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Effects of Plant Growth Regulators on Fruit Set and Fruit Shape of Parthenocarpic Apple Fruits

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We applied auxin, cytokinin, gibberellins, and gibberellin synthetic inhibitor to flowers of 'Ohrin', which is parthenocarpic, and 'Fuji', which is non-parthenocarpic, to elucidate the relationship between plant growth regulators and parthenocarpy in apple. The percentage of fruit set in gibberellin A_3 (GA_3) was about 60% in 'Ohrin' and about 7% in 'Fuji'. Forchlorfenuron (CPPU) combined with GA_3 treatment increased the fruit retention rate more than 14 days after treatment (DAT) than GA_3 alone. Dichlorprop (2,4-DP) combined with GA_3 slightly enhanced the gibberellin effect of inducing parthenocarpy. In 'Ohrin', GA_3 and gibberellin A_{4+7} (GA_{4+7}) showed a higher fruit retention rate before flowering than after. Parthenocarpic fruits were induced even when uniconazole (UCZ) was sprayed after flowering, but not before flowering. The calyx end was large in GA_{4+7}, and in GA_3 and CPPU singly, and GA_3 and CPPU combined treatments, which increased the percentage of fruit set. These results suggest that gibberellins before flowering trigger parthenocarpic apple fruits. Subsequent fruit growth requires cytokinins and auxin.

Key Words: apple, auxin, cytokinin, gibberellins, parthenocarpy.

Introduction

Parthenocarpy produces seedless fruits without fertilization, which is a very useful characteristic for fruit cultivation because it obviates hand pollination, the use of pollinating insects, and pollenizers. Seeds are usually formed, and the ovary and receptacle enlarge if pollination and fertilization occur normally in apples. Simultaneously, seeds produce indole-3-acetic acid (IAA) (Kondo et al., 1999), gibberellins (Dennis, 1976; Luckwill and Weaver, 1969; Oyama et al., 1996), and cytokinins (Ramirez, 2000) during growth. These plant growth regulators apparently control fruit growth. Several apple cultivars exhibit parthenocarpy. 'Megumi' reportedly shows percentages of fruit sets of 38–67% over four years with non-pollination (Saito et al., 1978). The percentages of fruit set with non-pollination have been reported as 72% in 'Ohrin' (Saito et al., 2007) and 48% in 'Spencer Seedless' (Tanaka et al., 2004); however, the relationship between endogenous plant growth regulators and the growth of seedless fruits, which have no seeds to function as a source of plant growth regulators, remains unclear. Gibberellins are closely related with parthenocarpy in apples: application of gibberellins (Bukovac, 1963; Bukovac and Nakagawa, 1967; Davison, 1960; Nakagawa et al., 1967) and the combination of gibberellins with cytokinins (Bangerth and Schröder, 1994; Williams and Letham, 1969) can induce parthenocarpic fruiting. Exogenous plant hormone application to unfertilized ovaries might serve as a switch that starts the continuing autonomous development of the fruit (Bangerth and Schröder, 1994), and controls the continuity of the flow of assimilates and nutrients required for fruit growth (Treharne et al., 1985). Genetic parthenocarpic cultivars might also have switch-induced autonomous development of the fruit, although the time at which this switch acts in naturally occurring parthenocarpic fruits remains unclear.

To elucidate which plant growth regulators act at which time to induce parthenocarpy in apple, we applied plant growth regulators to two apple cultivars that display different parthenocarpy: 'Ohrin' and 'Fuji'. We then investigated their fruit set and shape.

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Materials and Methods

1. Effects of single and combined treatment of plant growth regulators on the fruit set and shape of parthenocarpic apple fruits

