Monoploid Peaches, *Prunus persica* Batch: Description and Meiotic Analysis[1]

Claron O. Hesse[2]
University of California, Davis

Abstract: Two monoploid peaches are described. Analysis of one showed that the chromosomes were distributed at random in the first meiotic division, and that some irregularities occurred because chromosomes are occasionally excluded from the spindle, divide precociously, or show other irregular behavior. Only one bivalent was observed in 236 division figures. The second division was normal. Due to varying orientation of the second division spindles a significant percentage of diad sporads formed, which yielded normal, haploid isogenic gametes. Some pollen germinated and fruit was set following pollination of diploid plants with pollen from the monoploid.

Kimber and Riley (16) reviewed the literature on haploid angiosperms and indicated the several areas of interest associated with these abnormal plants. Over 42% of the haploids listed by Kimber and Riley originated following interspecific hybridization, where recognition of the haploids was aided by their resemblance to one of the parental species. The second most frequent source, 26.7%, were those of spontaneous intraspecific origin. An additional 18% originated as one of spontaneous twin seedlings; experimental treatments, often irradiation, accounted for the remaining 13%.

Three haploids have been reported in the Rosaceae: one in *Fragaria vesca* [King, E. E., in (6)], one in hybrid plums (25) and another in *Prunus persica* by Pratassenja (27).[4]

The theoretical possibility of securing homozygosity through colchicine doubling of the haploid genome has been attained for many haploids (7, 32) and it remains one of the intrinsically appealing potentials for haploids in plant breeding (12, 21, 23, 32).

Abnormal chromosomal behavior within a genus or species is also of interest *per se* and haploids provide an extreme instance of such irregularity. Cryptic homologies (3, 9, 19, 22), gene controlled synapsis (30), and evidence for evolutionary history (4, 22) have been revealed through studies of haploids.

In this report a brief description of 2 spontaneous monoploid[3] peach clones is given, and the meiotic behavior of one is studied in some detail. A previously undescribed mechanism for the production of viable, presumably isogenic gametes by the monoploid is described.

Materials and Methods

The monoploids arose in the peach breeding program of the Pomology Department, University of California, Davis. They were recognized in the orchard following a year in the nursery. The 2 nearly identical plants were designated GH 4-11 and GH 15-57.

Both monoploids were derived from a cross between selection 7-5 x 'Cortez'. The female parent 7-5 is a freestone nectarine of complex pedigree; 'Cortez' is a non-melting clingstone peach. Presumptive evidence for the merogenous origin of the monoploids was: 1) both are nectarines, as is the female parent; the male parent is a peach. Nectarine is recessive to peach. 2) Both have showy flowers, as does 7-5; 'Cortez' has non-showy flowers. Showy flowers is recessive; 'Cortez' is known to be a homozygous non-showy dominant. Genetic markers have been used by others to indicate the merogenous (8) or androgenous (2, 17) origin of haploids, or even to detect haploids in plant cultures (5).

Measurements or tests to characterize the monoploids were made on each of the monoploids and the female parent 7-5, as follows:

Length and width of 15 leaves taken at random from mid-shoot positions were measured and the length/width (L/W) ratio calculated. The length of 5 stomatal guard cells in each of 5 ramdom fields of 5 leaves was measured with an ocular micrometer. Size of approximately 100 pollen grains was measured from acetocarmine squashes of nearly mature anthers. Pollen stainability was recorded. Germination *in vitro* was determined by germinating pollen on 15% sucrose hanging drops. Two peach clones were pollinated with pollen from the 2 monoploid plants.

Meiosis in GH 15-57 was followed in aceto-carmine smears of anthers collected at appropriate times and fixed in Carnoy fluid (3:1:4). Slides were made permanent by the method of Bradley (1).

Results

Plant Morphology and Pollen Fertility

The monoploids are small in all their parts compared to a normal diploid peach tree. Shoots are more slender, internodes shorter, and the leaves, flowers (Fig. 2A) and fruits smaller than those of the parental diploid. With age the branches of the monoploids develop internal necrotic lesions and swellings (Fig. 2B), which eventually girdle the limbs or trunk. They may be maintained by periodic propagation.

