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Review

Studies on Pistil Doubling and Fruit Set of Sweet Cherry in Warm Climate

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Recently, attempts have been made to produce sweet cherry in the warm region of Japan, although the occurrence of double fruit, resulting from double pistil formation, and poor fruit set due to frequent physiological fruit drop have become serious problems preventing stable production; however, the inducing factors have not been clarified. Therefore, we investigated the effects of environmental and nutritional conditions on the occurrence of double pistils and poor fruit set. Based on those results, we studied the regulation of pistil doubling and fruit set in this region. In this review, we summarize our studies on the reproduction of sweet cherry in a warm climate.

Key Words: double fruit, phytohormone, *Prunus avium*, reproductive organ, temperature.

Introduction

Sweet cherry (*Prunus avium* L.) has been mainly produced in the cool areas of northern Japan. Currently, however, attempts are being made to produce sweet cherry in the warm areas of the southwestern part of the country, such as Kagawa, Kochi, Hiroshima, Okayama, and Wakayama, in order to harvest the fruits earlier than in the northern major production areas and to supply local markets (Beppu and Kataoka, 2006).

In these warm areas, however, many problems arise, such as poor fruit set, occurrence of malformed fruit, including double fruit, poor coloration, fruit cracking, early defoliation, and the frequent appearance of pests and diseases, which prevent stable production of sweet cherry (Beppu and Kataoka, 1998; Kataoka et al., 1996). Among these problems, poor fruit set and the frequent occurrence of double fruit are the most serious problems directly relating to productivity. In cultivation in these areas, fruit set is very unstable, and severely fluctuates yearly and locally. In some years and orchards, trees seldom set fruit even after bearing many flowers. On the other hand, double fruit is a morphological disorder, resulting from double pistil formation (Fig. 1). In some years and cultivars, more than half of all flowers have double pistils in this region; however, environmental and nutritional factors inducing poor fruit set and pistil doubling have not been determined, and effective methods for overcoming them have not been found.

Therefore, to gain basic knowledge for the stable production of sweet cherry in warm regions, the aspects of double pistil formation and fruit set under natural growing conditions in this region were investigated initially. Then, the mechanisms of the occurrence of double pistils and poor fruit set were clarified by experimentation under controlled conditions. Based on these results, the regulation of pistil doubling and fruit set in this region was studied. In this review, we summarize our studies on the reproduction of sweet cherry in a warm climate.

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1. Process of flower bud formation and occurrence of double pistils in warm region

1) Process of flower differentiation

Double pistil formation is due to abnormal differentiation of pistil primordia in the process of flower bud formation, which occurs in the previous growing season. In cherries, floral initiation begins with flattening of the meristem, on which individual flower primordia are formed, then sepal, petal, stamen, and pistil primordia differentiate sequentially (Diaz et al., 1981; Guimond et al., 1998; Watanabe, 1982).

In the main production area, the Yamagata Basin, floral initiation reportedly occurred in late July, and then sepal and pistil primordia differentiated in late August and late September, respectively (Noguchi et al., 1999; Watatabe and Umetsu, 1980). In our observation in the Shonai Plain of Yamagata, the process was slightly earlier; floral initiation occurred in late June, and pistil primordia were formed in early September (Beppu, 2000). In Yamagata, pistil differentiation occurred when temperatures had decreased relative to mid-summer (Fig. 2).

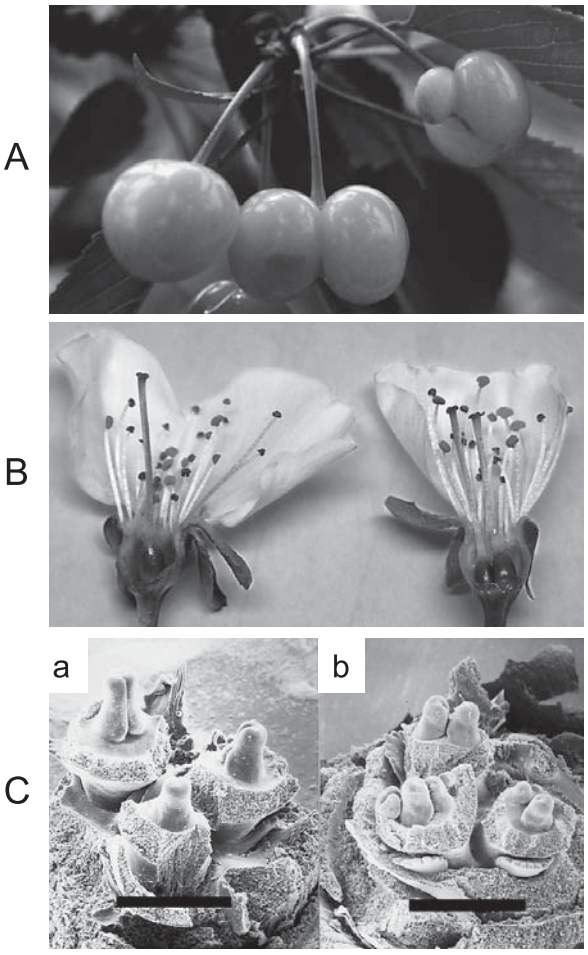

Fig. 1. Normal (left) and double (right and center) fruits (A), normal (left) and double (right) pistils (B) and normal (a) and double (b) pistil premordia (C).

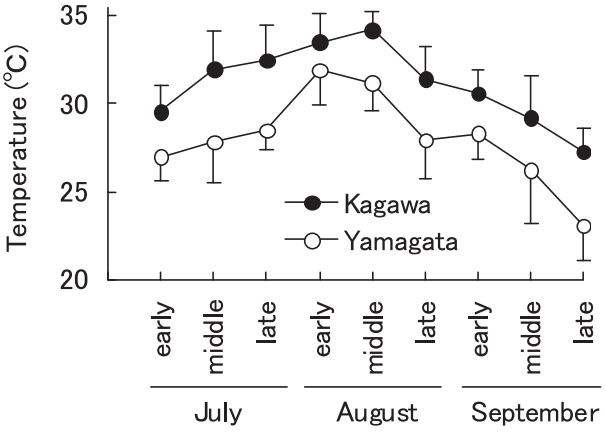

Fig. 2. Average (2005–2009) of 10-day mean daily maximum temperature from July to September in Yamagata (Yamagata City) and Kagawa (Takamatsu City). Data were obtained from the climate statistics of the Japan Meteorological Agency. Bars = SD (n = 5).

In the warm area of Kagawa, however, we observed that floral differentiation initiated in early to mid-June, and then sepal and pistil primordia differentiated in early to mid-July and early to late August, respectively (Table 1). Sepal to pistil differentiation occurred in the hottest period in that year (Beppu, 2000).

2) Occurrence of double pistils

Double pistil formation occurred more frequently in Kagawa than in Yamagata. The temperatures during flower bud formation were much higher in Kagawa, where the daily maximum temperatures frequently exceeded 30°C (Beppu, 2000). As for regional differences, Southwick et al. (1991) found that hot regions without coastal influences tended to have more double fruits than regions with climates moderated by coastal breezes in California. Roversi et al. (2008) also observed in Italy and Slovenia, that doubling occurred more frequently in areas where the previous summer was hotter than in the other regions.

Regarding the yearly variation, it was shown that doubling occurred more severely in the spring following hotter weather in the previous summer (Engin and Ünal, 2008; Roversi et al., 2008; Tucker, 1934, 1935). We also observed that the higher the temperature from mid-July to mid-August in the previous year, the higher the frequency of doubling (Beppu, 2000) (Fig. 3).

The position in the tree canopy also reportedly affected the frequency of doubling; doubling was more severe in the south and top parts than in the north and bottom parts (Philp, 1933; Southwick et al., 1991; Tucker, 1934, 1935). In our observations also, the frequency of double pistils was higher in the southern and top parts of the canopy, and locally in the spurs of top and southern sides of horizontal limbs (Beppu, 2000), implying that doubling occurs more severely in areas where bud temperatures may be increased by high solar radiation.

Taken together, these field observations may suggest that high temperatures during flower bud differentiation are involved in the occurrence of double pistils.

The frequency of doubling was highly variable among cultivars; in several cultivars, such as ‘Choinook’, ‘Karabodur’, and ‘Bing’, 30% or more of fruit had double fruit, although in several other cultivars, such as ‘Jubilee’, ‘Cherie’, and ‘Takasago’, double fruit seldom occurred (Fukai, 1995; Micke et al., 1983; Roversi, 2001; Roversi et al., 2008; Tucker, 1934). In Kagawa, severe pistil doubling occurred in popular cultivars ‘Satohnishiki’ and ‘Napoleon’, whereas it occurred only slightly in ‘Takasago’ (Beppu, 2000). This suggests that it may be possible to breed cultivars with a low occurrence of double pistils, as well as with the trait of high fruit quality.

2. Factors in flower bud formation and pistil doubling

1) Temperature during flower bud differentiation

The observations under natural growing conditions indicated the involvement of temperatures during flower bud differentiation in the occurrence of double pistils in

Table 1. The progression of flower bud formation in 'Satohnishiki' sweet cherry grown in Kagawa (Beppu, 2000).

Year	Sampling date	Buds (%) without apparent signs of floral initiation	Flowers (%) at the stage of differentiation of:					
			bract primordium	flower primordium	sepal primordium	petal primordium	stamen primordium	pistil primordium
1995	May 29	100.0	—	—	—		—	—
	June 14	50.0	50.0	—	—		—	—
	July 1	—	6.7	93.3	—		—	—
	July 15	—	—	21.4	78.6[z]		—	—
	July 30	—	—	40.0	50.0[z]		10.0	—
	Aug. 9	—	—	—	5.9[z]		70.6	23.5
	Aug. 19	—	—	—	—		35.3	64.7
	Aug. 29	—	—	—	17.6[z]		23.5	58.8
	Sep. 10	—	—	—	6.7[z]		—	93.3
1999	May 24	100.0	—	—	—	—	—	—
	June 8	90.0	10.0	—	—	—	—	—
	June 24	40.0	60.0	—	—	—	—	—
	July 8	—	6.9	69.0	24.1	—	—	—
	July 24	—	—	3.2	6.5	71.0	19.4	—
	Aug. 8	—	—	—	3.0	12.1	51.5	33.3
	Aug. 24	—	—	—	—	—	2.8	97.2
	Sep. 8	—	—	—	—	—	—	100.0

[z] Data include petal primordium in 1995.

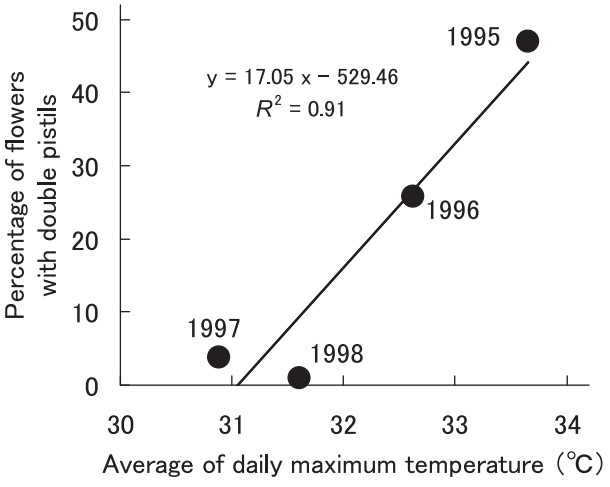

Fig. 3. Relationship between temperature from July 15 to August 14 and the frequency of double pistils in buds of 'Satohnishiki' sweet cherry trees grown in the reseach field of Kagawa University (Beppu, 2000).

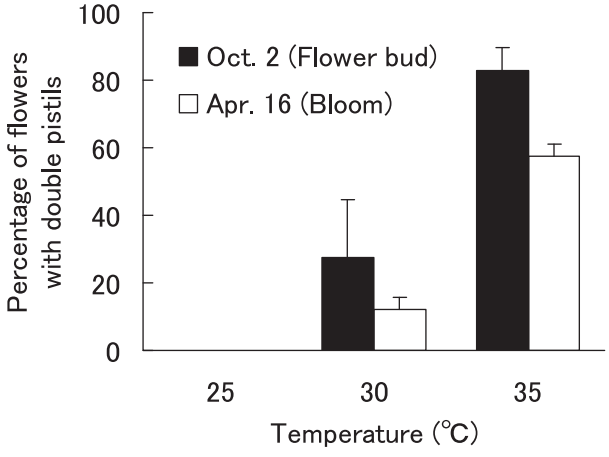

Fig. 4. Effect of temperature on the occurrence of double pistils in 'Satohnishiki' sweet cherry. Trees were grown from July 23 to September 5 in 1996 at the indicated temperature during the day and at 25°C (Beppu and Kataoka, 1999a). Bars = SE (n = 3).

sweet cherry as described above. To demonstrate this and to clarify the critical temperature inducing pistil malformation, we conducted experiments under controlled temperatures using sunlit growth chambers. High temperature treatments during flower bud formation delayed the progression of flower differentiation, thereby prolonging the period during which floral primordia could be subjected to further high temperatures. At 25°C, all of the flower buds had a single pistil, whereas above 30°C, the percentage of flowers with double pistils increased markedly (Fig. 4). This result clarified that high temperature above 30°C is a critical factor in the formation of double pistils (Beppu and Kataoka, 1999a). The frequency of double pistils at anthesis was considerably lower than that observed in the buds in autumn, suggesting that the more inferior of the two pistils sometimes degenerated before anthesis.

Exposure time to high temperatures during flower differentiation also influenced the occurrence of double pistils. High temperature induced pistil doubling most severely in buds that contained sepal and petal primordia at the beginning of treatment, and the frequency of occurrence of double pistils was slightly lower in buds treated at the earlier stage of flower differentiation. On

Table 2. Progression of flower bud formation at the onset of the high temperature treatment and effect of the treatment on the occurrence of double pistils in 'Satohnishiki' sweet cherry (Beppu et al., 2001a).

Period of high temperature treatment	Stage of flower differentiation at the onset of treatment						Percentage of flowers with double pisitils
	Bract primordia	Flower primordia	Sepal primordia	Petal primordia	Stamen primordia	Pistil primordia	
June 21–July 5	100.0[z]	—	—	—	—	—	4.8 ± 3.2[y]
July 6–July 20	28.6	42.9	12.2	16.3	—	—	5.9 ± 2.5
July 21–Aug. 4	6.4	21.3	55.3	17.0	—	—	8.8 ± 3.4
Aug. 5–Aug. 19	—	—	21.2	21.2	34.6	23.1	2.9 ± 0.7
Aug. 20–Sep. 3	—	—	—	—	24.0	76.0	2.2 ± 1.3
Control							1.7 ± 1.0

[z] Data show the percentage of flower buds at each stage of differentiation.
[y] Mean ± SE (n = 3).

the other hand, high temperature had little effect on pistil doubling in buds with differentiated stamen and pistil primordia (Table 2). These results suggest that buds are most sensitive to the induction of double pistils at high temperatures at the transition stage from sepal to petal differentiation (Beppu et al., 2001a).

In the warm area of Kagawa, flower buds form sepal and petal primordia in early to mid-July, when temperatures increase rapidly after the rainy season (Beppu, 2000); therefore, pistil doubling in this area seems to be caused not only by extremely high temperatures but also by the correspondence of the sensitive period of differentiation with the high temperature season.

2) Soil moisture

It has been experienced that postharvest water stress in regions with hot summers increased the occurrence of double pistils in peach, nectarine, and apricot buds (Johnson et al., 1992; Naor et al., 2005; Ryugo, 1988; Tukey, 1954). Thus, in sweet cherry also, drought during the floral initiation stage had been thought to induce doubling (Fukai, 1995). In our experiment, however, severe water stress by restricting watering hardly affected the frequency of double pistils (Beppu and Kataoka, 1999a). Engin and Ünal (2008) and Engin et al. (2009) also observed that drought stress treatments did not increase doubling, suggesting that water stress is not involved in doubling in sweet cherry. In plum, it was also reported that double fruit formation was not increased by postharvest water stress in summer (Johnson et al., 1994; Naor et al., 2004); therefore, the susceptibility to pistil doubling may be different among *Prunus* fruit tree species.

3) Phytohormones

High temperatures induced not only the formation of double pistils but also that of pistil-like appendages which replaced anthers (Beppu and Kataoka, 1999a; Philp, 1933; Roversi, 2001; Ryugo, 1988). These symptoms may reflect the feminization of the flower. Many researchers have observed the influences of phytohormones on flower sexuality (Chailakhyan and Khrianin, 1987; Sedgley and Griffin, 1989). The occurrence of double pistils in sweet cherry also may be controlled by endogenous phytohormones; thus, we investigated the involvement of phytohormones in the formation of double pistils.

Table 3. Effect of growth regulators on the occurrence of double pistils in 'Satohnishiki' sweet cherry (Beppu et al., 2007b).

Year	Treatment (ppm)[z]		Percentage of flowers with double pistils
1996	Control		15.1 ± 0.6[y]
	GA_3	(100)	7.8 ± 4.0
	ABA[x]	(100)	7.0 ± 4.7
	NAA	(100)	4.2 ± 3.1
	BA	(100)	27.2 ± 12.3
1997	Control		0.3 ± 0.3
	ABA[x]	(100)	0
	NAA	(100)	0
	Ethephon	(100)	6.4 ± 4.9
	BA	(100)	11.2 ± 7.6
1999	Control		1.0 ± 1.0
	Ethephon	(100)	12.1 ± 4.6
	Ethephon	(200)	10.0 ± 0.7
2005	Control		13.5 ± 2.8
	Ethephon	(100)	23.1 ± 6.7

[z] Aqueous solutions were applied to spurs by a hand sprayer in late July to early August.
[y] Mean ± SE [n = 3 (1999), 4 (1996, 1997, 2005)].
[x] Mixed isomers.

The application of benzyle adenine (BA) and ethephon increased the percentage of flowers with double pistils (Beppu et al., 2007b) (Table 3). It is known that cytokinin and ethylene induce flower feminization in Cucurbitaceae (Chailakhyan and Khrianin, 1987; Suge, 1980). Ethephon treatment delayed the progression of flower differentiation similar to that due to high temperature. Ethylene production by the spurs of trees grown in growth chambers controlled at 20°C, 30°C, and 35°C increased with the temperature. