Production of Tetrahaploids in the Cultivated Strawberry[1]

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Abstract. **Octoploid strawberry, *Fragaria* x *ananassa* Duch., (2n = 56), pollinated by *Potentilla anserina* L. (2n = 28) and *P. fruticosa* L. cv. Golddrop (2n = 14) gave high achene set but germination was low and seedling lethality high in both cases. Most offspring from *P. anserina* died by 6 weeks with 1% survival of germinating seed at 34 weeks; offspring from *P. fruticosa* pollination showed gradual mortality with 7% surviving at 34 weeks. Surviving offspring from *P. anserina* pollinations were either 28 chromosome tetrahaploids (5 plants) or 56 chromosome octoploids (4 plants). Surviving offspring from *P. fruticosa* pollinations included tetrahaploids (2 plants), octoploid (1 plant), or 35-chromosome pentaploid intergeneric hybrids (9 plants). Tetrahaploids are weak with small leaves and two that have flowered are pollen and pistil sterile.**

Although there have been many attempts at chromosome reduction in strawberry only two "haploids" have been reported in *Fragaria*. Islam (7) obtained a single haploid (2n = x = 7) from pollinations of *F. vesca* L. (2n = 14) with *F. ananassa* cv. Bradley (2n = 56) and recently Barrientos and Bringhurst (3) report a single "polyhaploid"[3] (2n = 28) from pollinations of the 'Tioga' strawberry with *P. anserina*.

Successful achene set from strawberry have been reported with a number of *Potentilla* species including *P. indica* (8), *anglica, execta,* and *reptans* (2), *fruticosa* (2, 5) *palustris* (5), *rupestris* (1), *glandulosa* (2, 9), and *anserina* (3). Most of these are sublethal because while some germination is observed, seedlings generally do not survive. Intergeneric hybrids surviving to flowering have been observed with *P. fruticosa* (2, 5), *palustris* (5), and *glandulosa* (2). The suggestion of Barrientos and Bringhurst in 1973 (3) for using the sublethal screen provided by intergeneric pollinations to obtain strawberry "haploids" prompted this study.

Two potentilla species were used: *P. fruticosa* cv. Golddrop, an ornamental shrubby species in the Purdue University collection originally obtained from Appalacian Nurseries, and *P. anserina,* obtained from Royce Bringhurst, University of California, Davis. The *P. anserina* clone was the same one used in the paper of Barrientos and Bringhurst (3). Chromosome counts of root tips indicated that the *P. fruticosa* clone was diploid (2n = 14) and the *P. anserina* clone was tetraploid (2n = 28). The chromosome number of this clone was unknown (R. Bringhurst, personal communication). Tetraploid clones of *P. fruticosa* and hexaploid clones of *P. anserina* (2n = 42) have been reported (4).

Three clones of the cultivated strawberry were used as seed parents, 'Surecrop'. Purdue 11-44, and Md-US 3699. Ud-US 3699 obtained from D. H. Scott, U. S. Department of Agriculture, Beltsville, Maryland, is a pistillate clone with only rudimentary anthers. Emasculation of this clone is unnecessary and unpollinated flowers in isolation never produce viable achenes; in contrast contamination after emasculation of 'Surecrop' is often observed (6). 'Surecrop' and Purdue 11-44 are perfect flowered.

Berry set with *P. anserina* pollen ranged from 67-78% which compared with an average of 93% for the controls (selfs of 'Surecrop' and Purdue 11-44 and Md-US 3699 x 'Surecrop') as shown in Table 1. *P. fruticosa* pollen gave berry set ranging from 10-63%. Achene set was variable; achenes per berry averaged 23.8 with *P. anserina* pollen and 22.3 with *P. fruticosa* as compared to 68.1 for controls. A total of 4423 achenes was obtained from *P. anserina* crosses and 2547 from *P. fruticosa*.

Seed was germinated in petri dishes containing sterile sand in a growth chamber, 16 hr photoperiod (13 klx) at

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[3]As the cultivated strawberry is octoploid, "haploids" (2n = 28) are tetraploid and the term tetrahaploid would be technically precise. The term polyhaploid was used by Barrientos and Bringhurst (3).