Table 1 shows leaf length (L), leaf width (W), and the L/W ratio for the parental clone and the 2 monoploids. Both monoploids have shorter and narrower leaves than the parental clone, and differ from each other. The L/W ratio of both monoploids was greater than that of 7-5, but did not differ from each other. The flower petals showed the same significant reduction in size and shape, and were also proportionately narrower than petals from diploid flowers.

The length of the stomatal guard cells, in ocular micrometer units, is shown in Table 1. In accordance with many other reports; e.g., Nordenskiold (24); stomatal size of the

Table 1. Average leaf length (cm), leaf width (cm), length/width ratio, and stomatal length (ocular micrometer units) of the parental peach diploid 7-5 and 2 monoploid derivatives GH 15-57 and GH 4-11.

	Length (cm)	Width (cm)	Length/ width	Stomatal length
7-5	10.88 a[1]	3.34 a	3.31 a	14.09 a
GH 4-11	8.06 b	1.88 b	4.30 b	10.16 b
GH 15-57	5.95 c	1.35 c	4.42 b	10.50 b

[1]In each column values followed by different letters are significantly different at the 5% level; t test.

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[2]Present address: San Joaquin Valley Research & Extension Center, 9420 So. Riverbend Avenue, Parlier, California.

[3]Monoploid is used to define a haploid of a basic diploid species, in this case x = n = 8 (20).

[4]T. K. Toyama, ARS, USDA, Prosser, Washington, has secured over a dozen peach monoploids, about half of which he has diploidized with colchicine (personal communication).

monoploids was significantly less than that of 7-5, but did not differ significantly from each other.

Figure 1 shows the distribution of pollen sizes for the 3 clones, and the percentage of pollen classified as viable by their staining reaction. The parental clone 7-5 had a low percentage of shriveled pollen (2.91%), the remainder being plump, stained, and presumably viable (97.09%). A small percentage of the stained pollen (1.94%) fell into a distinctly larger size class, and is presumed to be diploid. Diploid pollen grains have been found in peach clones (28). GH 4-11 and GH 15-57 clones had a high percentage of shriveled pollen, 89.00% and 93.14%, respectively. The remainder was in the size class of the normal pollen of the parental clone (Fig. 1), and was stained. From its size, stainability, and normal appearance it was assumed that at least some of such grains contained the haploid chromosome complement, n = 8, and should be functional.

Anthers of the monoploid do not dehisce; pollen was obtained by crushing the dried anthers. Although germination percentages could not be obtained because many of the degenerate spores adhered to the crushed anther walls, Figs. 2C

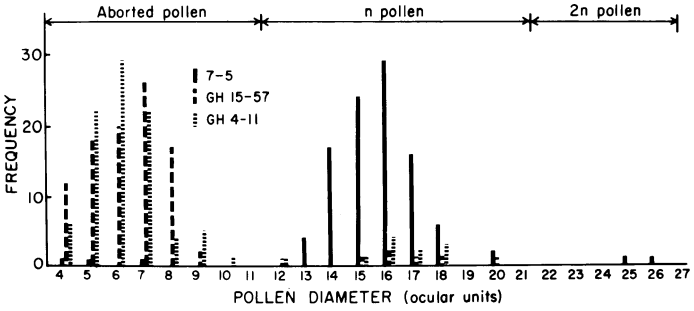

Fig. 1. Distribution of pollen grain size of the diploid clone 7-5 and the monoploids GH 4-11 and GH 15-57.

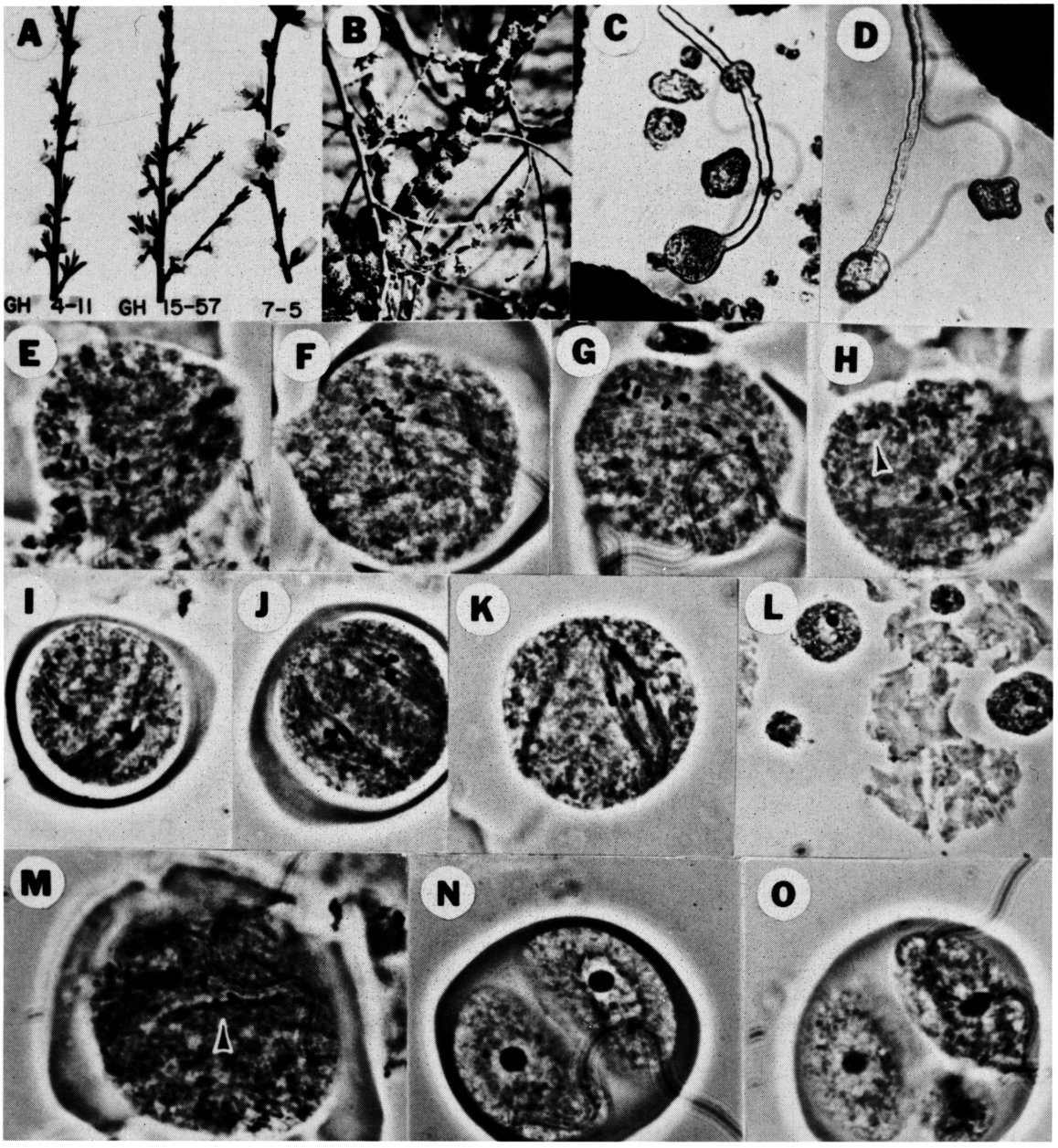

Fig. 2. A. Twigs and flowers of the monopolids and 7-5. B. Limb of GH 15-57, with abnormal growth pattern. C-D. Germinating pollen, GH 15-57 and GH 4-11. 8-hr cultures. E-N. All GH 15-57. E. MAI with 8 I's distributed at random. F. MAI segregated 6-2. G. MAI segregated 3-2-3. H. MAI with 7 I's at random and 1 I off the spindle (arrow). I. MII with spindles at right angles; 5 and 3 segregation at MAI, focused to show spindles. J. MII with spindles parallel, 4-4. K. Angular orientation of spindles which may result in a triad, 6-2, focus to show spindles. L. Tetrad, probably from a 6-6 + 2-2 AII. M. MAI with 6 I's and 1 II (arrow). N. Diad. O. Triad.

and 2D demonstrate that at least some of this pollen was germinable.