This study examined three 15-year-old apple trees each of 'Ohrin' (*Malus pumila* Mill.), which is a parthenocarpic cultivar, and 'Fuji', which is a non-parthenocarpic cultivar, both grafted on M. 26 rootstock, growing at Takizawa Farm at the Field Science Center of Iwate University, Japan. Lateral flowers were removed. The petals, pistils, and stamens of the central flower on the apical buds of 'Ohrin' and 'Fuji' trees were removed immediately before flowering, respectively, on May 14 and May 19, 2005. The following were applied to the cut surface by hand spraying immediately after removal of the flower parts: 4.5 ppm Dichlorprop (2,4-DP), 5 ppm Forchlorfenuron (CPPU), 500 ppm gibberellin A_3 (GA_3), 500 ppm GA_3 combined with 4.5 ppm 2,4-DP (GA_3 + 2,4-DP), 500 ppm GA_3 combined with 5 ppm CPPU (GA_3 + CPPU), and 500 ppm GA_3 combined with 4.5 ppm 2,4-DP and 5 ppm CPPU (GA_3+2,4-DP+CPPU). Removal of flowers only, with no spray treatment, was used as a control; hand pollination was also established. No flowers were covered with paper bags. Each treatment used 10 flowers per tree: 30 fruits for each cultivar. Until harvest, the dropped fruit from two trees were counted; their percentages were calculated. 'Ohrin' and 'Fuji' were harvested respectively on November 9 and 10. Their fruit set, fruit weight, and fruit shape were measured. In 'Ohrin', each length of vertical section was measured, as depicted in Figure 1. The presence of seeds in harvested fruits was confirmed to judge whether they were parthenocarpic: all harvested fruits, except for those with pollination treatment, were parthenocarpic.

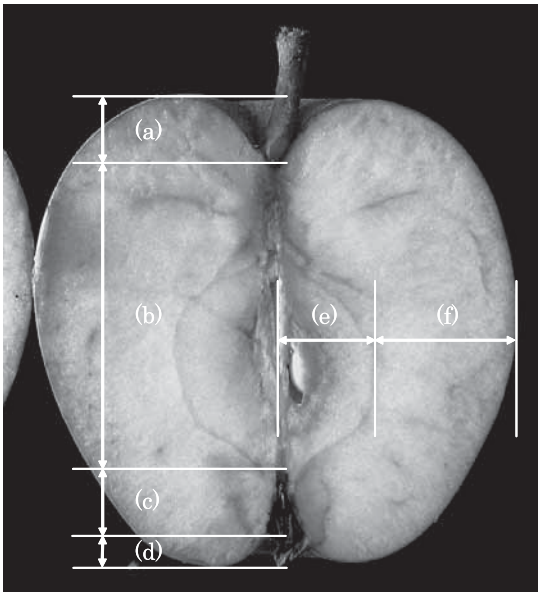

Fig. 1. Longitudinal cross-section of an apple, indicating stalk cavity (a), core length (b), basal calyx end (c), apical calyx end (d), core width (e), and cortex (f).

2. Effects of plant growth regulator treatment time on fruit set and shape of parthenocarpic apple fruits

In 2006, this study examined four 16-year-old apples trees each of 'Ohrin' and 'Fuji', all grafted onto M. 26 rootstock, growing at Takizawa Farm. In both cultivars, the central flowers of the apical bud on spurs were selected and petals, pistils, and stamens were removed before flowering. Each plant growth regulator was applied for 'Ohrin' on May 12 (4 days before flowering, –4 DAF), May 15 (1 day before flowering, –1 DAF), May 18 (2 days after flowering, 2 DAF), and May 22 (6 days after flowering, 6 DAF), and for 'Fuji' on May 17 (1 day before flowering, –1 DAF), May 20 (2 days after flowering, 2 DAF), and May 24 (6 days after flowering, 6 DAF). Then, 45 ppm 2,4-DP, 5 ppm CPPU, 500 ppm GA_3, 500 ppm gibberellin A_{4+7} (GA_{4+7}), and 500 ppm uniconazole (UCZ) were hand sprayed to the cut surfaces of five flowers per tree: 20 fruits in all. The control flowers were untreated. No flowers were covered with paper bags. 'Ohrin' and 'Fuji' were harvested on November 6 and 13, respectively and then the fruit set and fruit weight were measured. In the cultivars, each length of the vertical section shown in Figure 1 was measured and the lengths and diameters of fruits were calculated. Only data for 'Ohrin' are shown. The presence of seeds in harvested fruits was confirmed to judge whether they were parthenocarpic: all harvested fruits, except for those with pollination treatment, were parthenocarpic.

3. Statistics

Data for fruit measurements of the same cultivars (Tables 1 and 2) and those for the same treatment of each cultivar (Tables 3 and 5) were compared using Tukey-Kramer HSD at the 5% level. The control was common to each treatment in Tables 3 and 5.

Results

1. Effects of single and combined treatment of plant growth regulators on the fruit set and shape of parthenocarpic apple fruits

The fruit retention rate in 'Ohrin' decreased from 8 DAT in the control, 2,4-DP-alone, and CPPU-alone treatments, and from 14 DAT in the GA_3-alone and GA_3-combined treatments (Fig. 2A). The wave pattern of fruit drop in 'Ohrin' was bimodal in control, 2,4-DP-alone, CPPU-alone and GA_3 + 2,4-DP treatments, and was uni-modal in the GA_3-alone and GA_3 + 2,4-DP + CPPU treatments. Regarding 'Fuji', the control, 2,4-DP-alone, and CPPU-alone treatment showed a fruit retention rate decrease from 6 DAT (Fig. 2B). The fruit retention rate of GA_3-alone and GA_3+2,4-DP treatments decreased from 21 DAT; that of CPPU-combined treatments decreased from 28 DAT. 'Fuji' showed one

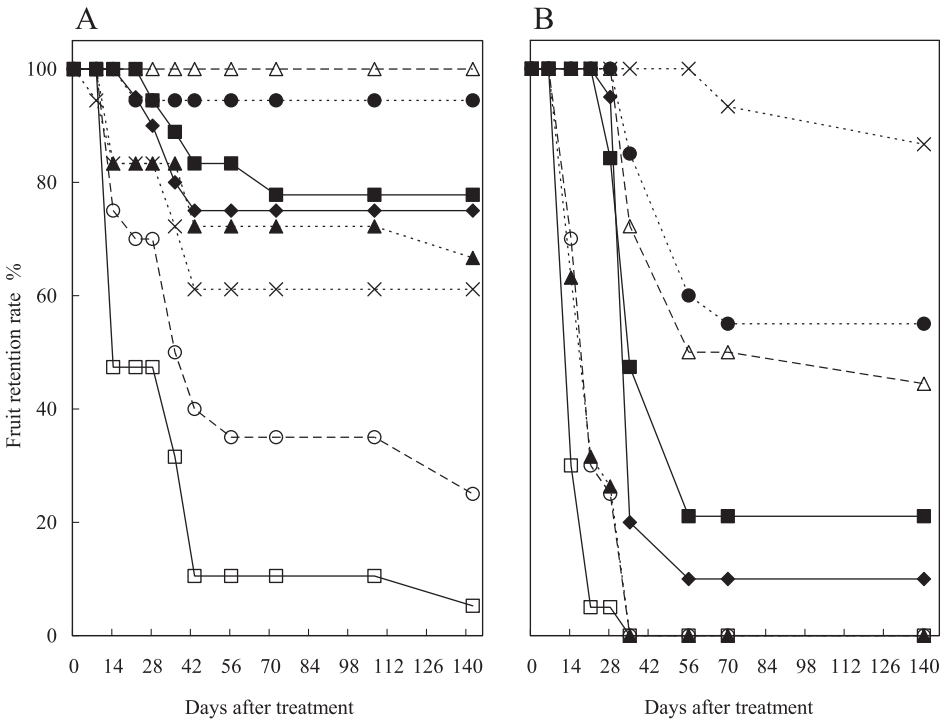

Fig. 2. Effects of plant growth regulators on retention of parthenocarpic fruits in 'Ohrin' (A) and 'Fuji' (B) in 2005. Data are the percentage of fruit retention for 20 fruits. □: Control, ○: 2,4-DP, ▲: CPPU, ◆: GA_3, ■: GA_3 + 2,4DP, △: GA_3 + CPPU, ●: GA_3 + 2,4DP + CPPU, ×: Pollination.

Table 1. Effects of plant growth regulators on fruit set, fruit weight, and fruit shape of parthenocarpic fruits in 'Ohrin' and 'Fuji' in 2005.

Clutivars	Treatments	Fruit set[z] (%)	Fruit weight (g)	Fruit length (mm)	Fruit diameter (mm)	L/D ratio[w]
Ohrin	Control	24.1	167[y] b[x]	68.8 d	68.9 b	1.00 b
	2,4-DP	31.0	168 b	68.8 d	69.3 b	0.99 b
	CPPU	64.3	225 b	85.1 b	73.3 b	1.16 a
	GA_3	60.0	189 b	78.2 bc	69.0 b	1.14 a
	GA_3 + 2,4-DP	69.0	205 b	81.7 b	70.3 b	1.16 a
	GA_3 + CPPU	86.7	313 a	91.9 a	82.1 a	1.12 a
	GA_3 + 2,4-DP + CPPU	89.3	314 a	92.5 a	82.5 a	1.12 a
	Pollination	64.3	209 b	74.6 cd	74.3 b	1.01 b
Fuji	Control	0	—	—	—	—
	2,4-DP	0	—	—	—	—
	CPPU	0	—	—	—	—
	GA_3	6.7	197 b	70.9 bc	73.1 bc	0.97 ab
	GA_3 + 2,4-DP	21.1	177 b	69.1 bc	65.3 c	1.07 a
	GA_3 + CPPU	50.0	291 ab	83.3 ab	80.2 ab	1.04 a
	GA_3 + 2,4-DP + CPPU	40.0	367 a	91.2 a	86.7 a	1.05 a
	Pollination	76.0	232 b	71.2 c	78.7 b	0.90 b

[z] Percentage of harvested fruits to 30 fruits.
[y] Data are the means of harvested fruits.
[x] Different letters within columns of each cultivar indicate significant differences, as indicated by Tukey-Kramer HSD ($P < 0.05$).
[w] Values show the ratio of fruit diameter to fruit length.

peak period of fruit drop, except for the control.

The percentages of fruit set in the control were 24% in 'Ohrin' and 0% in 'Fuji' (Table 1). For both cultivars, the percentage of fruit set was higher in the GA_3-alone treatment than in the control. Furthermore, 2,4-DP or CPPU combined with GA_3 treatment showed a higher percentage of fruit set. Additional effects of CPPU were higher than those of 2,4-DP in the GA_3 + 2,4-DP and GA_3 + CPPU treatments. Furthermore, 2,4-DP-alone and CPPU-alone treatments in 'Ohrin' showed about 31%

and 64% fruit set, respectively, whereas all fruit dropped in 'Fuji'. 'Ohrin' showed a higher percentage of fruit set than 'Fuji' in all treatments, except for pollination. Regarding 'Ohrin', the harvested fruits were significantly longer and the L/D ratios were significantly higher in CPPU-alone, GA_3-alone, and combined GA_3 treatments than in the control. In addition, GA_3 + CPPU and GA_3 + 2,4-DP + CPPU treatments showed heavier fruits with the larger diameter than the control. For 'Fuji', fruit were heaviest, longest, and showed the largest

Table 2. Effects of plant growth regulators on morphology of parthenocarpic fruits in 'Ohrin' in 2005.

Treatments	Stalk cavity (%)	Core length (%)	Calyx end (%)		Core width (%)	Cortex (%)
			basal	apical		
Control	14.6[z] a[y]	61.2[z] ab	18.0[z] a	3.3[z] c	34.5[x] ab	65.5[x] ab
2,4-DP	13.1 a	62.8 a	18.0 a	4.1 bc	33.9 ab	66.1 ab
CPPU	12.9 a	58.5 ab	18.6 a	5.6 bc	35.5 ab	64.5 ab
GA_3	13.9 a	59.0 ab	18.9 a	6.3 b	38.9 ab	61.1 ab
GA_3 + 2,4-DP	12.8 a	57.0 bc	18.6 a	6.4 b	34.7 ab	65.3 ab
GA_3 + CPPU	14.5 a	56.6 bc	17.8 a	8.2 a	39.3 a	60.7 b
GA_3 + 2,4-DP + CPPU	14.6 a	53.8 c	18.6 a	9.0 a	34.6 b	65.4 a
Pollination	12.9 a	61.2 a	16.6 a	5.5 bc	36.9 ab	63.1 ab

[z] Values represent the ratio of that part to fruit length.
[y] Different letters within columns indicate significant differences by Tukey-Kramer HSD ($P < 0.05$).
[x] Values represent the ratio of that part to fruit diameter.

Table 3. Effects of treatment time of plant growth regulators on fruit set, fruit weight, and fruit shape of parthenocarpic fruits in 'Ohrin' in 2006.