Table 1. Results of intergeneric crosses of cultivated strawberry pollinated by *Potentilla anserina* and *P. fruticosa.*

Seed parent	Pollen parent	No. pollination	% fruit set	Seeds per fruit	No. seed	% germination[z]	No. germinated seed	Plant survival (no.) growth chamber 2 wk	growth chamber 6 wk	greenhouse 18 wk	greenhouse 24 wk	greenhouse 34 wk	No. of plants 2n=28 Tetrahaploid	2n=35 Intergeneric hybrid	2n=56 Octoploids
Intergeneric crosses															
Purdue 11-44	*P. anserina*	50	76.0	29.7	1130	30.8	293	287	31	0	0	0	–	–	–
Surecrop	*P. anserina*	108	66.7	14.0	1010	9.0	84	73	6	2	2	2	0	0	2
US-Md 3699	*P. anserina*	96	79.2	30.0	2283	15.4	319	296	13	9	7	4	5	0	2
Total or (avg.)		*254*	(73.2)	(23.8)	*4423*	(17.6)	*696*	*656*	*50*	*11*	*9*	*6*	*5*	*0*	*4*
Purdue 11-44	*P. fruticosa*	57	49.1	18.5	519	70.3	83	72	33	28	20	8	–	–	–
Surecrop	*P. fruticosa*	143	10.5	2.9	44	25.0	11	9	2	2	0	0	–	–	–
US-Md 3699	*P. fruticosa*	112	63.4	27.9	1984	17.0	338	318	287	50	22	2	2	9	1
Total or (avg.)		*312*	(36.5)	(22.3)	*2547*	(24.8)	*432*	*399*	*322*	*214*	*70*	*30*	*2*	*9*	*1*
Controls															
Purdue 11-44	self	5	100	89.4	447	63.6	196	48[y]	42	42	42	42	0	0	2
Surecrop	self	5	80	58.3	233	57.5	65	10[y]	10	9	9	9	–	–	–
US-Md 3699	Surecrop	5	100	54.6	273	76.8	86	35[y]	32	31	31	31	–	–	–
Total or (avg.)		*15*	(93)	(68.1)	*953*	(65.1)	*347*	*93*	*84*	*82*	*82*	*82*	*0*	*0*	*2*

[z]Based on uncontaminated seed; about 18% of seed lots were discarded because of fungal growth.
[y]Only 48 seedlings of Purdue 11-44, 10 of Surecrop and 35 of US-Md 3699 x Surecrop were transplanted.

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25°C (day) 20° (night), and surviving seedlings transplanted to the greenhouse after 6 weeks.

There were differences in germination and seedling lethality depending on the pollen parent. Germination was 24.8% from *P. fruticosa* pollinations and 17.6% for *P. anserina* compared to 65.1% for the controls. After 34 weeks only 1% of seedlings derived from *P. anserina* survived and 7% from *P. fruticosa* as compared to 88.2% for the controls. In *P. anserina* the mortality was greatest by 6 weeks while in *P. fruticosa* death occurred continuously over the 34 week period (Fig. 1).

After 18 weeks, the 225 survivors of *Fragaria-Potentilla* crosses separated into at least 2 morphological groups: 5 normal-appearing seedlings and 220 plants of moderate to very weak vigor, with small to medium leaves, many of which appeared abnormal.

Chromosome counts of the 5 normal appearing plants showed them to be octoploid with 56 chromosomes. Chromosome counts of the weak group proved difficult due to poor growth but were completed for 16 plants. Two counts were found: 2n = 28 and 2n = 35.

After 24 weeks, 9 plants remained from the *P. anserina* pollinations and chromosome counts were obtained from all of them. Of these, 2 were derived from 'Surecrop' and 7 from Md-US 3699. The 2 'Surecrop' seedlings were octoploid and may be contaminants as indicated by studies of wide crosses involving 'Surecrop' by Fowler and Janick (6). Of the 7 plants from Md-US 3699, 2 were octoploid (2n = 56) and 5

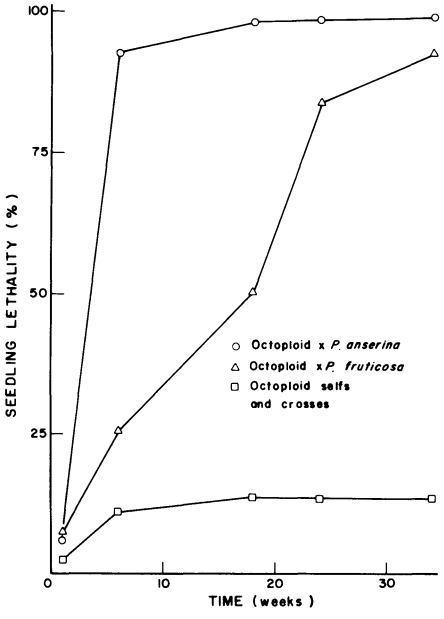

Fig. 1. Lethality of selfs and crosses within cultivated strawberry as compared to offspring derived from pollinations by *Potentilla anserina* and *P. fruticosa*.