Flowers of diploid peach trees were emasculated and pollinated with pollen from the monoploids. Frosts on the nights of April 27 and 28 reduced the apparent set markedly, but 1 fruit formed from 90 pollinations with GH 4-11 and 4 from 54 pollinations with GH 15-57, and support the evidence for the production of viable pollen indicated above from size measurements, stainability, and germination *in vitro.* Viable pollen from monoploids and polyhaploids has been reported repeatedly (9, 18).

The percentage of haploid pollen formed remains questionable. GH 15-57 produced 6.86% good pollen, as indicated by size and stainability. This is approximately half of that which should have been produced on the basis of the 25.6% diads observed at the end of the second division. The latter counts may have been in excess because of the greater probability of disrupting sporads of higher microspore count in the squashing technique used. On the other hand, the 6.86% good pollen observed may be less than would be expected from diad formation due to degeneration of some haploid or nearly haploid microspores in some anthers.

Cytology

Prophase was particularly poor in the monoploid; no information was available until the chromosomes reached metaphase. At first metaphase the chromosomes did not congress on the equatorial plate, but were distributed along the spindle between the poles in a manner characteristic of haploids with a low percentage of bivalent formation. Person (26) has termed this stage meta-anaphase (MAI). At MAI the distribution of chromosomes can be categorized as: 1) those more or less evenly and randomly distributed between the poles (31.1% of 193 MAI's and AI's) (Fig. 2E); 2) those separated into 2 polar groups yielding various distributions from 0-8 to 4-4 (40.4%) (Fig. 2F, 6+2); 3) those distributed into more than 2 groups, usually 1 to 3 at each pole with the remainder near the equator (20.7%) (Fig. 2G); and 4) a low percentage (7.7%) of misdivisions--one or more chromosomes excluded from the spindle (Fig. 2H), precocious second division of a chromosome, syncitial cells with n = 16, etc.

Interkinetic nuclei do not form nor does cytokinesis occur between divisions I and II; the chromosomes enter the second division directly from TI. Nearly all second divisions are highly regular, and it is inferred that, except for irregular divisions, categories 1 and 3 above represent sequential stages of MAI, with the inclusion of the 8 chromosomes into 2 TI groups being the usual final condition. Due to the regularity of the second division, the final distribution of chromosomes at TI could be inferred from the number on each second division spindle, as 5-5 + 3-3 at MII and AII would follow from a 5-3 distribution at TI, for example Fig. 2I.

Of the 236 MAI, TI, MII, and AII divisions 98 (41.5%) could be accurately scored for distribution (Table 2). Random distribution of the chromosomes at the first division of meiosis is probable.

Table 2. Distribution of chromosomes in meiosis of GH 15-57.

	Distribution					
	4-4	5-3	6-2	7-1	8-0	Total
Obs.	30	38	22	8	0	98
Exp.	26.80	42.88	21.44	6.12	0.76	98

$X^2 = 0.79$, df = 3, P = >.50

At MII spindle orientation was not regular. Spindles were observed at right angles to each other (Fig. 2I), parallel (Fig. 2J), or at an angle to each other (Fig. 2K). The meiotic cells of the monoploid were small; when the second division spindles were oriented parallel or nearly so, the chromosomes from each spindle often lay close to each other at either pole at the end of the second division. In such cases they could be reconstituted into a single nucleus at each polar position. Following cytokinesis, a diad would be formed in such instances (Fig. 2N), and each microspore would contain a complete genome, n = 8. Since this genome resulted from a single mitotic division of the chromosomes, the microspores so formed would be normal haploid gametes, all alike insofar as their genetic constitution is concerned. No bivalent formation, and presumably no crossing-over, occurred.

