be emphasized that this feature was not assumed to be the cause of sterility, but only an association. Therefore, recombinants in the F_2 and backcross progeny were possible. That is, sterile plants which were not cleistogamous, and fertile plants which displayed cleistogamy, would occur if recombination took place. This could not be foretold from the previous observations with sterile plants only. Similarly, the position of the stigma in relation to the anther column was observed when scoring for sterility. If a plant displayed consistent deviation from the expected (cleistogamous flowers with inserted stigmas or non-cleistogamous flowers with exerted stigmas [assuming an association between stigma exertion and cleistogamy]), a recheck would be made to definitely establish or disprove the presence of recombinants

with regard to these two factors. Although this mutant did not affect leaf shape, as did bn, prior to flowering plants were scored for cut leaf or potato leaf shape. Fruit color was determined by artificial pollination of the sterile plants. Thus, it could be determined if gene cl_3 assorted independently of genes c and u. Shape of the fruit was scored when the fruit was approximately 20-30 mm in diameter. This characteristic (beak-like protuberance at the stylar end and style retention) was not a feature of either the variety Red Jacket or Rutgers.

Since sterility might be caused by a premature abscission of the flower, as in the mutant Cl_1Cl_1 reported by Rick and Robinson (43), the number of days from anthesis to abscission on both fertile and sterile segregates was determined. Individual flowers were tagged and daily observations were made. Anthesis in fertile flowers was based on both petal position and anther color (reflexed petals and bright yellow anthers). The former feature could not be used with sterile plants; therefore it was necessary to depend upon anther color.

To relate the degree by which fertile and sterile plants differed in the amount of stigma exertion, and the barrier this presented to self-pollination, the stigma was examined for the presence or absence of pollen. Measurements of sepal, petal, stamen, and pistil lengths were then taken of both the fertile and sterile flowers. After the stigma had been examined for pollen, and the floral organs measured, the staminal column was carefully removed to learn whether or not pollen could be found on the style. The presence

of pollen on the style would not prove conclusively that dehiscence had taken place, since removal of the anthers could have ruptured the stromial cells. On the other hand, an absence of pollen would indicate that the pollen had not been shed. Rick and Robinson (43) found this method more satisfactory than sectioning the flowers. All examinations were made at anthesis, and the date of examination was recorded. The base of the ovary served as the starting point for all measurements. Difficulty was encountered only with measurements of the calyx and corolla in fertile plants due to the reflexed nature of these organs at anthesis. Measurements were made in mm, using a caliper. The fate of the pollen was determined by use of a dissecting microscope (20x).

In a further effort to learn more concerning the possible cause or causes of sterility, the following tests were conducted: (1) sterile plants were artificially self-pollinated; (2) pollen from plants of known fertility was used in pollinating $\underline{cl}_3$ flowers; (3) pollen from $\underline{cl}_3$ flowers in turn was used to pollinate flowers of plants of known female fertility; and (4) $\underline{cl}_3$ flowers were tagged (on the same dates as these other operations were performed) based on the position of the stigma (inserted or exerted) at anthesis. The number of fruits set and an approximation of the seed number were recorded for the above tests.

Mutant s1(?)

Description

The similarity of this mutant to the stamenless mutants, previously cited by Bishop (3) and Hafen and Stevenson (15, 18), led to the use of the symbol s1. Until two questions are settled conclusively, however, the mutant must tentatively be assigned a question mark in place of a numerical subscript (provided a subscript is necessary). Although five non-allelic stamenless mutants are listed, strong evidence is now available that they may be allelic (see page 9). Consequently, a test of allelism between s1(?) and a member of the allelic series would next be in order.

The original s1(?) plant possessed only six fruits and as a consequence was vigorously vegetative in habit. An examination of the flowers revealed a stamenless condition. The effect of s1(?) was not confined to the androecium (Figure 12) but also extended to the corolla. The petals were attenuate, somewhat shorter than in S1(?), generally in a vertical-horizontal position to the floral axis at anthesis, and were a green-yellow in color. The pistil was usually unaffected, but occasionally the style was grooved and the stigma rough or notched (Figures 3 and 12). Fruit and seed production was normal when male-sterile flowers were pollinated with fertile pollen. Sepals of s1(?) showed no significant deviation from those of S1(?).

Expression of this stamenless condition was somewhat variable. Stamen production was usually greater than that in bn. On the basis of approximately one year's observation, the range in stamen number

during most of the year was from a stamenless condition to flowers with two or three stamens. Therefore little self-pollination occurred, as measured in terms of fruit set. However, during mid-late spring (month of May principally) stamen number and appearance approached that of Bn segregates which is apparent from flowers photographed at that time (Figure 13). Another interesting feature of these s1(?) flowers shown in Figure 13, was the increase in stamen number with a corresponding decrease in flower size (age). Adnation between the stamens and pistil occasionally occurred, but to a lesser degree than observed with bn. When adnation did take place a scarring of the fruit resulted.

A slight delay in flowering (average of two and one-half days) was observed with s1(?) plants. This possibly resulted from a slower rate of development of s1(?) flowers from the time the petals separated until full bloom was reached. Since the difference was slight, a difficulty in determining the exact day on which anthesis occurred might provide another explanation.

There was no observable effect of the s1(?) gene on the vegetative parts of the plant. A comparison may be made of s1(?) and bn flowers from Figure 3.

Methods

The inheritance test with s1(?) was conducted in the following manner. A saving in time and space could be achieved by assigning to the cross s1(?)s1(?) by bn F_1 (Table 1), a dual purpose. This cross would serve as the s1(?) and bn allelism test, as well as the parental cross for the F_2 and backcross segregation studies with s1(?), provided

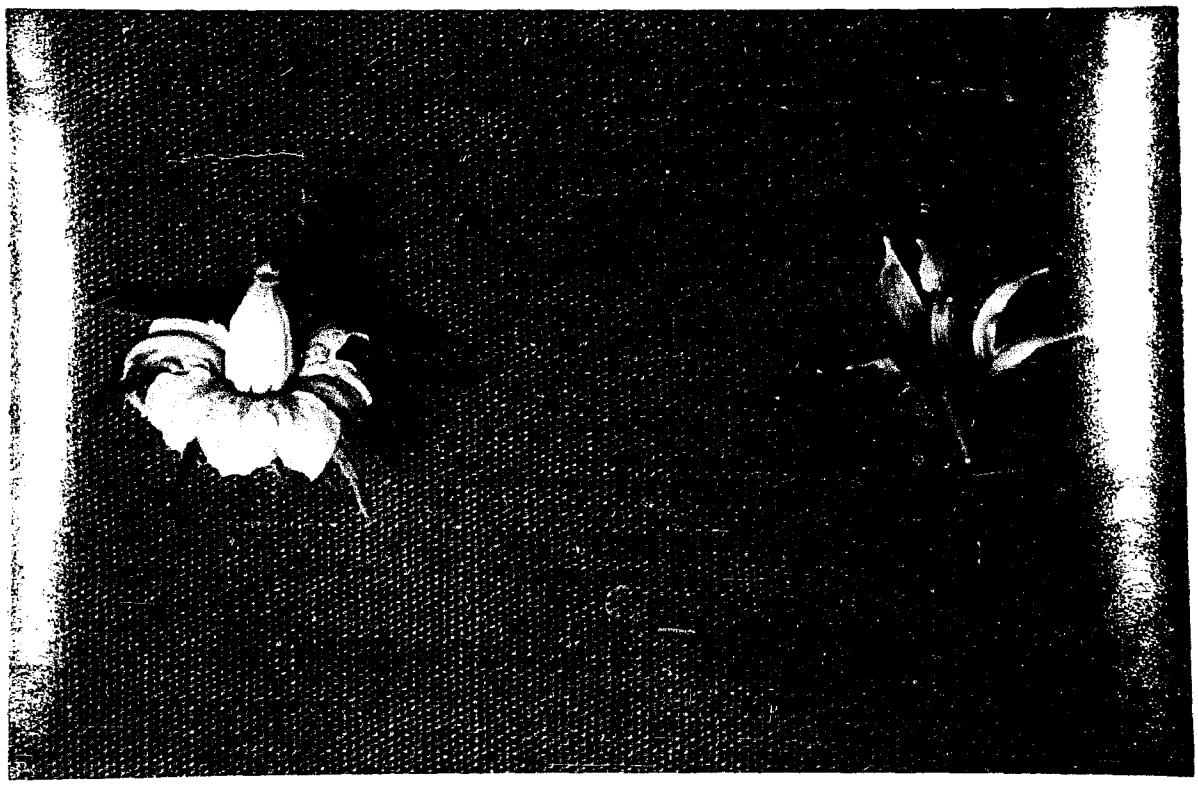


Figure 12. Flower from S1(?) plant on left and flower from mutant s1(?) plant on right. X2

Figure 13. Flowers from mutant $\overline{s1}$(?) plant with flower from S1(?) plant on extreme left. X1½

the progeny of this cross were all fertile, indicating a separate identity of sl(?) and bn. No time would be lost if they proved to be allelic since the crosses of sl(?)sl(?) by cl_3F_1 or by ps(?) F_1 could be used as a substitute (Table 1). The progeny of this cross, sl(?)sl(?) by bnF_1, (if non-allelic) would, therefore, serve as the F_1 generation in so far as the gene sl(?) was concerned. Fruits from individual F_1 plants were harvested, seed of which produced the F_2 generation. Further advantage would be realized, from this procedure, by the introduction of the genes bn, c, and u from the male parent (bn F_1). The effect upon the phenotype of the double homozygous sl(?)bn genotype could be evaluated in the F_2 generation (provided seed was sown from an F_1 plant heterozygous for the gene bn). Furthermore, an F_2 family segregating for both sl(?) and bn would provide further evidence of an allelic or non-allelic relationship between sl(?) and bn. In addition, if found to be non-allelic, then the F_2 progeny could be tested for goodness of fit to a 9:3:3:1 dihybrid F_2 ratio, to determine if sl(?) and bn assort independently. The backcross would be made in the usual way using sl(?) as the female parent and sl(?) F_1 as the male parent. Both the F_2 and backcross generations were greenhouse grown in the spring of 1958. See Figure 14 for the genotypes of the plants involved in the above discussion.

For the same reason and in a similar manner as with bn, the stamen number was determined for sl(?), in the field in 1957. Observations relating to stamen production in this mutant during the greenhouse studies will be elaborated upon in the next section (Presentation of Results).

Fruitfulness was determined in the greenhouse, using the back-cross progeny. Fruits and pedicels of the first cluster only were counted in each plant. An estimation of the seed number per fruit was also made.

Mutant ps?

Description

A resemblance to the previously reported ps mutant was so great that only a brief description need be given. Also for this reason, no test was conducted to determine the cause of sterility. The most obvious characteristic was that of the unopened, urn-shaped, corolla which appeared normal in size. An additional feature of the corolla, displayed by these sterile plants and designated as ps?, was described by Larson and Paur: "The interlocking of the petals of the mutant form and the uneven constrictive force they apply to the exterior of the anther cone results in a convoluted petalous condition giving the appearance of extremely irregular growth."

Before removal of the stamens considerable pollen was observed within the anther cone and occasionally covering the stigmatic surface. This was especially true of the original sterile plants and to a lesser extent with those grown in the greenhouse. To intact stromial cells, thus preventing dehiscence of the pollen in the mutant ps, Larson and Paur attributed the possible cause of sterility. Natural self-pollination, to a considerable degree, has been reported

in this mutant in certain environments, especially toward the latter part of the growing season.

Methods

Four unfruitful plants resembling the previously reported ps mutant were found in a total of fifteen Big Early Hybrid plants. Fruits which set naturally were collected. Artificial cross-pollination with sibling fertile plants were made. Progeny from these two sources were greenhouse grown and fruits collected from the fertile plants. The progeny of these greenhouse individuals were planted in the field in 1957. Assuming heterozygosity of these greenhouse plants for the sterility gene, a goodness of fit to a 3:1 ratio was tested. Fruitfulness of both fertile and sterile progeny was determined in the same manner as with the previous mutants. Thus, a comparison could be made of the fruitfulness of this mutant to that of the new mutants in this study. Also, the fruitfulness of ps under Ohio conditions could be evaluated, provided ps? mutants were identical with the previously reported ps mutant.

Allelism Test

All crosses, to which the allelism test was applied, were made in the greenhouse during the spring of 1957. The crossing scheme is given in Table 1. The progeny of these crosses were transplanted to the field on two dates, July 10, 1957 and August 26, 1957. At least one family of each test was transplanted on each of the above dates with the

TABLE 1

Parents of Crosses to Test the Identity of Mutants *bn*, *cl*$_3$, *sl*(?), and *ps*?

Female Parent (Sterile)	Male Parent		
	bn F_1	*cl*$_3$ F_1	*ps*? F_1
sl(?)	x	x	x
ps?	x	x	
cl$_3$	x		

exception of *sl*(?) x *cl*$_3$ F_1 (August 26, 1957 only). Flowers of all plants were checked periodically for those characteristics associated with the two sterile mutants involved in any one cross. If the gene was known to modify parts other than the flower (*bn*, *cl*$_3$), such associated characteristics were also sought in the offspring. Finally, the fruitfulness or unfruitfulness of each plant was determined. On the basis of these data the plant was then designated as sterile or fertile.

PRESENTATION OF RESULTS

The fruitfulness of each mutant and their respective fertile segregates can be compared in Table 2. Mutant bn exhibited 0 per cent fruitfulness in the four plants examined on September 19, 1957. To assess the accuracy of these fruitfulness determinations on the basis of only four plants, a comparison was made between these results and those acquired three weeks later from an examination of all 275 mutants in the 1957 population. A total of 96 fruits were found. Fruit set occurred in 0.17 per cent of the flowers for all mutants. Therefore it can be concluded that the percentage stated in Table 2 was an accurate estimate of the fruitfulness of bn in 1957.

Mutant cl_3 demonstrated a low natural fruit set (September 19, 1957) although higher than that of bn. Of the 11 fruits found on the four plants which were examined, none exceeded 45 mm in diameter and most were considerably less than this in size. Again at the conclusion of the season a cursory examination was conducted of all 268 mutant plants. The few fruits that were found were also of small size. Larger fruits would most certainly have been seen even in this cursory observation; therefore indicating that the few fruits which were formed by this mutant were mainly set toward the latter part of the growing season.

Seed of ps? was sown about two weeks later than the above two mutants. Fruit and pedicel counts therefore were taken approximately three weeks following those for bn and cl_3. The percentage of

TABLE 2

Fruitfulness of Male-Sterile Mutants bn, cl_3, ps?, and sl(?) and Their Respective Fertile Siblings

Mutant and Fertile Sibling	Plant Number	Number of Pedicels	Number of Fruits	Percentage of Flowers that Set Fruit	Mean Percentage
Bn	12-1	135	39	28.89	
	12-3	138	38	27.54	
	12-9	157	42	26.75	
	12-28	210	56	26.67	27.34
bn	12-4	273	0	0	
	12-14	184	0	0	
	12-26	161	0	0	
	12-43	342	0	0	0
Cl_3	42-2	166	45	27.11	
	42-3	155	33	21.29	
	42-4	159	35	22.01	
	42-7	135	33	24.44	23.74
cl_3	41-10	294	2	0.68	
	42-5	215	6	2.79	
	42-12	182	0	0	
	42-34	338	3	0.89	1.07
Ps?	56-15	104	21	20.19	
	56-28	80	23	28.75	23.91
ps?	56-22	112	9	8.04	
	56-31	151	22	14.57	11.79
Sl(?)	28*	158	67	42.41	42.41
sl(?)	30*	173	23	13.29	13.29

*Number of plants examined (first cluster only).

flowers which set fruit was extremely high (11.79), thereby casting doubt as to the value of ps? as a female parent in hybrid seed production under Ohio conditions.

Before evaluating the fruitfulness of s1(?) (Table 2), it is necessary to consider factors which were peculiar to this mutant at the time of the fruitfulness determinations. The plants were grown in the greenhouse. Fruit and pedicel counts were made during the month of June at which time only the first cluster was available. The stamen number varied considerably between flowers, but those which approached the S1(?) type were most common. (See Figure 13, second and third flowers from the left.) During the remainder of the year (in the greenhouse) an occasional near normal flower could be found, but it was the rare specimen. An increase in stamen number also occurred in the spring of 1957, beginning about the middle of March and reaching a peak in May. However, the common flower type in the spring of 1957 consisted of flowers with three, and occasionally four stamens, which were bent and twisted. Self-pollination would therefore be limited, which was borne out by the few fruits which were set. The plants in the 1957 study were six feet or over in height in comparison to those in 1958, in which only the first cluster had developed sufficiently to set fruit by the month of June. Therefore, in addition to possible environmental differences, the plants also differed in age. The fact remains, stamen number in s1(?) for both 1957 and 1958 was greater during the spring months, especially the month of May, than during the remainder of the year.

Although the only record available of stamen number in field grown plants of sl(?) was taken from the first and second clusters of young plants late in the growing season, these data should be mentioned. The average stamen number per flower in these field grown plants (Table 3) was 0.76 and 1.60 on two examination dates. This was considerably less than the stamen number in the flowers from which the fruitfulness determination were obtained for sl(?). Limited observation, therefore, indicated that field conditions in 1957 were less conducive to stamen production in sl(?) than greenhouse conditions, especially greenhouse conditions in late spring.

Unfruitfulness in these mutants was accompanied by the expected vigorous vegetative growth which is evident from the greater pedicel number in sterile plants as compared with their fertile sibling (Table 2). Examination of the pedicel number of the four bn plants, disclosed considerable variation in number between plants. However, an increased node number between flower clusters was a characteristic of bn. Also the degree to which the node number was increased was variable. Therefore, a difference in the number of flower clusters between bn plants would probably account for the difference in pedicel number. In addition, the vegetative vigor resulting from unfruitfulness in bn plants would in all probability be partially masked, if based upon pedicel number.

Following the plan which was utilized in the Material and Methods, the data will be presented for each mutant individually, and in the following order: bn, cl_3, sl(?), and ps?.