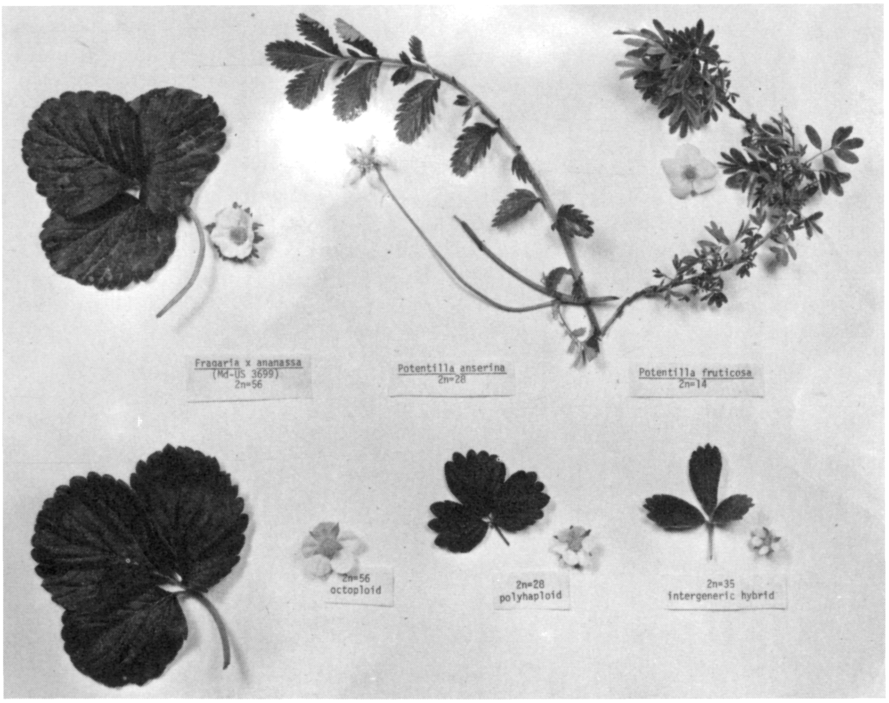

Fig. 2. Leaves and flowers of the cultivated strawberry, *Potentilla* species (top row) used as pollinators, and representative offspring (bottom row). All 3 chromosome types were produced with *P. fruticosa* pollinations; only octoploids and tetrahaploids were produced from *P. anserina* pollinations. Flowers of *P. anserina* and *P. fruticosa* are yellow; Md-US 3699, and octoploid and tetrahaploid offspring are white; 35 chromosome intergeneric hybrids are pale yellow.

were tetrahaploid (2n = 28). No plants were observed with 42 chromosomes which would indicate viable intergeneric hybrids.

After 24 weeks 50 plants survived from the *P. fruticosa* pollinations of which 12, all derived from Md-US 3699, were counted. One of these was octoploid (2n = 56), 9 were pentaploids (2n = 35) and thus intergeneric hybrids, while 2 were tetrahaploids (2n = 28).

Offspring from intergeneric pollinations and controls were rated for plant vigor, and morphology of leaves and flowers. Octoploids could be distinguished easily from intergeneric hybrids and tetrahaploids on the basis of high plant vigor, large leaf size, and large flower size (Table 2, Fig. 2). Because of their great variability it was difficult to separate haploids and intergeneric hybrids before they flowered. In general, leaves of intergeneric hybrids tended to have a greater length/width ratio with serrations confined to the apex than leaves of tetrahaploids. A clear cut distinction appears possible at flowering: petals of 2 flowering tetrahaploids were white while petals of one confirmed 35 chromosome intergeneric hybrids was pale yellow, most clearly visible immediately after opening.

By 34 weeks, 7 of the remaining 21 "unknowns" from *P. fruticosa* pollinations flowered; all had pale yellow flowers and are therefore assumed to be intergeneric hybrids. All tetrahaploids and intergeneric hybrids

Table 2. Plant vigor and leaf morphology in offspring of intergeneric crosses.

Chromosome number	No. of plants	Plant[z] vigor	Leaf morphology[y] Length (mm)	Width (mm)	L/W	Serration index[x]
Controls						
56	20	4.8	52	45	1.17	.31
Intergeneric crosses						
56	5	4.8	45	43	1.09	.39
35	9	3.2	31	26	1.21	.57
28	7	3.3	34	32	1.06	.47
Unknown	21	2.7	31	26	1.23	.56

[z]Ratint scale: 1 (weak) to 5 (vigorous).

[y]Measurements were made of the central blade of each of 3 trifoliate leaves per plant.