If the spindles at MII were oriented at right angles to each other, 4-spored tetrads formed, according to expectation, but each contained fewer than 8 chromosomes. The microspore nuclei and nucleoli appeared proportional in size to the number of chromosomes contained, and nuclei and spores of 2 complementary sizes usually formed (Fig. 2L).

Occasionally sporads with unusual numbers of spores formed; 3 or 5 or more were observed. Triads were believed to form when the second division spindles lay at such an angle that the end products at one pole were close to each other and were reconstituted into a haploid gamete with 8 chromosomes. Those at the opposite poles lay far enough apart to form 2 nuclei, each with a reduced chromosome number. One of the microspores in triads is of a size similar to those formed in diads, whereas the other 2 are of reduced size (Fig. 2O). Sporads with more than 4 microspores are believed to have originated from exclusion of chromosomes from the MAI spindle or other irregularities in division. All microspores with reduced chromosome numbers are believed to be nonfunctional and degenerate. Those with a normal chromosome complement, as from diads or the large microspore of a triad, were probably functional in at least a moderate percentage of cases. They were presumed to be isogenic, since they are constituted of a complete genome derived through a simple, albeit 2 partite, mitotic division.

A single bivalent (Fig. 2M) was observed in one of the division figures analyzed.

Discussion

Haploid plants have been observed to arise spontaneously and following a variety of treatments. The 2 peach monoploids reported here arose spontaneously, as did the peach monoploid reported by Pratassenja (27). Chase (7) reported that certain parents were more liable to yield haploids than others. Because both of those reported here came from the same parent, genetic control may be involved. The numbers are too small to be more than suggestive.

The physical characteristics of the monoploids, smaller and with finer plant parts than the normal diploid, were similar to those reported for other haploid plants (16, 24). The reduced stomatal sizes were in conformity with those of other haploids investigated.

While most authors have have studied the fertility of haploids have found them to be less, usually considerably less, fertile than the normal diploid, some fertility is usually encountered (6, 8, 9, 18). For example, Smith (31) secured 90 plants, 88 of which were diploid, from self-pollinated einkorn monoploid plants. Pollen fertility in haploids has usually been related to restitution nuclei forming at some stage of meiosis, usually at the first division.

Diad formation resulting from reconstitution of second division products into 2 nuclei usually infers that the products of each spindle are recombined. This invariably would result in unbalanced gametes. The unique feature observed in GH 15-57 was the recombination of the complementary products of the 2 second division spindles due to their parallel orientation which regularly resulted in 2 nuclei, each containing a complete genome. When cytokinesis follows the first division haploid gametes will form only if all the chromosomes migrate to the same pole. Regular bivalent formation also will yield unbalanced products. Katamaya (14) correctly pointed out in his

Aegilotricum haploid that cytokinesis followed division I and the irregular distribution of chromosomes of the respective parental genomes at AI normally would not yield viable pollen. In his *Triticum monococcum,* haploid, cytokinesis precluded haploid gametes forming from second division restitution nuclei. The same was true for the haploid *Hordeum distichum* studies by Tomentorp (33) and the haploid rye studied by Nordenskiold (24). In the GH 15-57 peach monoploid cytokinesis does not occur between division I and II, and bivalent formation is very rare.

Restitution nuclei at the first division can lead to the formation of haploid gametes whether or not bivalent formation is rare. Thus Hakansson (11) found as many as 70% diads in *Godetia whitneyi* due to the many restitution nuclei formed after the first division. Tomentorp (33) observed about 9% diads, presumably with the normal chromosomal complement of n = 7, which was attributed to the omission of the first division, the second being mitotic and hence yielding diads with the normal chromosomal complement.

A second possible source of diads for all haploids, but especially for monoploids of low chromosome numbers, would follow from first divisions in which all chromosomes moved to one pole at AI. This would account for less than 0.8% diads if n = 8 and chromosome segregation was random, and seems unlikely to be a significant mechanism for their production.