Treatments	Time (DAF)	Fruit set[z] (%)	Fruit weight (g)	Fruit length[w] (mm)	Fruit diameter[v] (mm)	L/D ratio[u]
Control	–4	30	167[y] a[x]	77.5 a	77.2 a	1.01 b
	–4	70	211 a	83.5 a	61.1 b	1.37 a
GA_3	–1	70	197 a	81.1 a	58.7 b	1.38 a
	+2	30	171 a	76.9 a	57.3 b	1.34 a
	+6	55	186 a	79.3 a	63.1 b	1.26 a
Control	–4	30	167 a	77.5 b	77.2 a	1.01 c
	–4	80	195 a	83.0 b	61.1 b	1.36 ab
GA_{4+7}	–1	60	211 a	86.7 b	61.6 b	1.41 a
	+2	55	192 a	99.2 a	80.5 a	1.23 abc
	+6	60	202 a	102.9 a	86.0 a	1.20 bc
Control	–4	30	167 ab	77.5 ab	77.2 a	1.01 a
	–4	0	—	—	—	—
UCZ	–1	0	—	—	—	—
	+2	25	135 b	71.7 b	70.7 a	1.01 a
	+6	20	180 a	88.7 a	87.2 a	1.02 a
Control	–4	30	167 a	77.5 ab	77.2 ab	1.01 a
	–4	35	195 a	94.5 a	84.9 ab	1.11 a
2,4-DP	–1	50	197 a	93.8 a	87.7 a	1.07 a
	+2	15	219 a	96.1 a	91.9 a	1.05 a
	+6	15	164 a	69.5 b	64.4 b	1.08 a
Control	–4	30	167 b	77.5 b	77.2 a	1.01 b
	–4	45	240 ab	106.5 a	88.2 a	1.21 a
CPPU	–1	50	260 a	108.4 a	85.9 a	1.26 a
	+2	25	213 ab	98.0 a	82.8 a	1.18 a
	+6	40	258 ab	103.8 a	88.0 a	1.18 a

[z] Percentage of harvested fruits to 20 fruits.
[y] Data are the mean of harvested fruits.
[x] Different letters within columns for each treatment indicate significant differences by Tukey-Kramer HSD ($P < 0.05$).
[w] Fruit length was calculated as (a) + (b) + (c) + (d) (Fig. 1).
[v] Fruit diameter was calculated as (e) + (f) (Fig. 1).
[u] Values represent the ratio of fruit diameter to fruit length.

diameter in GA_3 + 2,4-DP + CPPU treatments; those characteristics were intermediate in GA_3 + CPPU treatment, and smallest in GA_3-alone and GA_3 + 2,4-DP treatments.

Regarding 'Ohrin', the ratios of the stalk cavity and basal calyx end to fruit length were not significantly different among treatments (Table 2). The ratio of the core length to fruit length was significantly smaller in the GA_3 + 2,4-DP + CPPU treatment than in the control. The ratio of the apical calyx end to fruit length in each of the GA_3-alone and GA_3 + 2,4-DP treatments was significantly larger than in the control; the respective ratios of GA_3 + CPPU and GA_3 + 2,4-DP + CPPU treatments were more increased; however, no treatment showed a significantly different ratio of the cortex or core width to the diameter.

2. Effects of the treatment time of plant growth regulators on fruit set and shape of parthenocarpic apple fruits

The percentage of fruit set of 'Ohrin' was 30% in the control (Table 3). Results for 2,4-DP treatment after flowering showed a lower percentage of fruit set: half that of the control. CPPU treatment before flowering showed a higher percentage of fruit set: 45–50%. The percentage of fruit set with GA_3 treatment before flowering was 70% and 55% at 6 DAF. All GA_{4+7} treatments showed better fruit set percentages than the control: the percentage of fruit set was 80% for −4 DAF. The percentage fruit set with UCZ treatment was 0% before flowering, but 20–25% after flowering. In 'Fuji', the percentage of fruit set was highest with GA_{4+7} treatments (25–35%) among all treatments, and 0% in the control (Table 4). The percentage of fruit set was 10% with GA_3 treatment at −1 DAF and 2 DAF. CPPU treatment applied after flowering displayed better fruit set (15%) than treatment with application before flowering (0–5%). For 2,4-DP treatment, fruit set was observed only at −1 DAF (5%). No fruit set occurred in any UCZ treatment. A slight effect among all treatments was apparent for the fruit weight of 'Ohrin' (Table 3). Fruit length increased significantly in all CPPU treatments compared to the control and decreased significantly in all GA_3 and GA_{4+7} treatments before flowering, thereby increasing the L/D ratio. The fruit length and diameter were significantly larger in GA_{4+7} treatments after flowering than before flowering. The 2,4-DP treatment showed significantly longer fruit length and diameter at 6 DAF than at any other time.