TABLE 3

Stamen Number in Mutant s1(?)
(Field 1957)

Date Examined	Total Number of Flowers Examined	Total Number of Stamens*	Average Number of Stamens Per Flower	Number of Flowers with Stamens	Percentage of Flowers with Stamens
9/23/57	22	17	0.76	10	45
9/27/57	80	128	1.60	49	61

*Resembled a normal stamen sufficiently to be judged capable of bearing viable pollen.

Mutant bn

Genetic Studies

The progeny in the 1956 F_2 inheritance studies were obtained from 26 F_1 plants. The families in 1957 and 1958, both field and greenhouse, came from 51 F_1 plants. All F_1 plants displayed normal and fertile flowers, a cut leaf, and non-uniform ripening of the fruit. Evidence of vegetative abnormalities, previously described as associated with bn plants, were absent from all F_1 plants. To the bn phenotype therefore is ascribed a recessive gene(s).

All phenotypic characteristics definitely associated with bn (abnormal floral structure, foliar modifications, increased node number, and delay in flowering) without exception were found inherited as a unit in all progeny, both F_2 and backcross generations. This was true irrespective of location, year, or the male parent used in the parental cross. Two plants in the 1957 F_2 field tests classed as cut leaf were late in flowering (increased node number), had floral modifications characteristic of bn, and thus appeared to be cross-over types. These two plants displayed rather severe symptoms of the tobacco mosaic virus. Later in the season, however, they produced leaves which displayed the bn leaf type. Therefore these plants were not recombinants but merely appeared as such, as a result of a leaf shape modification produced by the tobacco mosaic virus.

As a means of introduction to the manner in which the data in the inheritance studies are to be presented, a rather detailed description will be given to the bn F_2 data.

To test the mode of inheritance of the mutant bn, the F_2 and backcross generations were subjected to a chi-square analysis for goodness of fit of bn to a 3:1 F_2 ratio and 1:1 backcross ratio. The first such test to be presented included all families (progeny) irrespective of the year tested, location, or the male parent used. Table 4 contains the results from all mutants, but to bn only, will reference be made at present. All 43 F_2 families were treated as one large sample in the pooled chi-square test. Utilizing the chi-square table a value of 0.9709 was obtained. Thus the

TABLE 4

Chi-Square Analysis for Goodness of Fit of bn, cl_3, sl(?), and ps? to a 3:1F_2 Ratio and 1:1 Backcross Ratio*

Mutant	Generation	Total			Pooled			Heterogeneity		
		df**	x^2	P	df	x^2	P	df	x^2	P
bn	F_2	43	45.6689	0.30-0.40	1	0.9709	0.30-0.40	42	44.6980	0.30-0.40
	BC	5	4.5068	0.40-0.50	1	2.1600	0.10-0.20	4	2.3468	0.60-0.70
cl_3	F_2	14	12.5405	0.50-0.60	1	3.5856	0.05-0.10	13	8.9549	0.70-0.80
	BC	5	1.4526	0.90-0.95	1	0.3636	0.50-0.60	4	1.0890	0.80-0.90
sl(?)	F_2	4	7.1616	0.10-0.20	1	6.0651	0.01-0.02	3	1.0965	0.70-0.80
	BC	4	8.6334	0.05-0.10	1	1.0274	0.30-0.40	3	7.6060	0.05-0.10
ps?	F_2	13	15.9159	0.20-0.30	1	2.1688	0.10-0.20	12	13.7471	0.30-0.40

*All data pooled irrespective of male parent, location, or year tested.

**Degrees of freedom and number of families.

probability of equaling or exceeding this chi-square attributable to errors in random sampling would be expected in from 30-40 per cent of similar tests. If the test were to be concluded at this point, many questions concerning the individual families would be left unanswered. The first question to be considered would be, do the individual families show a significant chi-square value? To answer this question the chi-square value for each family must be calculated. Each family chi-square could then be presented in tabular form. Not only would this method of presentation be a cumbersome one but also time-consuming for the reader, without a return in information commensurate with his effort expended. Therefore, information of nearly equal value can be revealed by the total of the individual family values, since they distribute according to chi-square, with, in this case (all 43 bn F_2 families) 43 degrees of freedom. From the two chi-square values presented (pooled and total) the heterogeneity chi-square value can easily be calculated by subtracting the pooled chi-square value from the total. In this example, therefore, the heterogeneity chi-square was 44.6980 with 42 degrees of freedom. This value (heterogeneity chi-square) is extremely important for it will reveal whether or not the individual families are deviating consistently in the same direction (excess or deficiency of mutants) from the hypothesized ratio, as was the pooled data. Establishing a significant deviation from the hypothesized ratio at or below the 5 per cent level, the heterogeneity value in this case would not reach significance (30-40%). Therefore, since the data as a whole

(pooled) satisfactorily fitted the 3:1 ratio, and since the individual families consistently (heterogeneity test) followed the direction in which the pooled data deviated, there appears no valid reason not to accept the hypothesis, that a single recessive gene, bn, determined the mode of inheritance of this mutant.

At this time it should be pointed out that although this level of 5 per cent for significance will be used, it will be done in a rather flexible manner. That is to say, if during the course of the experiment an observation of sufficient import was made to cause doubt as to the validity of the data or indicated a need for further information, a value as high as, for example, 30-40 per cent would not be automatically accepted as non-significant. Conversely, a value of 2 per cent will not be accepted as unquestionable proof that the hypothesis should be rejected. If, however, there is no valid reason to question the value which is stated (which usually will be the case) the 5 per cent level will be utilized in determining significance. Therefore, on the basis of the information found in Table 4, the hypothesis will be accepted, that a single recessive gene, designated as bn, represents the mode of inheritance of this mutant.

With all mutants a complete chi-square analysis, such as this, will be supported by an accompanying table such as Table 5 headed "Segregation--," which will include total plant numbers, number of mutants (or recessive genes tested such as c or

TABLE 5

Segregation of bn, cl_3, sl(?), and ps?
in the F_2 and Backcross Generation*

Mutant	Generation	Expected Ratio	Total Number Plants	Observed Number Mutant Plants	Expected Number Mutant Plants	Deviation	Pooled		
							df	x^2	P
bn	F_2	3:1	2,365	612	591.2500	+20.7500	1	0.9709	0.30-0.40
	BC	1:1	150	66	75.0000	- 9.0000	1	2.1600	0.10-0.20
cl_3	F_2	3:1	839	186	209.7500	-23.7500	1	3.5856	0.05-0.10
	BC	1:1	44	20	22.0000	- 2.0000	1	0.3636	0.50-0.60
sl(?)	F_2	3:1	343	66	85.7500	-19.7500	1	6.0651	0.01-0.02
	BC	1:1	219	117	109.5000	+ 7.5000	1	1.0274	0.30-0.40
ps?	F_2	3:1	535	119	133.7500	-14.7500	1	2.1688	0.10-0.20

*All data pooled irrespective of male parent, location, or year tested.

u), and the number of mutants which deviated (+ or - direction) from the number which was expected.

Since this mutant (bn) was observed in different years and locations, the families were divided according to these factors and analyzed (Tables 6 and 7). It might be questioned at this point why this was necessary, since Table 4 showed no obvious evidence of suspect. The author possessing information that the population was heterogeneous with regard to location, year, and male parent used (and corresponding differences in family number in these sub-groups), concluded that a significant difference in any one sub-group which was made up of a small number of families, might be masked in the total analysis. This breakdown to sub-groups was, therefore, essential whether positive or negative results were obtained.

Although no value exceeded the 5 per cent level of significance, the 1956 field population (Table 6) raises some question not only with respect to the pooled data, but as to the consistency of the deviation of the families and their distribution as to chi-square. This would not be questioned too strongly had it not been for the fact that this population consisted of families in which different male parents were used in the original cross. In addition, this deviation was due to an excess number of mutants. More often when a deviation occurs, it results from a deficiency of recessive mutant types.

TABLE 6

Chi-Square Analysis for Goodness of Fit of bn to a 3:1 F_2 Ratio and 1:1 Backcross Ratio (Data Pooled According to Location and Year)

Location and Year	Genera-tion	Total			Pooled			Heterogeneity		
		df*	x^2	P	df	x^2	P	df	x^2	P
Field										
1956	F_2	20	26.8406	0.10-0.20	1	3.7421	0.05-0.10	19	23.0985	0.20-0.30
1957	F_2	20	17.1670	0.60-0.70	1	0.6689	0.40-0.50	19	16.4981	0.60-0.70
	BC	5	4.5068	0.40-0.50	1	2.1600	0.10-0.20	4	2.3468	0.60-0.70
Greenhouse										
1957-58	F_2	-	-	-	1	0.5536	0.40-0.50	-	-	-
1958	F_2	2	1.1077	0.50-0.60	1	0.4243	0.50-0.60	1	0.6834	0.40-0.50

*Degrees of freedom and number of families.

TABLE 7

Segregation of bn in the F_2 and Backcross Generation
(Data Pooled According to Location and Year)

Location and Year	Generation	Expected Ratio	Total Number Plants	Observed Number bn Plants	Expected Number bn Plants	Deviation	Pooled		
							df	x^2	P
Field									
1956	F_2	3:1	945	262	236.2500	+25.7500	1	3.7421	0.05-0.10
1957	F_2	3:1	1,148	275	287.0000	-12.0000	1	0.6689	0.40-0.50
	BC	1:1	150	66	75.0000	- 9.0000	1	2.1600	0.10-0.20
Greenhouse									
1957-58	F_2	3:1	118	33	29.5000	+ 3.5000	1	0.5536	0.40-0.50
1958	F_2	3:1	154	42	38.5000	+ 3.5000	1	0.4243	0.50-0.60
Total	F_2	3:1	2,365	612	591.2500	+20.7500	1	0.9709	0.30-0.40
Total	BC	1:1	150	66	75.0000	- 9.0000	1	2.1600	0.10-0.20

The 1956 population, therefore, was further subdivided according to the male parent used in the initial cross. Red Jacket, which was used in both the 1957 and 1958 studies, was added since it had not yet been analyzed as a unit. The pooled chi-square for both Heinz Marketer and WR-3 were significant at the 5 per cent level (as shown in Table 8) and deviated in the same direction (Table 9). Thus the original deviation in the direction of an excess number of mutants shown in Table 5 was contributed mainly by the progeny of the male parents Heinz Marketer and WR-3. Furthermore, this deviation was rather uniformly distributed among the progeny of Heinz Marketer but a lack of consistency characterized WR-3 (Table 8). The total chi-square value for WR-3 is 11.3398, and of the four families, one disproportionately contributed 10.6124. Such a high value limited to one family (fruit) could be attributed to possible contamination of seed from selfed bn fruits. The total and heterogenity chi-square values for Heinz Marketer on the other hand, indicated no such association with a single family. The possibility therefore exists that modifier genes were contributed by the male parent, Heinz Marketer. It can be said with confidence that the significant deviation found in the pooled data for Heinz Marketer was not likely the result of misclassification of the F_2 progeny. To date, bn plants have been identified readily.

Although the backcross generation does show a slight defieiency of mutants (Table 5) this deficiency of mutant plants is not of sufficient magnitude to question the 1:1 hypothesized ratio

TABLE 8

Chi-Square Analysis for Goodness of Fit of bn to a 3:1 F_2 Ratio and 1:1 Backcross Ratio (Data Pooled on the Basis of the Male Parent)

Male Parent	Generation	Total			Pooled			Heterogeneity		
		df*	x^2	P	df	x^2	P	df	x^2	P
Heinz Marketer	F_2	9	11.4921	0.20-0.30	1	4.2135	0.02-0.05	8	7.2786	0.50-0.60
Marietta #1	F_2	-	-	-	1	0.3704	0.50-0.60	-	-	-
Purdue F_2	F_2	-	-	-	1	0.1852	0.60-0.70	-	-	-
Red Jacket	F_2	23	18.8283	0.70-0.80	1	0.0939	0.70-0.80	22	18.7344	0.60-0.70
	BC	5	4.5068	0.40-0.50	1	2.1600	0.10-0.20	4	2.3468	0.60-0.70
Urbana	F_2	5	3.4531	0.60-0.70	1	0.8519	0.30-0.40	4	2.6012	0.60-0.70
WR-3	F_2	4	11.3398	0.01-0.02	1	4.4641	0.02-0.05	3	6.8757	0.05-0.10

*Degrees of freedom and number of families.

TABLE 9

Segregation of bn in the F_2 and Backcross Generation
(Data Pooled on the Basis of the Male Parent)

Male Parent	Gener-ation	Expected Ratio	Total Number Plants	Observed Number bn Plants	Expected Number bn Plants	Deviation	Pooled		
							df	x^2	P
Heinz Marketer	F_2	3:1	445	130	111.2500	+18.7500	1	4.2135	0.02-0.05
Marietta #1	F_2	3:1	90	25	22.5000	+ 2.5000	1	0.3704	0.50-0.60
Purdue F_2	F_2	3:1	45	10	11.2500	- 1.2500	1	0.1852	0.60-0.70
Red Jacket	F_2	3:1	1,420	350	355.0000	- 5.0000	1	0.0939	0.70-0.80
	BC	1:1	150	66	75.0000	- 9.0000	1	2.1600	0.10-0.20
Urbana	F_2	3:1	207	46	51.7500	- 5.7500	1	0.8519	0.30-0.40
WR-3	F_2	3:1	158	51	39.5000	+11.5000	1	4.4641	0.02-0.05
Total	F_2	3:1	2,365	612	591.2500	+20.7500	1	0.9709	0.30-0.40
Total	BC	1:1	150	66	75.0000	- 9.0000	1	2.1600	0.10-0.20

(Table 4). Therefore from the results obtained in both the F_2 and backcross generations, it can be ooncluded that a single recessive gene determined the mode of inheritance of the mutant designated as bn.

The chi-square analysis that follow (Table 10) tests the goodness of fit of bn and c, bn and u to a 9:3:3:1 F_2 ratio and all three genes to a 27:9:9:9:3:3:3:1 F_2 ratio. The combination c and u were not tested in combination because they have been proven to be found in different chromosomes and linkage groups; c in chromosome 6, linkage group IV, and u in chromosome 10, linkage group VII. Placement of bn and c, and bn and u in two different locations in Table 10 was necessary because c and u were each tested separately with bn in families other than those of the trihybrid cross.

If linkage occurred both genes c and u would be in repulsion with bn. That is, bnbnCC and BnBncc would represent the parental types for bn and c, bnbnUU and BnBnuu the parental types for bn and u. Thus if a significant difference from the expected occurred, an excess in numbers should be found in these parental classes. A component test for each gene to a 3:1 ratio was computed. A deficiency of either gene, bn or c, or bn or u, in the dihybrid crosses, or of bn, c, or u in the trihybrid cross, would affect the number of individuals in any given phenotypic class of these crosses. Therefore the "interaction" chi-square value for each cross was calculated, which would give a true picture of the number of individuals in each phenotypic class, by accounting for the deviation from that expected in the monohybrid ratio.

TABLE 10

Chi-Square Analysis for Goodness of Fit of bn, c, and u to a 27:9:9:9:3:3:3:1 F_2 Ratio; of bn and c to a 9:3:3:1 F_2 Ratio; of bn and u to a 9:3:3:1 F_2 Ratio; and of bn, c, and u, Each to a 3:1 F_2 Ratio as Components in the Dihybrid and Trihybrid Crosses

Gene or Genes	Expected F_2 Ratio	Total			Pooled			Heterogeneity		
		df	x^2	P	df	x^2	P	df	x^2	P
bn and c	9:3:3:1	66	75.0112	0.20-0.30	3	1.8566	0.60-0.70	63	73.1546	0.10-0.20
bn	3:1	22	18.2747	0.60-0.70	1	0.0349	0.80-0.90	21	18.2398	0.60-0.70
c	3:1	22	26.1674	0.20-0.30	1	1.5576	0.20-0.30	21	24.6098	0.20-0.30
I**		22	30.5691	0.10-0.20	1	0.2641	0.60-0.70	21	30.3050	0.05-0.10
bn and u	9:3:3:1	9	5.2617	0.80-0.90	3	1.8288	0.60-0.70	6	3.4329	0.70-0.80
bn	3:1	3	1.6613	0.60-0.70	1	0.9608	0.30-0.40	2	0.7005	0.70-0.80
u	3:1	3	1.7700	0.60-0.70	1	0.1235	0.70-0.80	2	1.6465	0.40-0.50
I		3	1.8304	0.60-0.70	1	0.7445	0.30-0.40	2	1.0859	0.50-0.60
bn, c, and u	27:9:9:9									
	3:3:3:1	14	18.0810	0.20-0.30	7	7.1266	0.40-0.50	7	10.9544	0.10-0.20
bn and c	9:3:3:1	6	11.5314	0.05-0.10	3	3.4141	0.30-0.40	3	8.1173	0.02-0.05
bn and u	9:3:3:1	6	2.8850	0.80-0.90	3	1.0525	0.70-0.80	3	1.8325	0.60-0.70
bn	3:1	2	1.1077	0.50-0.60	1	0.4243	0.50-0.60	1	0.6834	0.40-0.50
c	3:1	2	2.5522	0.20-0.30	1	2.5021	0.10-0.20	1	0.0501	0.80-0.90
u	3:1	2	0.4028	0.80-0.90	1	0.3157	0.50-0.60	1	0.0871	0.70-0.80
I (bn and c)		2	7.8715	0.01-0.02	1	0.4877	0.40-0.50	1	7.3838	0.00-0.01
I (bn and u)		2	1.3745	0.50-0.60	1	0.3125	0.50-0.60	1	1.0620	0.30-0.40

*See corresponding segregation Table 11 for genotypic classes.