The assumption that gametes resulting from diad formation are isogenic in peach GH 15-57 is circumstantial. The less than 1% bivalent formation nearly precludes crossing-over following normal chiasma formation as a relevant factor. Nevertheless Reiger (29) and McClintock (20) have observed midprophase pairing in haploids (presumed monopolids), but the association did not last until metaphase. Whether or not crossing-over occurred was not determined. The evidence for crossing-over occurring in those instances where pseudobivalent formation occurs is even less compelling, Walters (34) and Person (26).

The distribution of the haploid set of chromosomes at AI if not random must be under some internal control. In the case of polyhaploids, the presence of pairing in greater or less amount can affect the regular distribution of chromosomes. In monoploids, where the production of bivalents is normally quite low, such interference with the random distribution of the chromosomes presumably is not present. Some investigators (14, 24, 33) indicate random distribution at AI. Others (26, 29) did not find distribution at MI to be random. Katamaya (14) did not find random distribution at MAI in monoploid *Triticum monococcum* but concluded that the distribution reported at MII and AII did indicate random distribution. He suggested that errors of classification at MAI may have been responsible for the non-random distribution.

Only one bivalent was included in the 193 MAI plates scored (Fig. 21). Bivalent formation in GH 15-57 is evidently a rare event, and one to which no particular significance can be credited. Bivalent formation in various monoploids has been observed to range from none to rather frequent--1 or more per cell. Examples are: none in *Crepis capillaris* (13), and *Nicotiana glutinosa* (15); less than 1% in *Godetia whitneyi* (11); 27.5% in rye (19); and 1 to 4 II per PMC in *Antirrhinum majus* (29). In all cases where an appreciable amount of bivalent formation has been observed synapsis has been considered evidence for cryptic homologies within the members of the genome, homologies which are inactive in the normal diploid where completely homologous chromosomes synapse. Catcheside (3) was able to demonstrate 6 such homologous sections in a monoploid *Oenothera,* and Reiger (29) concluded that 40% of the chiasmata in meiosis of a monoploid *Antirrhinum majus* resulted from 1 duplicated section in the monoploid.

The amount of bivalent formation may vary between haploids of a given species. For example, while Reiger (29) reported 1 to 4 II's per PMC in a monoploid *Antirrhinum majus,* Ernst (10) observed, in the same species, from 10.8 to 20.4% of cells with 8 I's. In some such cases the differences observed may be attributed to genetic control of pairing; thus Riley and Chapman (30) demonstrated that chromosome arm 5B of *Triticum aestivum* contained factors regulating pairing.

Doubling of the chromosome number of haploids produces homozygous clones which are true breeding, barring mutation. These could serve as "living yardsticks," as suggested by Meyer and Justus (21) in breeding lines, especially those designed to study genetic variability and heritability. Nei (23) showed in theoretical studies that the haploid method of breeding; e.g., doubling and selfing; may be advantageous if the number of genes is large and the number of favorable loci small, as would be the case in peach materials. Assuming that the pollen produced by these monoploid peaches is isogenic and that sufficient male fertility for fruit set is available, the necessity for doubling is eliminated if self-pollinated progeny are not required.

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Factors Affecting Pollination of Onions in Idaho During 1969[1]

W.P. Nye, G.D. Waller,[2] and Norman D. Waters[3]
Agricultural Research Service, U.S. Department of Agriculture
Logan, Utah and *Tucson, Arizona*

Abstract. The ratio of pollen-fertile to pollen-sterile rows was more than twice the recommended ratio. The activity of honey bees, *Apis mellifera* L., was largely limited to pollen collecting on the pollen parent rows; there was an average of 100 bees per 100 ft of row on the pollen-parent as compared with a maximum of 40 bees per 100 ft of row on the male-steriles. Bee activity and seed yields (which were generally unsatisfactory) decreased as the distance from the pollen rows increased. About half of the bees sampled at the hive entrances had pollen loads and about 8 percent of these were onion pollen. Samples from the pollen traps contained 6 percent onion pollen. Onion as a source of pollen is less attractive to honey bees than other sources in the area. The viability of onion pollen from flowers in the morning was 2 to 3 times greater than in the afternoon. Onion pollen taken from pollen traps did not germinate.

Inconsistent yields of onion seed have been reported in the 2 main areas of onion seed production in the United States (southern California and southwestern Arizona, and southwestern Idaho and eastern Oregon). Seed company representatives report increased difficulty in obtaining grower-contracts because of the risk of crop failure and the high cost of production.

Pollination and seed production in onion, *Allium cepa* L., are known to be affected by several cultural practices including irrigation (7,9), numbers of effective pollinators (1), use of N fertilizer (13), use of insecticides (8), row spacing (7), ratio of male-sterile (A-line) to male-fertile (C-line) rows in hybrid seed production fields (6), and the distance of A-line rows from C-line rows (5). Other important factors include synchrony in the blooming dates of parent lines, the period the pollen remains viable, and the length of time the stigma remains receptive.

Onion flowers normally produce enough nectar and pollen to attract insects, but growers sometimes have difficulty getting honey bees, *Apis mellifera* L., to work blossoming onion fields when other floral sources are available nearby. Also, in Idaho, Campbell et al. (4) observed "abnormal" florets in onion seed fields in which the ovary had started to develop but had failed to produce seeds. They did not determine whether this abnormality resulted from lack of pollination or from some unknown physiological stress on the plant. Brewbaker and Majumder (2), however, reported that viability of pollen and pollen tube growth are affected almost immediately by any irregularity in plant vigor. Also, the onion stigma is known to have a normal receptive time of 3 days (11), but germination is reduced as the pollen ages (10).

In 1969 a study was made by the Wild Bee Pollination Investigations Laboratory at Logan, Utah, the Bee Research Laboratory at Tucson, Arizona, and the University of Idaho, Branch Experiment Station, Parma, Idaho, to investigate the factors affecting yields of hybrid onion seed. The results reported here are based on a single study without replication in time or space.

Materials and Methods

A 35-acre field of commercial hybrid onion seed located about 14 miles southwest of Nampa in southwestern Idaho was used as the test area. The onions were planted seed-to-seed in 21-inch rows with a ratio of 24 A-line to 4 C-line rows. The pollen-sterile parents were MSU-2399A and MSU-1411A, and the pollen-fertile parent was MSU-611C (planted to produce hybrids MSU-2399 X 611 and MSU-1411 X 611). Sugar beets, barley, and potatoes were growing in fields adjacent to the onion seed field. Carrot and alfalfa seed fields were 1 mile to the northwest and alfalfa seed fields were about 3 miles to the east.

On June 10, 132 colonies of honey bees at the rate of 3.8 colonies per acre with an average of 600 sq inches of brood and bees to cover 16 frames were moved into the field. Approximately equal numbers were placed in each of the 4 corners.

Honey bee visitation within the field was recorded during the period of peak bloom (June 15 to July 7). Also, returning foragers were collected at the hive entrances between 2:00 and 4:00 PM with an aspirator made from a small automobile vacuum cleaner (Fig. 1) (12). Bees observed in the field and those caught at the hive entrances were classified as either pollen or nectar collectors. In addition, pollen samples were collected daily from pollen traps installed on 4 colonies. Pollen loads from the bees collected in the aspirator and from the pollen traps were identified as to plant source. Germination of onion pollen from freshly dehisced anthers and from older anthers was studied by taking samples from MSU-611C (growing in the study field) and from B-2215C and 'White Ebenezer' (growing in the area) at mid-morning and mid-afternoon and germinating them in a pollen culture medium recommended by Brewbaker and Kwack (3). Pollen from the pollen traps was also tested for germination. The concn of nectar sugar was determined by catching foraging bees, causing them to regurgitate the stomach contents and then using a hand refractometer to measure the total soluble solids. Seed yield

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[2]Entomology Research Division.

[3]University of Idaho, Branch Experiment Station, Parma, Idaho.