The ratios of stalk cavity and cortex to fruit length and the ratio of core width to diameter were little affected by the respective treatments (Table 5). The ratio of core length to fruit length was significantly lower in GA_3 and 2,4-DP treatments at −4 DAF, GA_{4+7} after flowering, and all CPPU treatments except for 2 DAF. Larger ratios of the basal calyx end to fruit length were apparent for earlier application of GA_3 and 2,4-DP and later application GA_{4+7}. The ratio of the apical calyx end to fruit length was larger in GA_{4+7} in 6 DAF and 2,4-DP treatments before flowering.

Table 4. Effects of treatment time of plant growth regulators on fruit set, fruit weight, and fruit shape of parthenocarpic fruits in 'Fuji' in 2006.

Treatments	Time (DAF)	Fruit set[z] (%)	Fruit weight (g)	Fruit length[x] (mm)	Fruit diameter[w] (mm)	L/D ratio[v]
Control	−1	0	—	—	—	—
GA_3	−1	10	98 ± 8[y]	53.4 ± 0.8	51.4 ± 0.3	1.04 ± 0.02
	+2	10	135 ± 38	62.0 ± 4.7	57.3 ± 5.5	1.08 ± 0.02
	+6	0	—	—	—	—
GA_{4+7}	−1	25	164 ± 60	68.5 ± 8.5	61.0 ± 9.1	1.12 ± 0.07
	+2	35	180 ± 56	68.2 ± 6.5	67.8 ± 7.7	1.01 ± 0.08
	+6	25	152 ± 25	63.8 ± 2.6	58.0 ± 5.1	1.10 ± 0.08
UCZ	−1	0	—	—	—	—
	+2	0	—	—	—	—
	+6	0	—	—	—	—
2,4-DP	−1	5	313	83.2	77.0	1.08
	+2	0	—	—	—	—
	+6	0	—	—	—	—
CPPU	−1	5	302	90.7	ND[u]	ND[u]
	+2	15	188 ± 55	71.9 ± 9.4	68.9 ± 7.4	1.04 ± 0.08
	+6	15	174 ± 22	63.3 ± 3.5	59.3 ± 7.3	1.07 ± 0.17

[z] Percentage of harvested fruits to 20 fruits.
[y] Mean ± SD; data are the mean of harvested fruits.
[x] Fruit length was calculated as (a) + (b) + (c) + (d) (Fig. 1).
[w] Fruit diameter was calculated as (e) + (f) (Fig. 1).
[v] Values represent the ratio of fruit diameter to fruit length.
[u] ND; no data.

Table 5. Effects of treatment time of plant growth regulators on morphology of parthenocarpic fruits in 'Ohrin' in 2006.