**"I" refers to the interaction chi-square value.

The pooled chi-square test (Table 10) does not show a single significant deviation from the hypothesized ratio. If a further examination were to be made, the rather low uniformity between families might be warranted with bn and c. Table 11 indicates a deficiency in the "parental" classes with one "cross-over" type to be much in excess. The deficiency in class bnbncc (Table 11) might partially be explained on the basis of a lower rate of viability often associated with double recessive homozygocity. The significant heterogeneity and total chi-square values found with bn and c in the trihybrid cross would be of importance if it were not based on but two families. Therefore, most emphasis must be placed on the dihybrid cross which contained 22 families. These families showed an excellent fit of the pooled data to a 9:3:3:1 F_2 ratio. Although the total and heterogeneity tests did show a considerable higher chi-squqre than the pooled, the values nevertheless were not significant. Considering the genes bn and u, Table 10 indicates an excellent fit to the proposed ratio. The heterogeneity test also shows that the small deviation from expected was consistent among the families (see Table 12 for the number of individuals in each class for segregation of bn and u, and Table 13 for the number in each class for the trihybrid cross). Therefore the conclusion can be drawn that dihybrid segregation (9:3:3:1 F_2 ratio) is indicated for bn and c, bn and u, and trihybrid segregation (27:9:9:9:3:3:3:1 F_2 ratio) for bn, c, and u.

TABLE 11

Segregation in the F_2 Generation for bn and c

Genotype	Expected F_2 Ratio	Number Observed in Each Class	Number Expected in Each Class	Deviation
bn and c				
Bn-C-	9/16	753	732.3750	+ 20.6250
Bn-cc	3/16	232	244.1250	- 12.1250
bnbnC-	3/16	243	244.1250	- 1.1250
bnbncc	1/16	74	81.3750	- 7.3750
bn				
Bn-	3/16	985	976.5000	+ 8.5000
bnbn	1/16	317	325.5000	- 8.5000
c				
C-	3/16	996	976.5000	+ 19.5000
cc	1/16	306	325.5000	- 19.5000

TABLE 12

Segregation in the F_2 Generation for bn and u

Genotype	Expected F_2 Ratio	Number Observed in Each Class	Number Expected in Each Class	Deviation
bn and u				
Bn-U-	9/16	148	151.8750	- 3.8750
Bn-uu	3/16	48	50.6250	- 2.6250
bnbnU-	3/16	52	50.6250	+ 1.3750
bnbnuu	1/16	22	16.8750	+ 5.1250
bn				
Bn-	3/4	197	204.0000	- 7.0000
bnbn	1/4	75	68.0000	+ 7.0000
u				
U-	3/4	200	202.5000	- 2.5000
uu	1/4	70	67.5000	+ 2.5000

TABLE 13

Segregation in the F_2 Generation for bn, c, and u

Genotype	Expected F_2 Ratio	Number Observed in Each Class	Number Expected in Each Class	Deviation
bn, c, and u				
Bn-C-U	27/64	61	64.1250	- 3.1250
Bn-C-uu	9/64	15	21.3750	- 6.3750
Bn-ccU-	9/64	26	21.3750	+ 4.6250
bnbnC-U-	9/64	24	21.3750	+ 2.6250
Bn-ccuu	3/64	9	7.1250	+ 1.8750
bnbnC-uu	3/64	6	7.1250	- 1.1250
bnbnccU-	3/64	6	7.1250	- 1.1250
bnbnccuu	1/64	5	2.3750	+ 2.6250
bn and c				
Bn-C-	9/16	76	86.6250	-10.6250
Bn-cc	3/16	36	28.8750	+ 7.1250
bnbnC-	3/16	31	28.8750	+ 2.1250
bnbncc	1/16	11	9.6250	+ 1.3750
bn and u				
Bn-U-	9/16	87	85.5000	+ 1.5000
Bn-uu	3/16	24	28.5000	- 4.5000
bnbnU-	3/16	30	28.5000	+ 1.5000
bnbnuu	1/16	11	9.5000	+ 1.5000
bn				
Bn-	3/16	112	115.5000	- 3.5000
bnbn	1/16	42	38.5000	+ 3.5000
c				
C-	3/16	107	115.5000	- 8.5000
cc	1/16	47	38.5000	+ 8.5000
u				
U-	3/16	117	114.0000	+ 3.0000
uu	1/16	35	38.0000	- 3.0000

Phenotypic Stability and Pleiotropism

Stamen Number

The range in stamen number found in bn flowers observed under both greenhouse and field conditions has been remarkably small. Seldom were flowers seen that produced two or three stamens, and even these were contorted, twisted, petaloid,or adnate to the pistil (occasionally the petals). Flowers which could be correctly classed as normal or even as near normal have yet to be seen in this mutant.

In Table 14 is shown the average number of stamens per flower, and the percentage of flowers with at least one stamen found in bn flowers on different dates of examination. These appendages, classed as stamens, were usually not completely normal in appearance, but sufficiently so to be judged capable of bearing viable pollen. A noticeable increase in stamen number was found under greenhouse conditions in the month of May, 1958. A breakdown of the 1957 and 1958 results according to the day of examination is presented in Table 15. Little variation can be found between the different dates of examination in the 1957 field data. The 1958 data were taken in a different manner from that in the field. The date given in 1958 corresponds to the date the first flower in the first cluster reached anthesis for an individual plant. The first three flowers in cluster one were examined on the day the first flower reached full bloom. An obvious line of demarcation can be drawn on the fifteenth day of the month. Prior to this date the number of stamens per flower, as well as the percentage of flowers with stamens was

TABLE 14

Stamen Number in Mutant bn
(Field 1956, 1957, and Greenhouse 1958)

Date Examined	Total Number of Flowers Examined	Total Number of Stamens*	Average Number of Stamens Per Flower	Number of Flowers with Stamens	Percentage of Flowers with Stamens
1956 September	130	39	0.30	37	28
1957 8/26-10/9	238	96	0.40	70	29
1958** 5/10-6/1	120	171	1.42	75	62

*Resembled normal stamens sufficiently to be judged capable of bearing viable pollen.

**First three flowers to reach anthesis in the first cluster examined in each of 40 plants.

TABLE 15

Stamen Number in Mutant bn According to Day of Examination
(Field 1957 and Greenhouse 1958)

Date Examined	Total Number of Flowers Examined	Total Number of Stamens*	Average Number of Stamens Per Flower	Number of Flowers with Stamens	Percentage of Flowers with Stamens
1957					
8/26	57	28	0.49	18	32
9/23	93	40	0.43	26	28
9/27	53	14	0.26	14	26
10/ 9	35	14	0.40	12	34
1958					
5/10	8	0	0	0	0
5/11	6	1	0.17	1	17
5/12	11	1	0.09	1	9
5/13	3	0	0	0	0
5/14	-	-	-	-	-
5/15	3	2	0.67	1	33
5/16	3	8	2.67	3	100
5/17	6	4	0.67	3	50
5/18	14	30	2.14	12	86
5/19	3	5	1.67	2	67
5/20	12	24	2.00	9	75
5/21	18	38	2.11	16	89
5/22	9	17	1.89	7	78
5/23	15	32	2.13	13	87
5/24	-	-	-	-	-
5/25	-	-	-	-	-
5/26	-	-	-	-	-
5/27	3	1	0.33	1	33
5/28	-	-	-	-	-
5/29	-	-	-	-	-
5/30	3	4	1.33	3	100
5/31	-	-	-	-	-
6/ 1	3	4	1.33	3	100
5/10-5/15	31	4	0.13	3	10
5/16-6/1	89	167	1.88	72	81

*Resembled normal stamens sufficiently to be judged capable of bearing viable pollen.

below that recorded under field conditions. However, from the sixteenth until the first of June when the last plant reached anthesis, a noticeable increase in stamen number (and the percentage of flowers with stamens) took place, both as compared with field data and especially as compared with the earlier blooming greenhouse progeny. Although it had previously been suspected that a slight increase in stamen number occurred at this time of the year, such an abrupt and noticeable line of demarcation had not been anticipated. Therefore the data necessary to interpret the cause of these observations are incomplete. In a later section factors that may have contributed to these differences will be considered but obviously no definite conclusions can be drawn. For valid conclusions to be reached one would necessarily require evidence from more than one year's data. If proven to consistently produce similar results, use might be made of this mutant in studies concerned with stamen initiation and development. Nevertheless one should not lose sight of the fact that from the standpoint of male sterility, this represents the maximum stamen production so far observed for this mutant. This is indeed a low rate of production and indicates phenotypic stability of a high order.

Node Number and Days to Anthesis

A description of bn was given in the Materials and Methods. Mutant bn was stated to be characterized by an increase in node number to the first cluster, similarly an increase from the first to the

second cluster, and consequently a delay in flowering. It is, therefore, the first purpose of this section to corroborate these statements with supporting data.

The mean difference in node number to the first cluster (not including plumule leaves) of Bn as compared to bn plants using the "t" test for significance is presented in Table 16 according to the treatment given and the cultural practice which was used. Two conclusions can be drawn from these data. The first is that the node number to the first cluster in bn was sufficiently greater than that of Bn to be significantly different at the 0.1 per cent level. The second is, irrespective of treatment, the level of significance remained at the 0.1 per cent level. This second conclusion, however, did not state that treatment had no effect; it only implies that if a modification had occurred it had not been of sufficient magnitude to reduce the level of significance to a point less than 0.1 per cent. Table 17 shows that the node number from the first to the second cluster in bn was greater than in Bn for all treatments at the 5 per cent level or beyond, and significantly different at the 0.1 per cent level in the "direct seeded" and the control. Another obvious difference was the rather large confidence interval displayed by bn. The marked delay in flowering exhibited by bn plants compared to Bn was significant in all treatments at the 0.1 per cent level (Table 18). It therefore appears quite certain that the increased node number to the first cluster in bn plants would account for the delay in flowering.

TABLE 16

Node Number to the First Cluster of Bn Compared to bn (by Treatment)
Using the "t" Test for Significance

Treatment	Mean of Bn	Mean of bn	Difference in Mean*** (bn-Bn)	df	"t" Value
Control	7.85 ± 0.56#	18.44 ± 0.55	+10.59	33	29.417
One application gibberellin	9.23 ± 0.37	19.13 ± 2.88	+10.10	32	14.638
Two applications "	9.44 ± 1.38	23.17 ± 1.39	+13.73	13	16.951
Three applications "	9.54 ± 1.09	23.17 ± 1.80	+13.63	15	16.635
"Direct seeded"	8.15 ± 0.37	13.13 ± 0.94	+ 4.98	47	10.596

***All differences significant at the 0.1 per cent level.

#Confidence interval at the 5 per cent level.

TABLE 17

Node Number from the First to the Second Cluster of Bn Compared to bn (by Treatment) Using the "t" Test for Significance

Treatment	Mean of Bn	Mean of bn	Difference in Mean (bn-Bn)	df	"t" Value
Control	4.38 ± 0.52#	11.33 ± 2.77	+ 6.95***	33	5.697
One application gibberellin	4.13 ± 0.51	12.33 ± 7.19	+ 8.20***	32	4.852
Two applications "	5.11 ± 1.04	19.83 ± 3.78	+14.72***	13	9.558
Three applications "	4.82 ± 0.98	11.67 ± 7.92	+ 6.85*	15	2.203
"Direct seeded"	3.20 ± 0.24	9.27 ± 2.14	+ 6.07***	47	6.070

*Difference significant at the 5 per cent level.

***Difference significant at the 0.1 per cent level.

#Confidence interval at the 5 per cent level.

TABLE 18

Days to Anthesis of Bn Compared to bn (by Treatment)
Using the "t" Test for Significance

Treatment	Mean of Bn	Mean of bn	Difference in Mean***	df	"t" Value
Control	86 ± 1.79#	108 ± 1.96	+ 22	33	18.033
One application gibberellin	88 ± 1.76	110 ± 1.76	+ 22	32	23.158
Two applications "	83 ± 4.04	115 ± 4.76	+ 32	13	12.549
Three applications "	83 ± 3.66	108 ± 3.98	+ 25	14	11.161
"Direct seeded"	68 ± 1.22	77 ± 2.64	+ 9	47	6.569

***All differences significant at the 0.1 per cent level.

#Confidence interval at the 5 per cent level.

Before the data in Table 19 can be interpreted correctly, the environmental conditions which prevailed at the time of the experiment must be known. This is especially important in that the "direct seeded" progeny would be at a different stage of growth than the plants constituting the gibberellin series, due to differences in seeding dates. Greenhouses were maintained at approximately 70° day and 55°-60° night temperatures (see Appendix Table 44). However, beginning approximately the first of March and continuing until the twenty-first the night temperatures were at about 50°. This produced two major effects. The gibberellin series was markedly checked in growth during this time (seeded January 31) and undoubtedly accounted in large measure for the delay in flowering of both Bn and bn in the gibberellin series. The series termed "direct seeded" as mentioned in the Materials and Methods was sown on February 28. According to Wittwer et al (49) tomato seedlings exposed to 50°-55° night temperatures during the time of node determination (shortly after cotyledon expansion and for approximately one to two weeks) produced fewer nodes to the first cluster than at higher night temperatures (certain varieties). These results also indicated short days in combination with low temperatures were more effective in reducing node number than long days (16 hour photoperiod). This information, therefore, may aid in interpreting the data with respect to these tests. Germination ("direct seeded") was completed on the 11th of March (in a 60° house) and immediately transferred to the 50° house. The seedlings were thereby exposed for the recommended length of time, proper stage of growth, and the night temperature

TABLE 19

Effect upon Node Number to the First Cluster, First to the Second Cluster, and Days to Anthesis of Bn and bn Plants Treated with One, Two, and Three Applications of Gibberellin (100 ppm/application), and Two Transplantings Compared with "Direct Seeding" ("t" Test for Significance)

Treatment Comparison	Node Number to First Cluster			Node Number to Second Cluster			Days to Anthesis		
	df	Difference in Mean	"t" Value	df	Difference in Mean	"t" Value	df	Difference in Mean	"t" Valu
Control versus one application gibberellin									
Bn	55	+1.38***	4.312	55	-0.25	0.714	55	+2	1.63
bn	10	+0.89	1.254	10	+1.00	0.485	10	+2	2.12
Control versus two applications gibberellin									
Bn	33	+1.59*	2.409	33	+0.73	1.431	33	-3	1.53
bn	13	+4.73***	8.017	13	+8.50***	4.474	13	+7**	3.44
Control versus three applications gibberellin									
Bn	35	+1.69**	3.018	35	+0.44	0.880	34	-3	1.6
bn	13	+4.73***	6.392	13	+0.34	0.103	13	0	0
Control (two transplants) versus "direct seeded"									
Bn	58	+0.30	0.938	58	-1.18***	4.538	58	-18***	16.9
bn	22	-5.31***	10.620	22	-2.06	1.321	22	-31***	13.9

*Difference significant at the 5 per cent level.
**Difference significant at the 1 per cent level.
***Difference significant at the 0.1 per cent level.

which might result in a reduced node number to the first cluster and possibly a reduction in the number of days to reach anthesis.

A comparison of the "direct seeded" progeny with that of the control (gibberellin series) (Table 19) shows that a highly significant (0.1 per cent) reduction in days to anthesis had occurred in both bn and Bn plants. Since these two progeny differed in a number of ways, it is necessary to evaluate the contribution made by each factor. Obviously one of the more important was the growth check received by the control plants from exposure to low night temperatures at one and one-half months of age, which would effect both bn and Bn.

A further difference resulting in the same effect, but from a different cause, was the check in growth of the controls resulting from two transplantings. As the name states, the "direct seeded" received no such treatment. It should be mentioned this group was titled "direct seeded" in that this was the original variable planned. The cold treatment was unplanned.

Another important factor was the difference in planting dates which subjected the "direct seeded" plants to the more favorable environment of early and late spring for a longer period and at a younger age. One of the most important of these environmental factors was the increased amount of radiant energy (both intensity and a photoperiod) received by the "direct seeded" plants. The rate of photosynthesis would be increased, and if other factors were not limiting would in turn produce a considerably greater continual carbohydrate supply than in the control. To be sure, during the period of cold treatment a check in growth occurred which resulted in

a build up of reserve carbohydrates in the controls. However, this supply was soon depleted after the night temperature was raised and was not replenished in sufficient amount due to the limited light conditions still in evidence at that time. Therefore, the aforementioned factors were those which contributed to the reduction in the number of days to anthesis in the "direct seeded" progeny as compared to the control.

Although the number of days to reach anthesis was reduced to a highly significant degree in both bn and Bn, a greater response was displayed by bn. It is quite obvious that the significant decrease in the node number with bn, which did not occur in Bn, would account for this difference. Assuming one or both of these treatments (cold or "direct seeded") was the cause of a reduced node number in bn, it is necessary to conclude that Bn and bn were at different stages of development, or an inherent difference in sensitivity to this (these) treatment(s) existed.

A detailed explanation with regard to Table 20 need not be given. Treatment in every case resulted in a significant increase (at the 0.1 per cent level) in node number to the first cluster. Whereas Bn responded only to the 5 per cent level as shown in Table 19 (two applications), this progeny showed a response of both bn and Bn at the 0.1 per cent level. Therefore, an actual difference between Bn and bn in this respect may or may not have existed. It, however, might be of interest to determine if one application of gibberellin, applied at the time the second would usually be given,

TABLE 20

Effect upon Node Number to the First Cluster and from the First to the Second Cluster of Bn and bn Plants (Progeny of bn by sl(?) Cross) Treated with Two Applications of Gibberellin (100 ppm/application) Using the "t" Test for Significance

	Mean Node Number to the First Cluster				Mean Node Number from First to the Second Cluster			
	Mean Number	Mean Difference	df	"t" Value	Mean Number	Mean Difference	df	"t" Value
Control								
Bn	8.70 ± 0.59#				3.60 ± 0.75			
bn	17.25 ± 0.80	+8.55***	12	23.750	11.33 ± 11.36	+7.73*	11	2.906
Treated								
Bn	11.45 ± 0.47				3.73 ± 0.67			
bn	20.89 ± 0.46	+9.44***	18	33.714	16.88 ± 1.99	+13.15***	17	14.775
Bn								
Control	8.70 ± 0.59				3.60 ± 0.75			
Treated	11.45 ± 0.47	+2.75***	19	8.333	3.73 ± 0.67	+0.13	19	0.289
bn								
Control	17.25 ± 0.80				11.33 ± 11.36			
Treated	20.89 ± 0.46	+3.64***	11	11.375	16.88 ± 1.99	+5.55	9	2.004

*Difference significant at the 5 per cent level.