[x]The serration index is a ratio of the nonserrated length (basal portion of leaflet) over total length; thus, the higher the index, the lower is the amount of leaf serration.

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are both pistil and pollen sterile as previously observed (3, 6).

Our results confirm the feasibility of the sublethal screen afforded by intergeneric hybridization to obtain chromosome reduction in strawberry. *P. anserina* is a useful choice as a pollen parent with cultivated strawberry because all intergeneric hybrids are lethal at an early stage. Results from *P. fruticosa* indicate that this species and undoubtedly others may be used. Tetrahaploids may have been previously overlooked from earlier *P. fruticosa* intergeneric crosses because of the difficulties in distinguishing them from intergeneric hybrids which survive at a much greater frequency. Our results suggest that tetrahaploids occur at an approximate frequency of 1 per 1000 seed in crosses of cultivated strawberry by *Potentilla anserina* and *P. fruticosa*. Further tetrahaploids will have to be produced to determine if fertility can be found in these types.

The octoploids derived from Md-US 3699 are very likely naturally-doubled tetrahaploids, and if so, suggest this technique may be useful for obtaining inbred lines directly in strawberry. Of the 3 octoploids derived from Md–US 3699 x *Potentilla*, 2 survived to flowering with one (derived from *P. anserina*) pistillate and the other (derived from *P. fruticosa*) hermaphroditic.

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Ethephon-induced Flowering in Apple Seedlings[1]

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Abstract. **(2-Chloroethyl) phosphonic acid (ethephon) applied as a foliar spray at 1000 and 2000 ppm to 3-, 4-, and 5-yr old nonbearing seedlings of apple (*Malus pumila* Miller) significantly increased the percentage of trees flowering for the first time and the total number of flower clusters per tree.**

Seedling apple trees generally retain their juvenile character for several years (2). These experiments were designed to determine if ethephon which stimulates flower bud initiation in mature apple trees (3) could also induce flowering in young seedling apple trees.

In 1969 and 1970 ethephon was applied as a foliar spray to 3-, 4-, and 5-yr old apple trees which had not yet flowered. Treatments consisted of ethephon at 0, 50, 100, 500, and 1000 ppm as single and repeat applications in 1969. Single sprays were applied June 10 and double sprays June 10 and June 30. The repeat applications were not made to 5-yr old trees. In 1970, ethephon was applied to previously untreated trees at 1000 and 2000 ppm as a single spray on May 26 and June 11, 2 and 5 weeks after normal full bloom respectively. Treatments were applied to 3-tree blocks and replicated 3 times, once in each of 3 age classes, making a total of 207 trees studied in 1969 and 90 trees in 1970. Trees from the same cross constituted a single replication. Treatments were applied to run off using a hand gun. Polyoxyethylene sorbitan (Tween 20) at 0.1% was used as a surfactant.

Ethephon, at 1000 ppm as a single application in 1969, significantly increased the % apple seedlings that flowered for the first time, at all ages the following year (Table 1). The mean no. of flower clusters per tree also increased with ethephon treatment (Table 2). Although 1000 ppm had the greatest influence on flowering response, there was no consistent pattern of response from concn or no. of sprays. In some cases less flowering occurred with the repeat applications.

Table 1. Effect of ethephon on the % flowering of young apple seedlings, 1970.

Ethephon Treatment (ppm)	Date of Application (June, 1969)	Trees Flowering (%) z/ Age of seedlings in 1969 3 yr	4 yr	5 yr	Mean
0	–	11	22	17	17
50	10	0	44	22	22
50	10 + 30	0	44	–	22
100	10	0	22	28	17
100	10 + 30	11	22	–	17
500	10	11	11	39	20
500	10 + 30	11	11	–	11
1000	10	56**	56**	50**	54
1000	10 + 30	11	44	–	28

* Statistical significance, P = 5%.
** Statistical significance, P = 1%.
z/ Data are means for 9 trees of each age class per treatment.

Table 2. Effect of ethephon on the number of flower clusters per tree on young apple seedlings, 1970.

Ethephon Treatment (ppm)	Date of Application (June, 1969)	No. flower clusters per tree z/ Age of seedlings in 1969 3 yr	4 yr	5 yr	Mean
0	–	7	11	8	9
50	10	0	42**	29**	24
50	10 + 30	0	28**	–	14
100	10	0	3	69**	24
100	10 + 30	14	5	–	10
500	10	15	10	55**	27
500	10 + 30	7	3	–	5
1000	10	56**	49**	148**	84
1000	10 + 30	18	12	–	15

* Statistical significance, P = 5%.
** Statistical significance, P = 1%.
z/ Data are means for 9 trees of each age class per treatment.

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