Treatments	Time (DAF)	Stalk cavity (%)	Core length (%)	Calyx end (%)		Core width (%)	Cortex (%)
				basal	apical		
Control	−4	14.8[z] a[y]	69.0[z] a	8.5[z] c	7.7[z] a	36.9[x] a	63.1[x] a
	−4	16.2 a	58.9 b	16.5 a	8.4 a	33.4 a	66.6 a
GA_3	−1	14.3 a	62.7 ab	15.2 a	7.8 a	31.3 a	68.7 a
	+2	16.3 a	61.5 ab	14.1 ab	8.1 a	34.3 a	65.7 a
	+6	16.0 a	65.0 a	10.6 bc	8.4 a	32.8 a	67.2 a
Control	−4	14.8 ab	69.0 a	8.5 c	7.7 bc	36.9 ab	63.1 ab
	−4	13.4 b	66.5 a	12.0 b	8.1 c	34.8 b	65.2 a
GA_{4+7}	−1	14.1 ab	63.7 ab	13.2 ab	9.0 abc	36.6 ab	63.3 ab
	+2	16.1 a	58.4 b	14.6 a	10.9 ab	40.7 a	59.3 b
	+6	14.9 ab	59.0 b	14.9 a	11.3 a	42.2 a	57.8 b
Control	−4	14.8 a	69.0 a	8.5 a	7.7 a	36.9 a	63.1 a
	−4	—	—	—	—	—	—
UCZ	−1	—	—	—	—	—	—
	+2	16.4 a	70.5 a	7.4 a	5.8 a	40.9 a	59.1 a
	+6	16.9 a	62.4 a	10.9 a	9.7 a	40.6 a	59.4 a
Control	−4	14.8 a	69.0 ab	8.5 ab	7.7 b	36.9 a	63.1 a
	−4	17.8 a	59.8 c	10.4 a	11.9 a	38.9 a	61.1 a
2,4-DP	−1	15.6 a	62.3 bc	10.2 a	11.9 a	40.6 a	59.4 a
	+2	17.9 a	60.6 abc	10.2 ab	11.3 ab	38.6 a	61.4 a
	+6	14.8 a	74.4 a	5.1 b	5.7 b	36.4 a	63.6 a
Control	−4	14.8 a	69.0 a	8.5 b	7.7 a	36.9 b	63.1 a
	−4	16.3 a	57.8 b	15.5 a	10.4 a	39.2 ab	60.8 ab
CPPU	−1	16.7 a	58.0 b	14.5 a	10.7 a	39.0 ab	61.0 ab
	+2	13.8 a	60.6 ab	14.4 a	11.2 a	41.9 a	58.0 b
	+6	15.0 a	58.9 b	15.6 a	10.6 a	38.5 ab	61.5 ab

[z] Values represent the ratio of that part to fruit length.
[y] Different letters within columns each treatment indicate significant differences by Tukey-Kramer HSD ($P < 0.05$).
[x] Values represent the ratio of that part to fruit diameter.

Discussion

In 2005, we conducted single and combined applications of GA_3, which can induce parthenocarpy (Bukovac, 1963; Bukovac and Nakagawa, 1967; Davison, 1960; Nakagawa et al., 1967), and of 2,4-DP and CPPU to confirm which plant growth regulators are closely related to parthenocarpy in apple. Then, we sprayed the following before or after flowering in 2006: GA_3; GA_{4+7}, which is more effective for inducing parthenocarpy (Nakagawa et al., 1967), UCZ; 2,4-DP, which was used at 10 times higher concentration than in 2005 and CPPU. We then further investigated the relationship between gibberellins and parthenocarpy to elucidate which plant growth regulators act at which time to induce parthenocarpy.

Physiological fruit drop in apple usually occurred immediately after flowering and in June. The respective main causes are respectively attributed to non-fertilization (Childers, 1976) and nutrient competition among fruits or between fruits and shoots (Makino et al., 1986). In our experiment, 2,4-DP and CPPU treatment showed no effects at the time of the first fruit drop, but GA_3-alone and GA_3-combined treatments delayed both cultivars. These results suggest that the effects of exogenous auxin and cytokinin on fruit retention are low during about a week after flowering in the early fruit development stage. It is noteworthy that parthenocarpic fruit in 'Ohrin' were induced even when UCZ was sprayed after flowering, but not before flowering (Table 3). Therefore, gibberellins that are present in the receptacle and/or ovary before flowering may be important to induce parthenocarpy; they play a minor role in fruit growth after flowering. Moreover, GA_3 + CPPU treatment increased the fruiting rate after 14 DAT, suggesting that cytokinins are responsible for fruit growth after induced parthenocarpy. This result supports the hypothesis (Bangerth and Schröder, 1994) that parthenocarpic fruits induced by gibberellins might be deficient in cytokinins and the synthetic cytokinin CPPU might then compensate for that.

The effects of GA_4 and GA_7 on induced parthenocarpy were stronger than that of GA_3 on 'Wealthy' (Nakagawa et al., 1967), 'Golden Delicious', and 'Jonagold' (Bangerth and Schröder, 1994). Both 'Ohrin' and 'Fuji' showed the same reaction exhibited in this study: GA_4

and GA_7 were closely associated with parthenocarpy in many apple cultivars; however, the relationship between parthenocarpy and the gibberellin variety remains unclear because GA_3 and GA_4 levels in exudates from developing apple fruits differed among apple cultivars, including parthenocarpic 'Spencer Seedless' in some studies (Prang et al., 1997), but not in others (Stephan et al., 1999).

In general, auxin is not active for induced parthenocarpy (Hayashi et al., 1968). Application of 2,4-DP alone in both cultivars only slightly affected fruit set; fruit set in the treatment after flowering was lower than that of the control for 'Ohrin'. The 2,4-DP might act as a flower-thinning agent (Yokota and Tsukahara, 2000); however, auxin combined with gibberellins slightly enhanced the effect of gibberellins on induced parthenocarpy. These results suggest that auxin alone cannot induce parthenocarpy, but it promotes fruit growth after parthenocarpy. This effect of 2,4-DP might be less than that of cytokinin.

Luckwill et al. (1969) reported that apple seeds, during their development within the fruit, produce different hormones sequentially, the appearance and disappearance of which are linked with successive stages in endosperm and embryo development. Gibberellins in apple seeds increased to a maximum concentration 6–10 weeks after full bloom and subsequently decreased quickly, disappearing completely by the time the seed was mature (Luckwill et al., 1969; Dennis, 1976; Ramirez, 1995). The amounts of cytokinins in seeds were higher 16 days after full bloom (Ramirez, 2000); IAA concentration in seeds peaked at about 11 weeks after full bloom (Kondo et al., 1999). In our experiments, parthenocarpy in the control was shown in 'Ohrin' (Tables 1 and 3), but not in 'Fuji' (Tables 2 and 4). In all treatments, the percentage of fruit set in 'Ohrin' was higher than that in 'Fuji'. Moreover, GA_3 and GA_{4+7} treatments presented a higher fruit retention rate before flowering than after flowering, and treatment with UCZ application before flowering did not induce parthenocarpy (Table 3); therefore, gibberellin concentrations in flowers might be higher in 'Ohrin' than 'Fuji' and higher before flowering than after flowering. Furthermore, the sensitivity to gibberellins of 'Ohrin' might be higher than that of 'Fuji' in the early stages before flowering. As a result, parthenocarpy ability might change in each cultivar. It is also likely that 'Ohrin' has high auxin and cytokinin concentrations in flowers compared to 'Fuji' because the fruiting rates in the treatment with gibberellin combined with 2,4-DP and/or CPPU are higher than in the gibberellin-alone treatment.

The most characteristic feature of GA-induced parthenocarpic apples is their larger polar diameter than that of seeded fruit; the transverse diameter of a parthenocarpic fruit is either equal to or slightly less than in seeded fruit (Nakagawa et al., 1967). Results of the present study showed that the effects of cytokinins on fruit length and diameter are independent of the application time. The effects on fruit shape changed according to the variety of gibberellins and the application time, which suggests that the fruit-growing stage alters the variety of endogenous gibberellins in parthenocarpic fruits. It is likely that the vigorous growth region of flowers changes by the growth stage, because the fleshy portion of the flower in the apple changed from flat to slender in stages from resting to bloom (Asada, 1980). The region with growth stimulated by gibberellin application might differ by the growth stage. The smaller cores in several treatments might be attributable to the increased stalk cavity and calyx end. Gibberellin and cytokinin treatments increased the apical calyx end compared to the control in 2005, but increased the basal calyx end in 2006. Saito et al. (2007) reported that the parthenocarpic fruit size in 'Ohrin' was affected by removal of the pistil. In the present study, the parthenocarpic fruit shape might have been affected by the time of petal, pistil, and stamen removal in each experiment. Reportedly, calyx end increase by gibberellins is dependent upon the cell number and cell size increase at the site of gibberellin application (Nakagawa et al., 1967), but parthenocarpic fruit naturally did not necessarily show increased calyx end size compared to seeded fruit. Nevertheless, it is interesting that the calyx end was large in high-fruit-set treatments, suggesting that endogenous gibberellin and cytokinin in the calyx end are related to parthenocarpy.

In conclusion, the results suggest that gibberellins applied before flowering induce parthenocarpic fruits. Cytokinins and auxin are necessary for fruit growth; however, no reports describing endogenous plant growth regulators in parthenocarpic apple fruits support these results. Future investigations must elucidate changes of endogenous gibberellins and sites of gibberellin production in parthenocarpic fruit.

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