**Difference significant at the 1 per cent level.

***Difference significant at the 0.1 per cent level.

#Confidence interval at the 5 per cent level.

would result in a difference in response. Such an experiment, and one in which the plants were subjected to cold treatment at various stages of growth, might indicate whether a genuine or spurious difference existed with respect to the stage at which node number was determined in Bn and bn plants.

Changes in leaf shape which had previously been reported by others, Bukovac et al. (5) and Rappaport (32), were also seen by the author. For example, leaves were observed which resembled a potato leaf type, margins of which were nearly entire, and also were more narrow and more tapered. The leaves and cotyledons were a light green in color and formed a more acute angle at the stem axis which became evident about one or two days following treatment. The gibberellin treated plants all exhibited a spindly growth and especially a thin stem in the region of rapid elongation. This latter characteristic might not have been as pronounced if grown during late spring and early summer.

In conclusion, a greater node number to the first cluster, a greater node number from the first to the second cluster, and a greater number of days required to reach anthesis were characteristic of bn plants as compared to Bn. These differences were significant at the 0.1 per cent level. The differences between bn and Bn were modified somewhat by treatment, but caused no difficulty in distinguishing bn from Bn. The experiments with the gibberellin and the "direct seeded" (cold treatment) series have also raised interesting

questions. By no means the least important of these would be, whether node number determination occurred at a different stage of development or whether an inherent difference in sensitivity to low temperatures and/or direct seeding existed between the mutant and fertile siblings.

Mutant *cl*$_3$

Genetic Studies

The male sterile parent *cl*$_3$ contributed the genes *cl*$_3$, *c*, and *u*. The variety Rutgers used as the male parent possessed the dominant alleles. Therefore, should linkage be shown to occur, these genes would be transmitted in coupling from parent to offspring. A total of 51 F_1 plants constituted the F_1 generation. All plants displayed the phenotypic characteristics of the dominant genes contributed by Rutgers. Therefore, a recessive gene(s) was indicated as responsible for the phenotype of *cl*$_3$.

A chi-square analysis similar to that described for *bn* was conducted with *cl*$_3$, which tested the goodness of fit of *cl*$_3$ to a 3:1 F_2 ratio and 1:1 backcross ratio. Table 21 contains the complete chi-square analysis for the total of all F_2 and all backcross families, and for the subdivisions in which families were grouped according to location and year of the test. Table 22 shows that a deficiency of mutants occurred but did not reach a level of significance in any category. A reduction in the viability of this mutant is indicated, not only from the pooled data, but from the

TABLE 21

Chi-Square Analysis for Goodness of Fit of $\underline{cl}_3$ to a 3:1 F_2 Ratio and 1:1 Backcross Ratio (Data Pooled According to Location and Year)

Location and Year	Generation	Total			Pooled			Heterogeneity		
		df*	x^2	P	df	x^2	P	df	x^2	P
Field										
1957	F_2	12	12.3325	0.40-0.50	1	3.7936	0.05-0.10	11	8.5389	0.60-0.70
	BC	5	1.4526	0.90-0.95	1	0.3636	0.50-0.60	4	1.0890	0.80-0.90
Greenhouse										
1957-58	F_2	-	-	-	1	0.1777	0.60-0.70	-	-	-
1958	F_2	-	-	-	1	0.0303	0.80-0.90	-	-	-
Total	F_2	14	12.5405	0.50-0.60	1	3.5856	0.05-0.10	13	8.9549	0.70-0.80
Total	BC	5	1.4526	0.90-0.95	1	0.3636	0.50-0.60	4	1.0890	0.80-0.90

*Degrees of freedom and number of families.

TABLE 22

Segregation of $\underline{cl}_3$ in the F_2 and Backcross Generation
(Data Pooled According to Location and Year)

Location	Generation	Expected Ratio	Total Number Plants	Observed Number $\underline{cl}_3$ Plants	Expected Number $\underline{cl}_3$ Plants	Deviation	Pooled		
							df	x^2	P
Field									
1957	F_2	3:1	620	134	155.00	-21.00	1	3.7936	0.05-0.10
	BC	1:1	44	20	22.00	- 2.00	1	0.3636	0.50-0.60
Greenhouse									
1957-8	F_2	3:1	120	28	30.00	- 2.00	1	0.1777	0.60-0.70
1958	F_2	3:1	99	24	24.75	- 0.75	1	0.0303	0.80-0.90
Total	F_2	3:1	839	186	209.75	-23.75	1	3.5856	0.05-0.10
Total	BC	1:1	44	20	22.00	- 2.00	1	0.3636	0.50-0.60

heterogeneity test which shows that the deficiency occurred consistently between families. The fact remains that even though a reduced viability is indicated, the chi-square analysis supports the hypothesis. Therefore, the mode of inheritance of the mutant $\underline{cl}_3$ was shown by these results to be determined by a single recessive gene.

A chi-square analysis was conducted with $\underline{cl}_3$ to test the goodness of fit of $\underline{cl}_3$ and $\underline{c}$, $\underline{cl}_3$ and $\underline{u}$, each to a 9:3:3:1 F_2 ratio, and all three genes to a trihybrid F_2 ratio. Also the male-sterile mutant $\underline{cl}_3$ was of the type that a backcross test for segregation of $\underline{cl}_3$ and $\underline{c}$ to a 1:1:1:1 ratio could be performed. The complete analysis of the F_2 progeny is shown in Table 23. Not a single value for any of the combinations approaches significance. Little further need be said concerning these data since all combinations and the respective components, including the interaction chi-square values, show a good fit to the hypothesized ratios. The segregation data for the dihybrid crosses are found in Tables 24 and 25 and that of the trihybrid cross in Table 26. Further confirmation for independent assortment between genes $\underline{cl}_3$ and $\underline{c}$ is available from the chi-square analysis of the backcross data in Table 27 and the corresponding segregation data in Table 28. These data (both F_2 and backcross) indicate independent assortment of $\underline{cl}_3$ and $\underline{c}$, and also between $\underline{cl}_3$ and $\underline{u}$. Therefore, $\underline{cl}_3$ would not be located in either chromosome 6 (linkage group IV) nor chromosome 10 (linkage group VII) or would be sufficiently removed in distance from either gene $\underline{c}$ or $\underline{u}$ to assort independently.

TABLE 23

Chi-Square Analysis for Goodness of Fit of $\underline{cl}_3$, $\underline{c}$, and $\underline{u}$ to a 27:9:9:9:3:3:3:1 F_2 Ratio; of $\underline{cl}_3$ and $\underline{c}$ to a 9:3:3:1 F_2 Ratio; of $\underline{cl}_3$ and $\underline{u}$ to a 9:3:3:1 F_2 Ratio; and of $\underline{cl}_3$, $\underline{c}$, and $\underline{u}$ Each to a 3:1 F_2 Ratio as Components in the Dihybrid and Trihybrid Crosses

Gene or Genes	Expected F_2 Ratio*	Total			Pooled			Heterogeneity		
		df	x^2	P	df	x^2	P	df	x^2	P
$\underline{cl}_3$ and $\underline{c}$	9:3:3:1	39	39.2786	0.40-0.50	3	3.9472	0.20-0.30	36	35.3314	0.40-0.50
$\underline{cl}_3$	3:1	13	12.3628	0.40-0.50	1	3.5091	0.05-0.10	12	8.8537	0.70-0.80
$\underline{c}$	3:1	13	11.6058	0.50-0.60	1	0.2897	0.50-0.60	12	11.3161	0.40-0.50
$\overline{I}$**	-	13	15.3100	0.20-0.30	1	0.1484	0.60-0.70	12	15.1616	0.10-0.20
$\underline{cl}_3$ and $\underline{u}$	9:3:3:1	6	3.3082	0.70-0.80	3	2.5403	0.40-0.50	3	0.7679	0.80-0.90
$\underline{cl}_3$	3:1	2	0.2080	0.90-0.95	1	0.1841	0.60-0.70	1	0.0239	0.80-0.90
$\underline{u}$	3:1	2	3.0093	0.20-0.30	1	2.3151	0.10-0.20	1	0.6942	0.40-0.50
$\overline{I}$	-	2	0.0909	0.95-0.97	1	0.0411	0.80-0.90	1	0.0498	0.80-0.90
$\underline{cl}_3$, $\underline{c}$, and $\underline{u}$	27:9:9:9: 3:3:3:1	-	-	-	7	6.9004	0.40-0.50	-	-	-
$\underline{cl}_3$ and $\underline{c}$	9:3:3:1	-	-	-	3	6.1763	0.10-0.20	-	-	-
$\underline{cl}_3$ and $\underline{u}$	9:3:3:1	-	-	-	3	0.2861	0.95-0.97	-	-	-
$\underline{cl}_3$	3:1	-	-	-	1	0.0303	0.80-0.90	-	-	-
$\underline{c}$	3:1	-	-	-	1	3.6667	0.05-0.10	-	-	-
$\underline{u}$	3:1	-	-	-	1	0.1649	0.60-0.70	-	-	-
$\overline{I}$ ($\underline{cl}_3$ and $\underline{c}$)	-	-	-	-	1	2.4793	0.10-0.20	-	-	-
I ($\underline{cl}_3$ and $\underline{u}$)	-	-	-	-	1	0.0909	0.70-0.80	-	-	-

*See corresponding segregation table for genotypic classes.

**"I" refers to the interaction chi-square value.

TABLE 24

Segregation in the F_2 Generation for $\underline{cl_3}$ and $\underline{c}$

Genotype	Expected F_2 Ratio	Number Observed in Each Class	Number Expected in Each Class	Deviation
$\underline{cl_3}$ and $\underline{c}$				
Cl_3-C-	9/16	418	404.4375	+13.5625
Cl_3-cc	3/16	143	134.8125	+ 8.1875
cl_3cl_3C-	3/16	115	134.8125	-19.8125
cl_3cl_3cc	1/16	43	44.9375	- 1.9375
$\underline{cl_3}$				
Cl_3-	3/4	561	539.2500	+21.7500
cl_3cl_3	1/4	158	179.7500	-21.7500
$\underline{c}$				
C-	3/4	533	539.2500	- 6.2500
cc	1/4	186	179.7500	+ 6.2500

TABLE 25

Segregation in the F_2 Generation for $\underline{cl_3}$ and $\underline{u}$

Genotype	Expected F_2 Ratio	Number Observed in Each Class	Number Expected in Each Class	Deviation
$\underline{cl_3}$ and $\underline{u}$				
Cl_3-U-	9/16	132	123.1875	+ 8.8125
Cl_3-uu	3/16	35	41.0625	- 6.0625
cl_3cl_3U-	3/16	42	41.0625	+ 0.9375
cl_3cl_3uu	1/16	10	13.6875	- 3.6875
$\underline{cl_3}$				
Cl_3-	3/4	167	164.2500	+ 2.7500
cl_3cl_3	1/4	52	54.7500	- 2.7500
$\underline{u}$				
U-	3/4	174	164.2500	+ 9.7500
uu	1/4	45	54.7500	- 9.7500

TABLE 26

Segregation in the F_2 Generation for $\underline{cl_3}$, $\underline{c}$, and $\underline{u}$

Genotype	Expected F_2 Ratio	Number Observed in Each Class	Number Expected in Each Class	Deviation
$\underline{cl_3}$, $\underline{c}$ and $\underline{u}$				
Cl_3-C-U-	27/64	41	41.7663	- 0.7663
Cl_3-C-uu	9/64	12	13.9221	- 1.9221
Cl_3-cc-U-	9/64	16	13.9221	+ 2.0779
cl_3cl_3C-U-	9/64	10	13.9221	- 3.9221
Cl_3-ccuu	3/64	6	4.6407	+ 1.3593
cl_3cl_3C-uu	3/64	3	4.6407	- 1.6407
cl_3cl_3ccU-	3/64	9	4.6407	+ 4.3593
cl_3cl_3ccuu	1/64	2	1.5469	+ 0.4531
$\underline{cl_3}$ and $\underline{c}$				
Cl_3-C-	9/16	53	55.6875	- 2.6875
Cl_3-cc	3/16	22	18.5625	+ 3.4375
cl_3cl_3C-	3/16	13	18.5625	- 5.5625
cl_3cl_3cc	1/16	11	6.1875	+ 4.8125
$\underline{cl_3}$ and $\underline{u}$				
Cl_3-U-	9/16	57	55.6875	+ 1.3125
Cl_3-uu	3/16	18	18.5625	- 0.5625
cl_3cl_3U-	3/16	19	18.5625	+ 0.4375
cl_3cl_3uu	1/16	5	6.1875	- 1.1875
$\underline{cl_3}$				
Cl_3-	3/4	75	74.2500	+ 0.7500
cl_3cl_3	1/4	24	24.7500	- 0.7500
$\underline{c}$				
C-	3/4	66	74.2500	- 8.2500
cc	1/4	33	24.7500	+ 8.2500
$\underline{u}$				
U-	3/4	76	74.2500	+ 1.7500
uu	1/4	23	24.7500	- 1.7500

TABLE 27

Chi-Square Analysis for Goodness of Fit of $\underline{cl}_3$ and $\underline{c}$ to a 1:1:1:1 Backcross Ratio, and of $\underline{cl}_3$ and $\underline{c}$ Each to a 1:1 Backcross Ratio as Components in the Dihybrid Backcross

Gene or Genes	Expected F_2 Ratio*	Total			Pooled			Heterogeneity		
		df	x^2	P	df	x^2	P	df	x^2	P
$\underline{cl}_3$ and $\underline{c}$	1:1:1:1	15	6.3463	0.95-0.97	3	1.2727	0.70-0.80	12	5.0736	0.95-0.97
$\underline{cl}_3$	1:1	5	1.4526	0.90-0.95	1	0.3636	0.50-0.60	4	1.0890	0.80-0.90
$\underline{c}$	1:1	5	2.1192	0.80-0.90	1	0.0908	0.70-0.80	4	2.0284	0.70-0.80
I**		5	2.7745	0.70-0.80	1	0.8183	0.30-0.40	4	1.9562	0.70-0.80

*See corresponding segregation table for genotypic classes.

**"I" refers to the interaction chi-square value.

TABLE 28

Segregation in the Backcross Generation for $\underline{cl_3}$ and $\underline{c}$

Genotype	Expected F_2 Ratio	Number Observed in Each Class	Number Expected in Each Class	Deviation
$\underline{cl_3}$ and $\underline{c}$				
Cl_3cl_3Cc	1/4	11	11	0
Cl_3cl_3cc	1/4	13	11	+ 2
cl_3cl_3Cc	1/4	12	11	+ 1
cl_3cl_3cc	1/4	8	11	- 3
$\underline{cl_3}$				
Cl_3cl_3	1/2	24	22	+ 2
cl_3cl_3	1/2	20	22	- 2
$\underline{c}$				
Cc	1/2	23	22	+ 1
cc	1/2	21	22	- 1

Phenotypic Stability and Pleiotropism

The phenotypic characteristics which had been associated with $\underline{cl}_3$ (cleistogamous flower, exerted stigma, and a protuberance on the stylar end of the fruit) were found to be inherited as a unit in all progeny, both F_2 and backcross generations. The cleistogamous condition of the mutant flower presented little difficulty in classification. Only toward the latter part of the growing season, and during mid-winter did the flowers open to any extent, and they did not approach normal anthesis with any regularity. An occasional flower could be found during mid-winter in which the petals were perpendicular to the floral axis, but by early spring such flowers could rarely be found. The aforementioned data were obtained by daily examination of the flowers from mid-winter until early spring. Late season field conditions produced an occasional partially open flower; however, by that time, through numerous previous examinations, plants had been firmly established as containing either normal or cleistogamous flowers. There can be little doubt as to the accuracy of classification for this feature.

An effort was made to classify the progeny by the degree to which the stigma was exerted. The main purpose was to determine if any recombinants between cleistogamy and stigma exertion of an obvious nature could be found. Dealing with such large numbers of plants a rather arbitrary method of classification was needed. The degree to which the stigma was exerted (or inserted) was variable from one examination date to the next. The method used in classification was to establish at each date a conception of the degree to

which the stigma was exerted for both fertile and sterile plants (cleistogamous condition used for this purpose) and note the relative difference in exertion between fertile and sterile plants. Then a sufficiently large number of flowers per plant was observed and a decision was reached as to the proper classification. A final decision would then be made on the basis of the periodic examination. Any questionable plants were rechecked. Unless the plants consistently exhibited recombinant characteristics they would not be classed as such in the final analysis. No such plants could definitely be said to have been found.

Fruit shape was determined by artificial pollination of all sterile plants. A slight degree of variability was experienced with the expression of fruit shape. However, on the basis of the percentage of fruits per plant falling into each group, an accurate means of classifying could be accomplished. The greenhouse 1957-58 progeny were used, and the results in Table 29 show the complete association of abnormal fruits with sterile plants, if plants containing at least 50 per cent abnormal fruits are classed as possessing this character. If a sufficient number of fruits are classed per plant the reverse could also be used, that is, plants with at least 50 per cent normal fruits classed as producers of normal shaped fruits. Actually one would not err in any large measure if plants were scored as abnormal, if they produced one or two abnormal fruits. If, however, the reverse method were used, i.e., classifying plants as normal, if they possessed one normal fruit, a greater error would be

TABLE 29

Fruit Shape in $\underline{cl_3}$

Genotype	Total Number of Plants	Average Number of Fruits Examined per Plant	At Least One Normal Fruit		At Least One Abnormal Fruit*		At Least 50% Normal Fruits per Plant		At Least 50% Abnormal Fruits per Plant	
			Number of Plants	Percentage of Plants	Number of Plants	Percentage of Plants	Number of Plants	Percentage of Plants	Number of Plants	Percentage of Plants
Cl_3-	92	5.40	92	100	2	2	92	100	0	0
cl_3cl_3	28	4.00	13	46	28	100	2**	7	28	100

*Abnormal refers to protuberance on stylar end similar to beaked fruits and/or a persistent style.

**Only two fruits present on each of these 2 plants.

encountered. Therefore, a plant homozygous for the $\underline{cl}_3$ gene would have a cleistogamous flower with an exerted stigma and a protruberance at the stylar end of the fruit and/or a persistent style.

Cause of Unfruitfulness

In an effort to resolve the cause or causes of unfruitfulness in the mutant $\underline{cl}_3$, two main areas were investigated. The first of these was to determine if the gynoecium and the androecium were capable of functioning. Secondly, to determine if any change in floral morphology occurred which would lower the rate of, or produce a barrier to, self-pollination.

The mutant Cl_1Cl_1 reported by Rick and Robinson (43) displayed a cleistogamous condition similar to $\underline{cl}_3$. The cause of unfruitfulness could be ascribed to a pre-mature abscission of the flower. Such a possibility with $\underline{cl}_3$ was, therefore, investigated. A comparison was made between Cl_3 and $\underline{cl}_3$ for a significant difference in the number of days from anthesis to abscission. This was accomplished by tagging individual flowers of both fertile and sterile plants, examining daily, and noting the day on which anthesis and abscission occurred. Either of two days could have been selected for $\underline{cl}_3$ as the day of anthesis. Table 30 shows that 3.95 days elapsed between anthesis and abscission for $\underline{cl}_3$. A difference of 1.05 days (when the second day was used as the day on which anthesis took place) was observed between Cl_3 and $\underline{cl}_3$, which was not a significant reduction at either the 1 or 5 per cent level. Had the first day been used this difference would, of course, have been

TABLE 30

Number of Days Following Anthesis on Which Abscission Occurred in Cl_3 Contrasted with $\underline{cl}_3$

Genotype	Total Number of Flowers Tagged	Number of Flowers Abscissed		Day on Which Anthesis Occurred (Average)	Mean Number of Days Between Anthesis and Abscission	Difference in Mean Number of Days from Anthesis to Abscission in Cl_3 and $\underline{cl}_3$	Difference Required for Significance	
		Stigma Not Exerted	Stigma Exerted				1% Level	5% Level
Cl_3	7	1	3	9/14	5.00	1.05	2.72	2.00
$\underline{cl}_3$	19	2	17	9/12-13	3.95			

only 0.05 days. It should be noted that this was not the first of such tests. Previously (latter part of August) many flowers were tagged but premature abscission took place with both Cl_3 and $\underline{cl}_3$, often occurring prior to, or at anthesis. Higher temperatures and drying winds were prevalent during that period and were probably responsible. At the time at which anthesis took place for the flowers in Table 30, the temperature did not exceed 85° F. (Appendix Table 43) and drying winds were not notably brisk immediately preceding, at, or following anthesis. Certainly a larger number of flowers, especially with Cl_3, would have been desirable. However, these results, and the fact that $\underline{cl}_3$ will set fruit (see data to follow), strongly indicate that premature abscission was not the cause or played only a small part in the unfruitfuoness which characterized $\underline{cl}_3$. An association between abscission and an exerted stigma is also indicated by the data in Table 30.

The percentage of normal appearing pollen grains for each mutant is given in Appendix Table 45. The pollen was scored (on various dates) according to their shape, size, and the intensity of the stain. The results obtained with each mutant can then be compared with the percentage of normal-appearing pollen grains recorded on the same dates from plants of proven fertility. All mutants (bn, $\underline{cl}_3$, and sl(?)) produced a high percentage of stainable, normal-appearing pollen. Had these data shown the mutants to produce pollen significantly lower than that of the fertile plants, inviability of the pollen grains would have been indicated at that point. However,

it would not be correct to assume that pollen grains which appeared normal were necessarily viable. Therefore, the following tests were conducted to determine if both the pollen and the gynoecium of $\underline{cl}_3$ were capable of functioning. Pollen of $\underline{cl}_3$ was artificially applied to the stigma's of emasculated flowers of the Rutgers variety. A high percentage of fruit was set (Table 31) which contained a good quantity of seed (insofar as this test is concerned). The F_2 generation from this cross segregated according to expectation for both the gene $\underline{cl}_3$ and $\underline{c}$, proving the seed to be of hybrid origin. Therefore, $\underline{cl}_3$ pollen was viable and cannot be considered the cause of sterility.

TABLE 31

Test of Pollen Viability of $\underline{cl}_3$ Using Rutgers Variety as the Female Parent (Artificially Cross-Pollinated)

Total Number of Flowers Pollinated*	Total Number of Fruits Set**	Percentage of Flowers Which Set Fruit
10	7	70

*Emasculated two days prior to pollination.

**Average of 60 seeds per fruit.

The next question to be answered concerned the fertility of the gynoecium. Pollen of known fertility was used to artificially cross-pollinate $\underline{cl}_3$ flowers. The date of pollination was noted, so that the effect of environment on fruit set could be taken into account. Although the pollen of $\underline{cl}_3$ was shown to be fertile (Table 32), the possibility of self-incompatibility yet remained. Therefore, in addition to the artificial cross-pollinations, $\underline{cl}_3$ plants were also artificially self-pollinated.

The results obtained by artificial self-pollination are found in Table 33. The first rather obvious feature in both Tables 32 and 33 would be the great difference in fruit set at the three dates of pollination. By referring to the temperature data in Appendix Table 43, these data in part can be explained. Stigmas exerted to 2.0 mm and beyond were relatively common on the 4th and 5th and presumably can be related to the high temperatures which prevailed just prior to these dates. Low temperatures (reaching a low of 47 on the 5th and 44 on the 6th) immediately following pollination would most certainly have reduced the rate of pollen tube growth. Perhaps most important of all in explaining the lack of fruit set on these dates (4th and 5th) was that at the time of pollination, warm, strong, drying winds were occurring. Favorable environmental conditions existed prior to, on the 9th, and immediately following pollination on this date, as compared with the earlier pollination dates. That is, there was no noticeable drying winds, temperatures were moderate

TABLE 32

Record of Fruit Set of $\underline{cl}_3$ Artificially Cross-Pollinated with Pollen from Plants of Known Fertility (Classed According to the Degree to Which the Stigma Was Exerted at the Time of Pollination)

Position of the Stigma (mm Exerted)	Total No. of Flowers Pollinated	Total No. of Fruits Set*	% of Flowers Which Set Fruit	Date Pollinated 9/4/57			9/5/57			9/9/57		
				No. of Flowers Pollinated	No. of Fruits Set	% of Flowers Which Set Fruit	No. of Flowers Pollinated	No. of Fruits Set	% of Flowers Which Set Fruit	No. of Flowers Pollinated	No. of Fruits Set	% of Flowers Which Set Fruit
0	11	2	18	2	0	0	6	0	0	3	2	67
0.1-1.0	7	2	29	2	0	0	2	0	0	3	2	67
1.1-2.0	7	0	0	2	0	0	2	0	0	3	2	0
2.1-3.0	4	0	0	2	0	0	2	0	0	0	-	-
3.0+	4	0	0	2	0	0	2	0	0	0	-	-
Total	33	4	12	10	0	0	14	0	0	9	4	44

*Average of 65 seeds per fruit.

TABLE 33

Record of Fruit Set of $\underline{cl}_3$ Artificially Self-Pollinated
(Classed According to the Degree to Which
the Stigma Was Exerted at the Time of Pollination)

Position of the Stigma (mm Exerted)	Total No. of Flowers Pollinated	Total No. of Fruits Set*	% of Flowers Which Set Fruit	Date Pollinated								
				9/4/57			9/5/57			9/9/57		
				No. of Flowers Pollinated	No. of Fruits Set	% of Flowers Which Set Fruit	No. of Flowers Pollinated	No. of Fruits Set	% of Flowers Which Set Fruit	No. of Flowers Pollinated	No. of Fruits Set	% of Flowers Which Set Fruit
0	8	2	25	3	0	0	2	1	50	3	1	33
0.1-1.0	11	2	18	6	0	0	2	0	0	3	2	67
1.1-2.0	10	1	10	5	0	0	2	0	0	3	1	33
2.1-3.0	7	0	0	3	0	0	2	0	0	2	0	0
3.0+	4	0	0	3	0	0	1	0	0	0	0	0
Total	40	5	12	20	0	0	9	1	11	11	4	36

*Average of 40 seeds per fruit.

(thus in part explaining the deficiency of flowers with stigmas exerted beyond 2.0 mm) but sufficiently high to be favorable for pollen tube growth and subsequent fertilization. On the morning of the 9th, 0.5" rainfall occurred. If, therefore, a water deficiency had been present, this, temporarily at least, would have been alleviated. Before proceeding to draw conclusions from these data it would be most helpful to know the amount of fruit set which took place on the same plants and on the same dates (especially the 9th) in flowers dependent entirely upon natural self-pollination (or natural cross-pollination). Table 34 shows that no fruit was set on September 5th or 9th with $\underline{cl}_3$ flowers which were dependent upon a natural means of pollen transfer. In view of this fact, and the data previously presented, it may therefore be concluded that neither pollen sterility nor a defective gynoecium would account (in any measurable degree) for the high level of unfruitfulness observed with the mutant $\underline{cl}_3$.

Thus, it became obvious a barrier was present which prevented or greatly reduced the transfer of pollen to the stigma in $\underline{cl}_3$. Consequently, the relationship between stigma exertion and self-pollination in both $\underline{cl}_3$ and Cl_3 was evaluated. From the number of flowers which fell into each designated class in Table 35, the percentages were determined and presented on a per date basis in Table 36. If dissection of the flower did not bring about dehiscence of the pollen in $\underline{cl}_3$, these data will immediately eliminate indehiscence of the pollen as the cause of unfruitfulness in $\underline{cl}_3$. The

TABLE 34

Record of Fruit Set of $\underline{cl}_3$ Plants Dependent Upon Natural Self-Pollination or Natural Cross-Pollination

Position of the Stigma	Total Number of Flowers Tagged	Total Number of Fruits Set	Percentage of Flowers Which Set Fruit	Date Tagged (Anthesis) 9/5/57			9/9/57		
				Number of Flowers Tagged	Number of Fruits Set	Percentage of Flowers Which Set Fruit	Number of Flowers Tagged	Number of Fruits Set	Percentage of Flowers Which Set Fruit
Not exerted	13	0	0	3	0	0	10	0	0
Exerted	20	0	0	10	0	0	10	0	0

TABLE 35

Relationship Between the Date of Examination, Stigma Position at Anthesis, and Self-Pollination in Flowers of Cl_3 and $\underline{cl}_3$

Date	Genotype	Total No. of Flowers Dissected	Number of Flowers Dissected with the Stigma Not Exerted				Number of Flowers Dissected with the Stigma Exerted			
			Total	Pollen on Stigma	Pollen on Style Only	No Pollen Shed	Total	Pollen on Stigma	Pollen on Style Only	No Pollen Shed
8/25/57	Cl_3-	13	4	2	2	0	9	1	8	0
	cl_3cl_3	13	0	0	0	0	13	0	12	1
8/26/57	Cl_3-	30	1	1	0	0	29	13	15	1
	cl_3cl_3	38	0	0	0	0	30	4	29	5
9/ 3/57	Cl_3-	24	8	6	1	1	16	3	12	1
	cl_3cl_3	27	0	0	0	0	27	0	25	2
9/ 4/57	Cl_3-	7	3	1	0	2	4	1	3	0
	cl_3cl_3	11	1	0	0	1	10	0	8	2
9/ 6/57	Cl_3-	6	2	1	1	0	4	1	1	2
	cl_3cl_3	6	2	0	0	2	4	0	3	1
9/ 7/57	Cl_3-	23	18	13	2	3	5	2	1	2
	cl_3cl_3	20	9	0	6	3	11	0	11	0
9/14/57	Cl_3-	35	23	21	2	0	12	4	7	1
	cl_3cl_3	35	6	5	0	1	29	2	25	2

TABLE 35 (contd.)

Date	Genotype	Total No. of Flowers Dissected	Number of Flowers Dissected with the Stigma Not Exerted				Number of Flowers Dissected with the Stigma Exerted			
			Total	Pollen on Stigma	Pollen on Style Only	No Pollen Shed	Total	Pollen on Stigma	Pollen on Style Only	No Pollen Shed
9/25/57	Cl_3-	20	11	8	2	1	9	4	4	1
	cl_3cl_3	20	9	6	3	0	11	2	6	3
5/ 6/58	Cl_3-	10	7	6	1	0	3	3	0	0
	cl_3cl_3	14	4	4	0	0	10	4	6	0
5/ 8/58	Cl_3-	10	7	5	1	1	3	1	2	0
	cl_3cl_3	11	4	4	0	0	7	2	4	1

TABLE 36

Relationship Between Date of Examination, Stigma Position at Anthesis, and Self-Pollination in Flowers of Cl_3 and $\underline{cl}_3$ (Percentage)

Date	Flowers with Stigma Not Exerted				Flowers with Stigma Exerted				Total Flowers Examined Which Shed Pollen of Genotype		Total Flowers Examined with Pollen on Stigma of Genotype	
	Total Examined of Genotype		Pollen Observed on Stigma of Genotype		Total Examined of Genotype		Pollen Observed on Stigma of Genotype					
	Cl_3	cl_3	Cl_3	cl_3	Cl_3	cl_3	Cl_3	cl_3	Cl_3	cl_3	Cl_3	cl_3
	%	%	%	%	%	%	%	%	%	%	%	%
8/25/57	31	0	50	-	69	100	11	0	100	92	23	0
8/26/57	3	0	100	-	97	100	45	11	97	87	47	10
9/ 3/57	33	0	75	-	67	100	19	0	92	93	38	0
9/ 4/57	43	9	33	0	57	91	25	0	71	73	29	0
9/ 6/57	33	33	50	0	67	67	25	0	67	50	33	0
9/ 7/57	78	45	72	0	22	55	40	0	78	85	65	0
9/14/57	66	17	91	83	34	83	33	7	97	91	71	20
9/25/57	55	45	73	67	45	55	44	18	90	85	60	40
5/ 6/58	70	29	86	100	30	71	100	40	100	100	90	57
5/ 8/58	70	36	71	100	30	64	33	29	90	91	60	55

fate of this pollen in reference to the position of the stigma at anthesis in both Cl_3 and $\underline{cl}_3$ will be presented referring at present only to the field data (August 25, 1957 through September 25, 1957).

The percentages of flowers of Cl_3 and $\underline{cl}_3$ with inserted stigmas differed greatly, excepting on the 6th, 7th, and 25th. These dates also correspond to the lowest temperature recorded for all examination dates. However, of these three dates only on the 25th was pollen in any measurable amount found on the stigmas of $\underline{cl}_3$. Although on the 14th a high percentage of the inserted flowers did contain pollen, only a small percentage of all $\underline{cl}_3$ flowers were inserted on this date. In contrast, Cl_3 had both a relatively high percentage of flowers with an inserted stigma and also a relatively high percentage which were pollinated. In reference to flowers with an exerted stigma, such a condition appeared to be an effective barrier to self-pollination in $\underline{cl}_3$. This, however, was not the case with Cl_3, which exhibited at all dates a fair amount of self-pollination. Three major conclusions may be drawn from these data. One, a significant difference in the percentage of flowers with an exerted stigma existed between fertile and sterile plants. This difference became less marked during what appeared to be conditions of lower temperatures and/or reduced light intensity and shorter photoperiods. Two, stigma exertion in $\underline{cl}_3$ presented a strong barrier to self-pollination which was not necessarily the case with the fertile segregates. Three, stigma insertion in $\underline{cl}_3$ did by no means insure self-pollination, only on the 25th were both the percentage of flowers with an inserted stigma and percentage

pollinated at a high enough level to indicate a fruit set of any possible significance. However, with Cl_3, insertion of the stigma resulted in a high percentage of flowers which were self-pollinated. An observation which did not lend itself to presentation in tabular form should be included. A noticeably smaller quantity of pollen was found on the stigmas of $\underline{cl}_3$, and quite often was restricted to the margin or "underside" of the stigma. These flowers were, nevertheless, categorized in the table as having been self-pollinated.

One should not lose sight of the fact that these data were by and large obtained toward the latter part of the season. Therefore, it may be assumed that the data from August 25, 1957 to September 4, 1957 are probably more indicative than those of September 25, 1957 of the amount of self-pollination which would occur in Cl_3 and $\underline{cl}_3$ during the early part of the season (prior to August 25, 1957). Also the data herein presented, which indicate a higher percentage of self-pollination in $\underline{cl}_3$ towards the latter part of the season are in agreement with the fruitfulness records presented at the beginning of this section which also indicated fruit set to be greater toward the end of the season.

The greenhouse data present a picture similar to the field data in regard to the increase in stigma exertion of $\underline{cl}_3$ as compared with Cl_3. However, the difference was not nearly so great, and of most significance would be the percentage of $\underline{cl}_3$ flowers which were self-pollinated, including a rather high percentage of flowers in which the stigmas were exerted. The cause of this decrease in stigma

exertion may hinge on several factors peculiar to the cultural practices which were used with greenhouse plants. Perhaps the most important are those factors which would tend to reduce the amount of readily available nitrogen. It may be recalled that these plants were grown in four-inch pots, with a 15-30-15 fertilizer applied periodically. As a consequence of a limited root system (pot size), a water deficit often occurred. Frequent watering could well have led to a substantial loss of nitrogen through leaching. Light intensity appeared favorable to a rapid rate of photosynthesis, thus leading to a substantial increase in the carbohydrate supply without a corresponding rapid rate of amino acid synthesis. The plants, in fact, did appear to be nitrogen deficient during the period of examination. Since a readily available supply of nitrogen would favor stylar elongation, the deficiency of nitrogen in all probability contributed in large part to a reduction in the degree of exertion in $\underline{cl}_3$, and for that matter of Cl_3.

A comparison may be made of the relative lengths of the floral parts of both Cl_3 and $\underline{cl}_3$ from Table 37. Whereas the corolla generally exceeded that of the sepals in Cl_3, the reverse relationship was found with $\underline{cl}_3$ flowers. It should be recalled that difficulty arose in measuring the lengths of the calyx and corolla of the fertile plants of Cl_3 due to the reflexed nature of these parts at anthesis. The corolla in all probability exceeded the sepals to a greater extent than is herein indicated. Nevertheless, the results do indicate the reverse relationship of petal to sepal length in $\underline{cl}_3$ as compared to

TABLE 37

Average Length of the Calyx, Corolla, Stamens, and Pistil in Flowers of Cl_3 and $\underline{cl_3}$ (Length in mm)*

Date Measured	Genotype	No. of Flowers Measured	Calyx Length	Corolla Length	Difference in Length (Corolla minus Calyx)	Stamen Length	Pistil Length	Difference in Length (Pistil minus Stamen Length)	Difference in Stigma Exertion (cl_3cl_3 minus Cl_3-)
9/ 3/57	Cl_3-	24	11.48	11.65	+0.17	10.21	11.12	+0.91	
	cl_3cl_3	27	10.61	10.24	-0.37	9.83	11.78	+1.95	+1.04
9/ 4/57	Cl_3-	7	10.50	11.29	+0.79	10.00	10.21	+0.21	
	cl_3cl_3	11	12.14	10.91	-1.23	10.05	12.23	+2.18	+1.97
9/ 6/57	Cl_3-	6	11.33	10.58	-0.75	9.42	10.00	+0.58	
	cl_3cl_3	6	11.33	10.08	-1.25	9.75	11.67	+1.92	+1.34
9/ 7/57	Cl_3-	23	10.57	11.28	+0.71	9.93	10.30	+0.37	
	cl_3cl_3	20	11.50	10.88	-0.62	10.28	11.12	+0.84	+0.47
9/14/57	Cl_3-	35				10.43	10.73	+0.30	
	cl_3cl_3	35				10.79	12.16	+1.37	+1.07
9/25/57	Cl_3-	20				10.15	10.88	+0.73	
	cl_3cl_3	20				10.62	11.90	+1.28	+0.55
5/ 6/58	Cl_3-	10	15.23	18.59	+3.36	13.19	13.28	+0.07	
	cl_3cl_3	14	16.45	15.75	-0.70	13.48	14.36	+0.88	+0.81
5/ 8/58	Cl_3-	10				12.63	12.87	+0.24	
	cl_3cl_3	11				12.82	13.36	+0.54	+0.30

*Average length computed on a per date basis. All measurements extended from the base of the ovary to the tip of the flower part.

Cl_3. Although the lengths of the stamen and pistil and the difference between the two are self-explanatory, it might be well to bring attention to the fact that, irrespective of the date of measurement (field), the mean maximum pistil length of Cl_3 at no time exceeded the mean minimum pistil length of $\underline{cl}_3$. In only one instance did the maximum difference in mean length (mean length of pistil minus mean length of stamen) of Cl_3 exceed (0.07 mm) that of the minimum amount of stigma exertion in $\underline{cl}_3$.

Before concluding this section, it should be pointed out that the length of the floral parts of both Cl_3 and $\underline{cl}_3$ were considerably greater, as shown in Table 37, under greenhouse conditions than those measurements obtained in the field.

Mutant sl(?)

The genotypes of the parents of the F_1 and of both the F_2 and backcross progeny involved in the inheritance studies of sl(?) are presented in Figure 14. A total of 294 plants constituted the F_1 generation which were found to contain normal and fertile flowers. The mutant can, therefore, be considered dependent upon a recessive gene(s).

Table 4 contains the complete chi-square analysis for goodness of fit of sl(?) to a 3:1 F_2 and 1:1 backcross ratio, and Table 5 the corresponding segregation data. The most obvious feature of the F_2

F_2	♀		♂
P_1	BnBn CC s1(?)s1(?) UU	X	P_2 Bnbn Cc S1(?)S1(?) Uu
		F_1 ⊗	
1/8		BnBn CC S1s1 UU	F_2 3 S1- +1 s1s1
1/8		BnBn CC S1s1 Uu	F_2 9 S1- U- + 3 S1- uu + 3 s1s1 U- + 1 s1s1 uu
1/8		BnBn Cc S1s1 UU	
1/8		Bn Bn Cc S1s1 Uu	F_2 27 C- S1- U- + 9 C- S1- uu + 9 C- s1s1 U- 9 cc S1- U- + 3 C- s1s1 uu + 3 cc s1s1U- + 3 cc S1- uu + 1 cc s1s1 uu
1/8		Bnbn CC S1s1 UU	F_2 9 Bn- S1- + 3 Bn- s1s1 + 3 bnbn S1- + 1 bnbn s1s1
1/8		Bnbn CC S1s1 Uu	
1/8		Bnbn Cc S1s1 UU	
1/8		Bnbn Cc S1s1 Uu	

Backcross	♀		♂
P_1	BnBn CC s1(?)s1(?) UU	X	F_1 Bn- C- S1(?) s1(?) U-
	1/2	Bn- C- S1s1 U-	
	1/2	Bn- C- s1s1 U-	

Figure 14. Inheritance studies with s1(?): genotype of parents, and genotype of F_1, F_2, and backcross progeny.

population is the highly significant pooled chi-square value resulting from a deficiency of mutant plants (Table 5). The heterogeneity test indicates a consistent deviation in this direction for the four families studied. However, not a single family deviated significantly from the stated hypothesis. A different picture is displayed by the backcross progeny. The pooled data show no such significant deviation from the expected as did the F_2. However, when the results of the heterogeneity test are examined, although by itself not significant, (5 per cent level) they show a rather high chi-square value when compared with the relatively low pooled chi-square. Thus the individual families were by no means consistent in the direction in which they deviated from the expected. The total chi-square approaches significance, thus indicating that the individual families were less than ideally segregating according to the hypothetical ratio. Table 5 also shows that the deviation of the pooled data was due to an excess of mutants, differing from the F_2 in this respect. There were two families in the backcross generation which contained an excess of mutants. It might then be asked, to what extent did these families contribute to the total chi-square? Of the 8.6334, 6.0796 was contributed by those two families. With two degrees of freedom this would be significant (5 per cent level). The possibility, therefore, exists that these two families are not representative of sl(?) segregation in the backcross generation. A deficiency of mutants did occur in the pooled backcross data of the two remaining families which would be in agreement with the F_2

data. These two families satisfactorily fit the expected 1:1 backcross ratio for all three tests (total, pooled, and heterogeneity test).

The important question now arises, whether to accept or rejedt the proposed hypothesis. The significant chi-square value found for the F_2 generation would lead to a rejection of the proposed hypothesis if no explanation could be offered. The previous mutants have all shown a deviation in the direction of a deficiency of the mutant gene (excess of bn in the F_2 has been accounted for). Such a deficiency of male-sterile mutants in segregating populations is not peculiar to this study. Larson and Paur (25), Rick (37), Rick and Robinson (43), to cite a few, recognized the rather common occurrence of reduced viability in recessive mutants including male-sterile mutants. Unfortunately segregation data with s1(?) was the latest to be acquired and viability tests were consequently not conducted. However, it is not presumptuous to suspect such a possibility (reduction in viability) in view of these studies with bn, cl_3, and ps?, and which is further supported by the results of the above workers with other male-sterile mutants.

Inheritance studies were conducted during the spring of 1958, during which time near normal flowers were found. It is, therefore, possible mutant types may have been classed as S1(?), thus leading to the deficiency of mutants. However, a sufficient number of flowers were examined and final classification was not made until relatively certain of its accuracy. Therefore, misclassification remains a possibility but it appears more likely the deficiency of mutants was

genuine, and possibly resulted from a reduced viability or a reduced vigor in the homozygous recessive individuals. If a reduction in vigor is characteristic of sl(?), selection of the more vigorous seedlings during transplanting would have increased the proportion of fertile to sterile plants. Thus the hypothesis that a single recessive gene determines the mode of inheritance of the mutant designated as sl(?) will be accepted but only on a tentative and qualified basis.

Since no recombinants were found, the floral characteristics associated with sl(?) (which were previously described as affecting the gynoecium, androecium, and corolla) appeared to be inherited as a unit.

As a result of using bn F_1 as the male parent in the original cross, the possibility existed that bn, c, and u might segregate with sl(?) in the F_2. Whether none, one, two, or three, or any combination of these genes segregated with sl(?) would depend upon the F_1 plant from which the fruit was harvested. The F_1 plants were all alike phenotypically, but would contain eight different genotypes, any one of which would have equal chance of being selected. The genotypes are given in Figure 14 with the expected ratios for the four F_2 families which were used. There was one chance in two of selecting any one of the three genes (bn, c, and u) in the heterozygous condition. Since each gene did segregate in at least one of the four families, sl(?) could then be tested with each of these for independent assortment.

The complete chi-square analysis for goodness of fit of sl(?) with bn, c, and u to a dihybrid segregation, and sl(?) with

c and u to trihybrid segregation ratio is presented in Table 38. All combinations tested, fit the hypothesized ratios satisfactorily and the interaction chi-squares are well below the significant level. In that the sl(?) and bn family is important from both the standpoint of allelism and independent assortment, a word should be said with reference to this family. Referring to Table 39, some question might be raised in that only four individuals are found in the double homozygous class. When the probability is low, all classes should contain at least five individuals for chi-square to be most accurate. A probability of 0.30-0.40 obtained with sl(?) and bn cannot be considered a questionable decision. However, due to the importance of this family to the allelism test, and furthermore, to eliminate any doubt in the reader's mind concerning this dihybrid chi-square value which he may entertain as a result of questioning the existence of a sufficient phenotypic difference in the class sl sl bn bn from either Sl-bnbn or sl sl Bn-, the following class groupings were made and the chi-square test was applied. The double homozygous recessive class could be grouped with either the sl(?) or bn class and tested for goodness of fit to a 9:3:4 F_2 ratio. If grouped with sl(?) the chi-square value is 1.2794 and with two degrees of freedom the probability of a greater chi-square value is 0.50-0.60, and if grouped with bn a value of 2.8032 with a probability of 0.20-0.30, again with two degrees of freedom. No question can, therefore, be raised concerning the satisfactory fit of these two genes to the dihybrid ratio or the

TABLE 38

Chi-Square Analysis for Goodness of Fit of sl(?), c, and u to a 27:9:9:9:3:3:3:1 F_2 Ratio; of sl(?) and bn to a 9:3:3:1 F_2 Ratio; of sl(?) and c to a 9:3:3:1 F_2 Ratio; of sl(?) and u to a 9:3:3:1 F_2 Ratio; and of sl(?), bn, c, and u Each to a 3:1 F_2 Ratio as Components in the Dihybrid and Trihybrid Crosses

Gene or Genes	Expected F_2 Ratio	Total			Pooled			Heterogeneity		
		df	x^2	P	df	x^2	P	df	x^2	P
sl(?), c, and u	27:9:9:9 3:3:3:1	-			7	5.2733	0.60-0.70	-		
sl(?) and c	9:3:3:1	-			3	3.7876	0.20-0.30	-		
sl(?)	3:1	-			1	1.7120	0.10-0.20	-		
c	3:1	-			1	0.2621	0.60-0.70	-		
I*	-	-			1	1.8135	0.10-0.20	-		
sl(?) and u	9:3:3:1	-			3	2.8899	0.40-0.50	-		
sl(?)	3:1	-			1	1.7120	0.10-0.20	-		
u	3:1	-			1	0.9352	0.30-0.40	-		
I	-	-			1	0.2427	0.60-0.70			
sl(?) and bn	9:3:3:1	-			3	3.1461	0.30-0.40	-		
sl(?)	3:1	-			1	0.0095	0.90-0.95	-		
bn	3:1	-			1	2.7524	0.05-0.10	-		
I	-				1	0.3842	0.50-0.60	-		
sl(?) and u	9:3:3:1	6	6.8654	0.30-0.40	3	4.2784	0.20-0.30	3	2.5870	0.40-0.50
sl(?)	3:1	2	3.5581	0.10-0.20	1	3.5572	0.05-0.10	1	0.0009	0.95-0.97
u	3:1	2	1.3268	0.50-0.60	1	0.0583	0.80-0.90	1	1.2685	0.20-0.30
I	-	2	1.9805	0.30-0.40	1	0.6629	0.40-0.50	1	1.3176	0.50-0.60

*"I" refers to the interaction chi-square value.

TABLE 39

Segregation in the F_2 Generation for sl(?) and bn

Genotype	Expected F_2 Ratio	Number Observed in Each Class	Number Expected in Each Class	Deviation
sl and bn				
Sl-Bn-	9/16	17	19.6875	- 2.6875
Sl-bnbn	3/16	9	6.5625	+ 2.4375
slslBn-	3/16	5	6.5625	- 1.5625
slslbnbn	1/16	4	2.1875	+ 1.8125
sl				
Sl-	3/4	26	26.2500	- 0.2500
slsl	1/4	9	8.7500	+ 0.2500
bn				
Bn-	3/4	22	26.2500	- 4.2500
bnbn	1/4	13	8.7500	+ 4.2500

modified dihybrid ratio. The segregation tables for the remaining tests are: Table 40 for sl(?) and u, and Table 41 for sl(?), c, and u.

These data indicate independent assortment between sl(?) and bn, sl(?) and c, and sl(?) and u. Therefore, sl(?) would not be located in the same chromosome as bn, c, or u, or if in the same chromosome would be sufficiently far apart to assort independently.

Mutant ps?

The greenhouse progeny were derived from two sources. The first resulted from artificial cross-pollination of sterile plants with pollen from fertile siblings, and the second, from seeds of fruits which were naturally set on the sterile plants. Both sources produced fertile and sterile progeny. This, therefore, did not indicate whether the gene was recessive or dominant. Fruits were collected from the fertile plants only, and if a dominant gene was involved all progeny would necessarily be fertile. If, however, a recessive gene governed sterility, the fertile plants would have been heterozygous and should have segregated three fertile to one sterile plants, provided a single gene was involved.

A total of 13 F_2 families were grown, each from a different F_2 plant. Eight of these families were derived originally by artificial cross-pollination and five families from fruits which were naturally set on the sterile plants. Since both these groups

TABLE 40

Segregation in the F_2 Generation for sl(?) and u

Genotype	Expected F_2 Ratio	Number Observed in Each Class	Number Expected in Each Class	Deviation
sl and u				
Sl-U-	9/16	123	115.8750	+ 7.1250
Sl-uu	3/16	44	38.6250	+ 5.3750
slslU-	3/16	30	38.6250	- 8.6250
slsluu	1/16	9	12.8750	- 3.8750
sl				
Sl-	3/4	167	155.2500	+11.7500
slsl	1/4	40	51.7500	-11.7500
u				
U-	3/4	153	154.5000	- 1.5000
uu	1/4	53	51.5000	+ 1.5000

TABLE 41

Segregation in the F_2 Generation for sl(?), c and u

Genotype	Expected F_2 Ratio	Number Observed in Each Class	Number Expected in Each Class	Deviation
sl c and u				
Sl-C-U-	27/64	40	43.4538	- 3.4538
Sl-C-uu	9/64	16	14.4846	+ 1.5154
Sl-ccU-	9/64	18	14.4846	+ 3.5154
slslC-U-	9/64	11	14.4846	- 3.4846
Sl-ccuu	3/64	7	4.8282	+ 2.1718
slslC-uu	3/64	6	4.8282	+ 1.1718
slslccU-	3/64	2	4.8282	- 2.8282
slslccuu	1/64	1	1.6094	- 0.6094
sl and c				
Sl-C-	9/16	58	57.9375	+ 0.0625
Sl-cc	3/16	25	19.3125	+ 5.6875
slslC-	3/16	17	19.3125	- 2.3125
slslcc	1/16	3	6.4375	- 3.4375
sl and u				
Sl-U-	9/16	60	57.9375	+ 2.0625
Sl-uu	3/16	23	19.3125	+ 3.6875
slslU-	3/16	13	19.3125	- 6.3125
slsluu	1/16	7	6.4375	+ 0.5625
sl				
Sl-	3/4	83	77.2500	+ 5.7500
slsl	1/4	20	25.7500	- 5.7500
c				
C-	3/4	75	77.2500	- 2.2500
cc	1/4	28	25.7500	+ 2.2500
u				
U-	3/4	73	77.2500	- 4.2500
uu	1/4	30	25.7500	+ 4.2500

segregated in this generation, the 13 families could be analyzed as if from the same source. The complete chi-square analysis is found in Table 4 and the accompanying segregation data in Table 5.

These results indicate a good fit to the hypothesized 3:1 ratio and it may, therefore, be concluded that a single recessive gene determines the mode of inheritance of the mutant designated as ps?. If this gene is identical to ps, it is located on chromosome 2 (linkage group I).

It should be pointed out that the original artificial cross-pollination was, in reality, a backcross, with the greenhouse progeny satisfactorily fitting a 1:1 ratio. Mutant ps? was found in Big Early Hybrid which was an F_1 hybrid. Since the fertile Big Early Hybrid plants were found to be heterozygous for the ps? gene, it appears likely that ps? had been used as the female parent in the parental cross. The occurrence of sterile plants probably resulted from unwanted self-pollination in the female parent. Therefore, it is presumed that ps? is actually ps and furthermore, did not originate by a second mutation at the Ps locus.

Allelism Test

The results which were obtained from the identity test involving the four mutants in this study are shown in Table 42. The only comment that need be made concerns the progeny of the cross, sl(?)sl(?) x Bnbn F_1. The two families containing 53 and 55 plants, which were

TABLE 42

Progeny of Crosses to Test the Identity of Mutants bn, cl_3, sl(?), and ps?

Female Parent (Sterile)	Male Parent (F_1)		
	Bnbn	Cl_3cl_3	Ps?ps?
sl(?)sl(?)	53F*	38F	56F
	55F		58F
	49F+1S**		
	45F**		
	44F+2S**		
	45F**		
ps?ps?	42F	25F	
	43F	43F	
	27F	52F	
cl_3cl_3	32F		
	30F		

*Progeny from one fruit (family). F denotes a fertile plant; S a sterile plant.

**Transplanted on 8/28/57, consequently only the first cluster was produced before frost, which displayed an occasional adnate or twisted stamen.

transplanted on the 10th of July, 1957 produced a very satisfactory quantity of fruit and seed, and in addition showed none of the characteristics associated either with sl(?), bn, or modifications thereof. The remaining four families were transplanted at the later date as indicated in Table 42. Although an occasional stamen was twisted and some adnation was evident between the stamens and pistil, no other characteristics associated with either of the mutants was noted (excepting in the three sterile plants). Had sl(?) and bn been allelic, the test cross should have produced offspring segregating 1 fertile : 1 sterile plant in all families. The three plants classed as sterile did produce stamens, but they were few in number, twisted, and associated with a green-yellow corolla, which closely resembled sl(?). Since sl(?) served as the female parent in this cross, possibly these three plants resulted from self-pollination; or they may have been genetically fertile, but sufficiently modified by environment so as to appear sterile. It, therefore, seems unlikely that an allelic relationship exists between sl(?) and bn. Conclusive proof of this was presented in one family of sl(?) F_2, which segregated for both sl(?) and bn and showed a good fit to a 9:3:3:1 (or 9:3:4) F_2 dihybrid ratio. (See Figure 14 for the genotypes of the parents and progeny, and Tables 38 and 39 for the chi-square analysis and segregation data.) Had bn and sl(?)

been allelic, a monohybrid ratio exhibiting either complete dominance of one mutant to the other (3:1) or incomplete dominance (1:2:1) should have resulted. A separate locus assigned to each of the four mutants (bn, cl, sl(?), and ps?) would be the only conclusion which could be justified by these results.

DISCUSSION

A single recessive gene was found to determine the mode of inheritance of each of the mutants in this study. As a result of the allelism test a separate locus was assigned to each of the four male-sterile mutants, designated by the symbols bn, cl_3, sl(?), and ps?. The progeny of each mutant were grouped according to the year and location in which they were tested, and with bn on the basis of the male-fertile parent used in the parental cross. Segregation in each group was tested for goodness of fit to a monohybrid ratio. A significant deviation occurred with the progeny of bn crossed with the male-fertile parent Heinz Marketer. Presumably modifier genes had been introduced by Heinz Marketer which resulted in this deviation from the expected ratio. The gene for potato leaf shape, c, and uniform unripe fruit color (u) are found in linkage group IV and VII, respectively (Rick and Butler, 42). This study indicated that bn, cl_3, and sl(?) assort independently of both genes c and u, and therefore were not found in linkage groups IV or VII, or were sufficiently far apart to assort independently. Furthermore, in a cross involving both bn and sl(?), in which the F_2 segregates were subjected to a goodness of fit test for an hypothesized dihybrid ratio, the conclusion was reached that bn and sl(?) also assort independently. Therefore, bn and sl(?) would be found in different linkage groups or would be located sufficiently distant from one another so as to assort independently.

These mutants should prove useful in further linkage studies due to an exposed stigmatic surface and the male sterile condition of each mutant, and in addition the crossing operation should be facilitated. The possibility also does exist that one or more of these genes may be located in a group which is in need of additional marker genes. Mutant bn, in which leaf shape was modified, should prove of special value since classification could be achieved at an early stage of growth which would result in a conservation of both time and space.

Mutant ps? appeared identical to the mutant ps reported by Larson and Paur (25). Rick (41) reported finding a mutant in the variety Pearson similar to the previously described ps. Since the mutant plant of Rick's came from an established variety and that an allelism test indicated it was the same gene, Rick concluded the mutant plant resulted from a new mutation at the Ps locus. Mutant ps? was found in Big Early Hybrid which was an F_1 hybrid. Since the fertile Big Early Hybrid siblings were found to be heterozygous for ps?, it appears likely that ps? had been used as the female parent in the parental cross. The occurrence of sterile plants probably resulted from unwanted self-pollination in the female parent. Therefore, it is presumed that the previously reported ps mutant had been used to produce the F_1 seed, and therefore ps? was actually ps, and furthermore did not originate by a new mutation at the Ps locus.

Multiple effects were observed with each of the new mutants reported herein. Flowers of mutant bn were usually stamenless. Occasionally a stamen with viable pollen was produced which was adnate

to the pistil. The gynoecium was defective. The style was contorted, twisted, and bent; the stigmatic area was reduced; and fewer ovules were found in the ovary. Broader and blunter petals and sepals were also associated with bn flowers. Rick and Robinson (43) described the mutants pi and cl_2 as affecting both floral and vegetative plant parts. Mutant bn can also be added to this list. The leaves were obovate in shape, the margins were nearly entire, and the leaves were more broad and more blunt resembling closely the leaves of mutant e (b), described by Butler (7). A delay in flowering was characteristic of bn and could be attributed to an increased node number to the first cluster. The affect upon the reproductive and the vegetative parts were found inherited as a unit, and thus appeared to be a pleiotropic effect of the gene bn. A less extensive effect was noted with cl_3 and sl(?). Mutant cl_3 plants had a cleistogamous flower, an exerted stigma, and a protuberance at the stylar end of the fruit and/or a persistent style. The effect of sl(?) was limited to the flower. The corolla was a yellow-green, and the stamens were either absent or reduced in number. No recombinants were found in the F_2 or backcross progeny of either cl_3 or sl(?). Multiple effects could be explained genetically in three possible ways. A group of closely linked genes could account for such an effect although it is improbable that a mutation would occur simultaneously in each of several genes. A chromosome deletion including those genes affecting the several associated characteristics, or a single gene with a pleiotropic effect could be responsible. Therefore multiple effects could be explained either by

a chromosome deletion or a pleiotropic gene, since no method is available to discriminate between the two types. In this paper multiple effects have been attributed to a pleiotropic gene, though at the same time recognizing the possibility that a chromosome deletion might have been responsible.

The stamenless condition of bn flowers proved, during the course of this study, to be remarkably stable. Maximum stamen number (Table 15) occurred during the latter part of May, 1958. The importance of the data taken in May, 1958 was the marked rise in stamen number which occurred at about the middle of the month. No definite conclusions can be reached on the basis of one year's data. If proven to consistently produce similar results, use might be made of the mutant in studies concerned with stamen initiation and development. Nevertheless, one should not lose sight of the fact that from the standpoint of male sterility the stamenless condition of bn was exceptionally stable.

Subjection of bn and Bn plants to 50° night temperatures and "direct seeding" as compared to 60° night temperatures and two transplantings, effected a reduction in the node number to the first cluster of bn plants without a similar response in Bn. Wittwer and Teubner (49) indicated that node number to the first cluster was determined at the time at which the cotyledons expanded. Whether an inherent difference in sensitivity to low temperatures and/or direct seeding existed between the mutant and fertile siblings, or whether node number determination occurred at different stages of development would require further study. It was not intended that such a question be answered in this study. In fact, the main purpose of the study

reported herein was to effect, if possible, a reduction in node number in bn, without a similar degree of response in Bn. This was accomplished with some indication as to the possible factors which may have played the major role(s). One should not, however, lose sight of the fact that although node number in bn was reduced to a highly significant degree, a highly significant difference in node number still existed between bn and Bn. This difference in node number was sufficiently great to serve as a means of distinguishing bn from Bn even after modification.

Rick and Robinson found that premature abscission of the flower in the mutant Cl_1Cl_1 was the cause of unfruitfulness. The number of days between anthesis and abscission did not differ significantly between Cl_3 and $\underline{cl}_3$ and was, therefore, concluded not to be the cause of unfruitfulness in $\underline{cl}_3$. Both the gynoecium and the androecium were then tested to determine if they were capable of functioning. Unfruitfulness could not be attributed to a defective gynoecium or androecium. Following this the fertile and sterile siblings were examined to determine if a difference in floral morphology existed which would present a barrier to self-pollination in $\underline{cl}_3$. If the method of dissection of the flower did not bring about dehiscence of the pollen, indehiscence was not the cause of unfruitfulness. The method of removal followed that of Rick and Robinson (43). They found that careful removal of the anther column more dependable than sectioning the flower. The possibility that the stigma in $\underline{cl}_3$ flowers was sufficiently exerted to drastically reduce the amount of

self-pollination was next investigated. Inasmuch as the degree to which the stigma would be exerted would depend upon both environmental conditions, as well as the genetic constitution of the plant, the day of examination and conditions prevailing at the time were recorded.

A short pistil in relation to the staminal column was shown by Howlett (20) to occur when only a moderate supply of readily available nitrogen was present. On the other hand, with an abundance of readily available nitrogen, the reserve carbohydrates became depleted (other factors not limiting) and maximum pistil length resulted. Inherently $\underline{cl}_3$ under similar conditions to that of Cl_3 was characterized by a longer pistil and consequently a stigma which was exerted to a greater degree. It is not said, however, that the degree to which the stigma was exerted was not governed by environmental factors. The differences in length of the pistil of both $\underline{cl}_3$ and Cl_3 on different days of examination (Table 37) indicated just such a response to environment. Although the length of the photoperiod was shorter during the latter part of September as compared to earlier in the month, the difference would not be great nor account for the differences in exertion as displayed during that time. The principal variable from one examination date to the next was that of temperature. In fact, the rather low temperatures on and preceding the 6th, 7th, and 25th correspond rather well with the pistil length and the degree to which the stigma was exerted on those days as compared with the other dates of examination. An association can also be made between the degree of stigma exertion

and the amount of pollination which took place as shown in Table 36. The question, therefore, remains as to how the temperature effected pistil length and the degree to which the stigma was exerted. First, a reduction in temperature in the range of this experiment would lower the rate at which the reserve carbohydrates would be utilized. Although nitrate absorption would probably not be reduced to any significant degree, the rate at which nitrate reduction occurred within the plant would be substantially reduced. Consequently, the rate of amino acid synthesis would in turn be lowered and in effect produce a nitrogen deficiency in so far as nitrogen utilization was concerned, thereby resulting in a build up of reserve carbohydrates. Thus the difference in stigma exertion and the amount of self-pollination which occurred on the different days of examination is interpreted as an effect of temperature upon the rate at which the reserve carbohydrates were depleted as a direct result of the rate of amino acid synthesis. Even though the degree to which the stigma was exerted and the amount of pollination which occurred did vary between the dates of examination, on any specific examination date $\underline{cl}_3$ always showed a greater degree of stigma exertion and a considerably less amount of self-pollination than did Cl_3.

Rick and Robinson (43) suggested Cl_1cl_1 might provide material for physiological studies, having observed that partial petal opening in the field, which distinguished it from Cl_1Cl_1, did not occur under midwinter greenhouse conditions, and therefore was indistinguishable from Cl_1Cl_1. Mutant $\underline{cl}_2$ in the same study responded in the opposite

direction from Cl_1cl_1 (greater petal opening occurring during midwinter than in midsummer). It may be recalled that $\underline{cl}_3$ showed a maximum flower opening during midwinter and in the field during the latter part of the growing season. Therefore, $\underline{cl}_3$ responded in the opposite direction to that of Cl_1cl_1 but similar to the mutant $\underline{cl}_2$. Following the suggestion of Rick and Robinson (43) a similar physiological study might also prove fruitful with $\underline{cl}_3$.

Since the suggestion by Barrons and Lucas (2) in 1942, that male-sterile mutants might be of value in reducing the cost of hybrid tomato seed, an extensive study with many male-sterile mutants has been conducted. The three new mutants reported herein will be evaluated with respect to their application to the production of hybrid tomato seed. Mutant bn would be extremely valuable since a bn plant could be identified by leaf shape. The need for securing large quantities of fruit and seed in hybrid seed production, and the difficulties that have been experienced in setting fruit and seed with bn, would in all likelihood exclude bn from further consideration. However, for use on a limited scale, especially in genetic and breeding experiments, it might be put to good use, inasmuch as fruit set could be increased by selecting flowers for pollination in which the pistils were least deformed. This would not be feasible on a commercial scale unless an environment were found in which such malformation were reduced to a minimum.

Use of $\underline{cl}_3$ would also be excluded from further consideration, since difficulty was experienced in setting a normal quantity of fruit

and seed. Possibly during the latter part of the season, when dessication of the stigma would be less likely to occur, fruit set could be increased. However, at that time self-pollination also would occur, so that contamination would, in all likelihood, then become a problem.

Although bn and $\underline{cl}_3$ are potentially valuable in other respects, sl(?) appeared to have the greatest prospect as a female parent in the production of hybrid tomato seed. Fruit set and seed set was normal with sl(?). Bishop (3) indicated that the stamenless mutants are of considerable value. Whereas the stigma of many of the pollen sterile mutants would be inaccessible without emasculation (thus eliminating the major reason for use of male-sterile mutants), the stigma of stamenless mutants would be exposed. The major concern appeared to be the stability of sl(?). It should, however, be recalled that self-pollination principally occurred during the month of May. Many of the mutants so far reported have been subject to self-pollination during certain seasons or under a specific set of environmental conditions. To name a few, Bullard and Stevenson (6) found a high rate of self-pollination in ps and $\underline{ms}_{16}$ during the summer months. Hafen and Stevenson also found stamen number to increase with sl mutants during the winter months. These same workers using one stamenless mutant and three pollen-sterile mutants showed that under field condition 50 per cent of the seed was the result of self-pollination. These examples have not been presented to justify the use of a mutant which self-pollinates to a high degree, but rather to indicate that if

self-pollination in sl(?) were shown to be limited to the afore mentioned spring conditions, a need might be filled until an exceedingly stable mutant was found. Before this mutant were to be used, adequate testing during a number of seasons and, if possible, in different localities, would be advisable. Currence (10) suggested use of a seedling marker such as potato leaf or green stem, to identify and rogue contaminants at an early age. This would provide an adequate means of identifying contaminants, but unfortunately would also require additional time and a larger number of plants to transfer a desirable mutant plus these two genes to the variety to be used. It is probably essential that this be done if self-pollination were expected, but should serve only as a temporary expedient until a stable mutant or a pleiotropic mutant affecting a seedling characteristic is found, such as that found by Allard (1) in the lima bean or bn in this study. Mutant sl(?) could be sexually propagated and thus would eliminate propagation by vegetative means or carrying the gene along in the heterozygous condition.

Rick (38) has shown promising results using natural cross-pollination for the production of hybrid tomato seed. Success depended in large part upon; the activity of insect vectors in the locality in which the crossing operation was to be performed (Rick, 38), the planting design (Rick, 36), and the variety to be used (Soost and Rick, 46). The cost of production could be greatly reduced if cross-pollination by natural means could be utilized. Since sl(?)

has a greenish-yellow corolla and lacks the bright-yellow-orange stamen color, it is improbable that natural cross-pollination would prove successful as a result of reduced insect activity in such flowers. For this reason use of natural cross-pollination with other stamenless mutants has not met with much success (Bullard and Stenson (6) and Hafen and Stevenson (17)). If, therefore, sl(?) proves to be phenotypically stable under field conditions, its principal value in the production of hybrid seed would be by hand cross-pollination. Even though other stamenless mutants are available, Rick (35) suggested that rather than transferring a male-sterile mutant to the variety to be used, much time could be saved by finding a mutant within the variety. Thus, sl(?) would be available for use in the new variety in which it was found, Ohio W-R Jubilee.

SUMMARY

A survey of the experimental tomato plots at the Northwestern substation and at the vegetable crops substation at Marietta was made to uncover mutants which resulted in unfruitfulness. Four male-sterile mutants, each from a different variety, were found: mutant bn in Purdue F_2; mutant cl_3 in Red Jacket; and mutant sl(?) in the new variety, Ohio W-R Jubilee. A fourth mutant (found in Big Early Hybrid) which appeared very similar to ps, and therefore designated as ps?, was discussed to a limited extent. The objectives of this study were: (1) To make a genetic study of each mutant; a) to determine the mode of inheritance; b) to establish or disprove a linkage relationship with the gene for potato leaf shape (c) and the gene for uniform unripe fruit color (u); and c) to conduct an allelism test among the male-sterile mutants to test their identity. (2) To obtain a detailed description and an account of the phenotypic stability of each mutant as expressed in both field and greenhouse tests. (3) To determine the cause of unfruitfulness, by studying the relationship between floral abnormality and unfruitfulness. (4) To evaluate each mutant from the standpoint of its use in hybrid seed production and/or its adaptability to other studies, as a result of a unique character possessed by the mutant.

A total of 5363 plants of the P_1, P_2, F_1, F_2, and BC generations were observed during the years 1956, 1957, and 1958 at the University

Greenhouses and the Horticultural Farm of The Ohio State University, Columbus, Ohio.

All F_1 plants contained normal, fertile flowers, cut leaves, and fruits that were a non-uniform unripe color.

1. Segregation in the F_2 and BC generations showed that each mutant was governed by a single recessive gene. There was no indication that an allelic relationship existed between the four mutants. However, it did appear that ps? was probably identical to the ps mutant reported by Larson and Paur.

2. Mutants bn, cl_3, and sl(?) were each found to assort independently of gene c and gene u, and would therefore not be located in linkage group IV or VII, or if in the same linkage group they would be sufficiently far apart to assort independently. Furthermore, dihybrid segregation was indicated for the male-sterile genes bn and sl(?).

3. A mutant bn plant usually contained flowers that were stamenless. Occasionally functional stamens were found, particularly toward the latter part of spring. They were often adnate to the pistil, and thus caused a scarring of the fruit. The gynoecium was defective, which, in part could be related to a twisted, grooved, and bent style with a reduced stigmatic surface. The leaves were obovate in form, the margins were nearly entire, and compared to the shape of either a cut leaf or a "potato" leaf, bn leaves were more broad and more blunt. A noticeable delay in flowering occurred

that resulted from an increased node number to the first inflorescence. A night temperature of 50 degrees Fahrenheit for one and one-half weeks following germination, and "direct seeding," effected a reduction in the bn node number. However, fertile and sterile plants were still distinguishable on the basis of a difference in node number.

4. Flowers of cl_3 plants did not open in the usual fashion; the petals were a pale yellow in color and remained in a vertical position to the floral axis. A prominent protuberance was also found on the stylar end of the fruit. The cause of unfruitfulness was attributed to a pre-mature exertion of the stigma, which produced a barrier to self-pollination. During the latter part of the growing season a partial breakdown of this barrier occurred, which was related to low temperature conditions.

5. The effect of sl(?) was confined to the flower. Stamen number was reduced and the petals were slender and a green-yellow in color. Although stamenless flowers were found, they were not as numerous as in bn.

6. The features which were associated with each of the three mutants were inherited as a Mendelian unit, and probably represented a pleiotropic effect of the mutant gene.

7. Further studies should be conducted with each of the mutants, to establish the linkage group to which each belongs. Both bn and cl_3 would be eliminated from a large-scale hybrid seed program on the basis of a low seed set. In a breeding program of limited size,

the blunt leaf shape of *bn* could prove valuable for an early identification of contaminants. Mutant *sl*(?), if proven to be phenotypically stable under field conditions, might be of use in a large-scale hybrid seed program. Mutant *cl*$_3$ would provide material for an interesting physiological study, since the cleistogamous condition of the flower was partially disrupted by the mid-winter greenhouse environment.

APPENDIX

TABLE 43

Daily Temperature (F.) Recorded at The Ohio State University Horticultural Farm

August 12, 1957 through October 12, 1957

Date	Average	Minimum	Maximum
1957			
8/12	77.5	69	86
8/13	70.5	55	86
8/14	78.5	62	95
8/15	81	70	92
8/16	73	66	80
8/17	71	62	80
8/18	68	54	82
8/19	-	62	-
8/20	-	-	82
8/21	70.5	58	83
8/22	70.5	57	84
8/23	72	54	90
8/24	73.5	62	85
8/25*	71.5	66	77
8/26*	72	60	84
8/27	69.5	60	79
8/28	67	60	74
8/29	79	64	94
8/30	79	66	92
8/31	77.5	64	91
9/1	81	68	94
9/2	81.5	69	94
9/3*	77.5	69	86
9/4*	70.5	64	77
9/5	60.5	47	74
9/6*	60.5	44	77
9/7*	62	54	70
9/8	62.5	47	78
9/9	69	56	82
9/10	70	65	75
9/11	70	56	84
9/12	73.5	65	82
9/13	68	65	71
9/14*	67.5	57	78
9/15	75.5	69	82
9/16	73	67	79

TABLE 43 (contd.)

Date	Average	Minimum	Maximum
9/17	64	52	76
9/18	66	54	78
9/19	74.5	67	82
9/20	74.5	70	79
9/21	76.5	69	84
9/22	72	70	74
9/23	61	50	72
9/24	55	40	70
9/25*	64.5	47	82
9/26	61.5	55	68
9/27	50.5	39	62
9/28	52	38	66
9/29	56	40	72
9/30	66	58	74
10/1	63	50	76
10/2	56	40	72
10/3	56.5	44	69
10/4	54.5	43	66
10/5	55.5	44	67
10/6	57	46	68
10/7	58.5	54	63
10/8	56.5	42	71
10/9	57.5	42	73
10/10	56	52	60
10/11	43	32	54
10/12	45.5	31	60

*Date flower measurements taken of Cl_3 and $\underline{cl}_3$.

TABLE 44

Daily Temperature (F.) Recorded at The Ohio State University Horticultural Greenhouses

Date	Average	Minimum	Maximum
1958			
3/12	65	50	80
3/13	-	-	-
3/14	71	50	92
3/15	61.5	46	77
3/16	59	48	70
3/17	61.5	49	74
3/18	69.5	52	87
3/19	66	54	78
3/20	68	58	78
3/21	72	64	80
3/22	68	58	78
3/23	73	58	88
3/24	65	60	70
3/25	66	60	72
3/26	67	60	74
3/27	74	60	88
3/28	66	58	74
3/29	71.5	59	84
3/30	71	60	82
3/31			
4/15	71	56	86
4/16	77	58	96
4/17	75.5	53	98
4/18	77	55	99
4/19	72	55	89
4/20	74	61	87
4/21	-	56	-
4/22	69	55	83
4/23	66.5	54	79
4/24	74.5	57	92
4/25	66.5	53	80
4/26	60.0	53	67
4/27	64	57	71
4/28	69	58	80
4/29	-	58	-
4/30	64.5	56	73

TABLE 44 (contd.)

Date	Average	Minimum	Maximum
5/1	70.5	57	84
5/2	65.5	55	76
5/3	69.5	61	78
5/4	66	62	70
5/5	68	58	78
5/6*	64	58	70
5/7	72	58	86
5/8*	72	57	87
5/9	70.5	58	83
5/10	71	57	85
5/11	73.5	62	85
5/12	74	58	90
5/13	73	57	89
5/14	75	57	93
5/15	72.5	56	89
5/16	73.5	57	90
5/17	-	-	-
5/18	-	-	-
5/19	-	-	-
5/20	-	-	88
5/21	70	50	90
5/22	65	52	78
5/23	69	56	82
5/24	73.5	56	91
5/25	74	60	88
5/26	72	58	86
5/27	74	56	92
5/28	76	64	88
5/29	69.5	49	90
5/30	73.5	54	93
5/31	76	60	92
6/1	79	68	90
6/2	-	60	-

*Date flower measurements taken of Cl_3 and $\underline{cl}_3$.

TABLE 45

Appearance of Mutant Pollen Grains Stained with Aceto-Carmine

Mutant	Date of Examination	Total Number of Pollen Grains	Number of Normal Pollen Grains	Number of Abnormal Pollen Grains	Percentage Normal Pollen Grains
Normal	2/ 7/56	500	484	16	97
	4/ 6/56	423	389	24	92
	4/26/56	250	249	1	100
	9/ /57	800	774	26	97
bn	2/ 7/56	600	514	86	86
	9/ /57	400	391	9	98
cl_3	2/ 7/56	400	385	15	96
	4/ 6/56	200	188	12	94
	4/26/56	265	153	112	58
	9/ 5/57	800	755	45	94
	5/ /58	400	388	12	97
sl(?)	9/ /57	435	369	66	85
	5/ /58	400	385	15	96

LITERATURE CITED

LITERATURE CITED

1. Allard, R. W. 1953. A gene in lima beans pleiotropically affecting male-sterility and seedling abnormality. Proc. Amer. Soc. Hort. Sci. 61:467-471.

2. Barrons, K. C. and H. E. Lucas. 1942. The production of first-generation hybrid tomato seed for commercial planting. Proc. Amer. Soc. Hort. Sci. 40:395-404.

3. Bishop, C. J. 1954. A stamenless male-sterile tomato. Amer. Jour. Botany 41:540-542.

4. Bohn, G. W. and T. W. Whitaker. 1949. A gene for male sterility in the muskmelon (Cucumis melo L.) Proc. Amer. Soc. Hort. Sci. 53:309-314.

5. Bukovac, M. J., S. H. Wittwer, and F. G. Teubner. 1957. Gibberellin and higher plants: VII. Flower formation in the tomato. Michigan Agr. Exp. Sta. Quart. Bul. 40:207-214.

6. Bullard, E. T. and E. C. Stevenson. 1953. Production of hybrid tomato seed. Proc. Amer. Soc. Hort. Sci. 61:451-458.

7. Butler, L. 1951. New linkage groups in the tomato. Jour. Hered. 42:100-104.

8. Butler, L. 1956. The importance of genetic background in the study of mutant characters. Tomato Genetics Coop. Rept. 6:11.

9. Crane, M. B. 1915. Heredity of types of inflorescence and fruits in tomato. Jour. Genet. 5:1-12.

10. Currence, T. M. 1944. A combination of semisterility with two simply inherited characters that can be used to reduce the cost of hybrid tomato seed. Proc. Amer. Soc. Hort. Sci. 44:403-406.

11. Gabelman, W. H. 1956. Male sterility in vegetable breeding. Brookhaven Symposia Biol. 9:113-122.

12. Gruneberg, H. 1938. An analysis of the "pleiotropic" effects of a new lethal mutant in the rat (Mus norvegicus). Roy. Soc., London, Proc., Ser. B. 125:123-144.

13. Hadfield, J. W. and R. A. Calder. 1936. Commercialization of hybrid vigour in the tomato. N. Z. Jour. Agr. 53: 139-146.

14. Hafen, L. and E. C. Stevenson. 1955. An attempt to induce male sterility in tomato by chemical treatments. Tomato Genetics Coop. Rept. 5:16-17.

15. Hafen, L. and E. C. Stevenson. 1955. New Male-sterile and stamenless mutants. Tomato Genetics Coop. Rept. 5:17.

16. Hafen, L. and E. C. Stevenson. 1956. The relative combining abilities of normal versus male-sterile mutants of tomatoes. Proc. Amer. Soc. Hort. Sci. 68:429-436.

17. Hafen, L. and E. C. Stevenson. 1956. Natural cross pollination in tomato using several male-sterile mutants. Proc. Amer. Soc. Hort. Sci. 68:433-436.

18. Hafen, L. and E. C. Stevenson. 1958. Preliminary studies of five stamenless mutants. Tomato Genetics Coop. Rept. 8:17-18.

19. Henderson, W. R. and W. N. Brown. 1958. Blunt (bn), a "stamenless" male-sterile mutant associated with a change in leaf shape. Tomato Genetics Coop. Rept. 8:20-21.

20. Howlett, F. S. 1939. The modification of flower structure by environment in varieties of Lycopersicum esculentum. Jour. Agr. Res. 58:79-117.

21. Jasmin, J. J. 1954. Male sterility in Solanum Melogena L. Preliminary report on a functional type of male sterility in eggplants. Proc. Amer. Soc. Hor. Sci. 63:443.

22. Jones, H. A. and S. L. Emsweller. 1936. A male-sterile onion. Proc. Amer. Soc. Hort. Sci. 34:582-585.

23. Larson, R. E. 1950. A comparison of combining abilities of a monorecessive male-sterile Earliana mutant and a normal Earliana tomato. Proc. Amer. Soc. Hort. Sci. 56:358-362.

24. Larson, R. E. and T. M. Currence. 1944. The extent of hybrid vigor in the F_1 and F_2 generations of tomato crosses. Minn. Agr. Exp. Sta. Tech. Bull. 164:1-32.

25. Larson, R. E. and S. Paur. 1948. The description and inheritance of a functionally sterile flower mutant in tomato and its probable value in hybrid tomato seed production. Proc. Amer. Soc. Hort. Sci. 52:355-364.

26. Lesley, J. W. 1924. Cross pollination of tomatoes. Jour. Hered. 15:233-235.

27. Lesley, J. W. and Margaret M. Lesley. 1939. Unfruitfulness in the tomato caused by male sterility. Jour. Agr. Res. 58:621-630.

28. Lesley, J. W. and Margaret M. Lesley. 1952. A cytogenetic study of a pleiotropic mutant of the tomato (Lycopersicon esculentum L.). Jour. Hered. 43:273-276.

29. Lesley, Margaret M. and J. W. Lesley. 1958. Male-sterile mutants in the tomato from seed treated with the radioactive isotope P32. Proc. Amer. Soc. Hort. Sci. 71:339-343.

30. Owen, F. V. 1945. Cytoplasmic inherited male sterility in sugar beets. Jour. Agr. Res. 71:423-440.

31. Pollack, B. L. 1956. Exerted, ex_1?. Tomato Genetics Coop. Rept. 6:22.

32. Rappaport, L. 1957. Giberellin effects, on growth, flowering, and fruiting of the Earlypak tomato, Lycopersicon esculentum. Plant Physiol. 32:440-444.

33. Rehm, S. 1952. Male-sterile plants by chemical treatment. Nature 170:38-39.

34. Rick, C. M. 1945. Field identification of genetically male-sterile tomato plants for use in producing F_1 hybrid seed. Proc. Amer. Soc. Hort. Sci. 46:277-283.

35. Rick, C. M. 1945. A survey of cytogenetic causes of unfruitfulness in the tomato. Genetics 30:347-362.

36. Rick, C. M. 1947. The effect of planting design upon the amount of seed produced by male-sterile tomato plants as a result of natural cross-pollination. Proc. Amer. Soc. Hort. Sci. 50:273-284.

37. Rick, C. M. 1948. Genetics and development of nine male-sterile tomato mutants. Hilgardia 18:599-633.

38. Rick, C. M. 1949. Rates of natural cross-pollination of tomatoes in various localities in California as measured by the fruits and seeds set on male-sterile plants. Proc. Amer. Soc. Hort. Sci. 54:237-252.

39. Rick, C. M. 1953. New male-sterile mutants. Tomato Genetics Coop. Rept. 3:19-21.

40. Rick, C. M. 1956. Two new male-sterile mutants in the variety Earlypak. Tomato Genetics Coop. Rept. 6:26.

41. Rick, C. M. 1958. New mutations at old loci. Tomato Genetics Coop. Rept. 8:31-32.

42. Rick, C. M. and L. Butler. 1956. Cytogenetics of the tomato. Advances in Genetics 8:267-381.

43. Rick, C. M. and Jeanette Robinson. 1951. Inherited defects of floral structure affecting fruitfulness in Lycopersicon esculentum. Amer. Jour. Bot. 38:639-652.

44. Scott, D. H. and M. E. Riner. 1946. Inheritance of male-sterility in winter squash. Proc. Amer. Soc. Hort. Sci. 47:375-377.

45. Shifriss, O. 1945. Male sterilities and albino seedlings in cucurbits. Jour. Hered. 36:47-52.

46. Soost, R. K. and C. M. Rick. 1957. Effect of varieties of pollen and ovule parents on natural cross-pollination of tomatoes. Proc. Amer. Soc. Hort. Sci. 70:357-365.

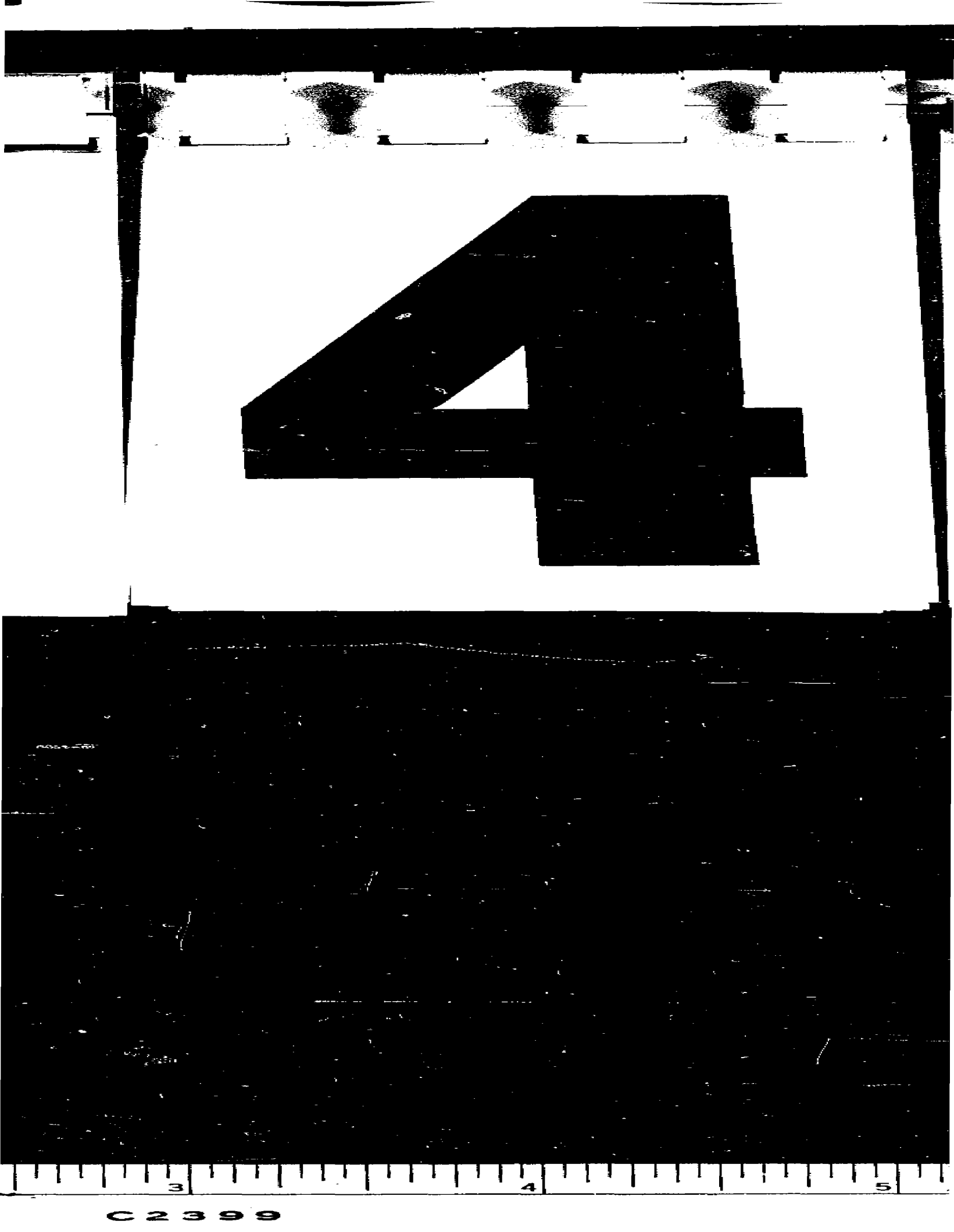
47. Welch, J. E. and E. L. Grimball, Jr. 1947. Male sterility in the carrot. Science 106:594.

48. Wellington, R. 1912. Influence of crossing in increasing the yield of tomato. N. Y. (Geneva) Agr. Exp. Sta. Bul. 346: 57-76.

49. Wittwer, S. H. and F. G. Teubner. 1956. Cold exposure of tomato seedlings and flower formation. Proc. Amer. Soc. Hort. Sci. 67:369-376.

AUTOBIOGRAPHY

I, Warren Robert Henderson, was born in Boston, Massachusetts, May 5, 1927. I received my secondary school education in the public schools of Boston, Massachusetts, and my undergraduate training at the University of New Hampshire, which granted me the Bachelor of Science degree in 1949. From Harvard University, I received the Master of Arts degree in 1951. I served as assistant to Professor Karl Sax at the Bussey Institution, Harvard University, from 1951 to 1954. During the summer of 1954, I was technician at the Muck Crops Substation, of the Ohio Agricultural Experiment Station. In October, 1954, I was appointed Assistant in the Department of Horticulture at The Ohio State University. I have held this position while completing the requirements for the degree Doctor of Philosophy.



C2399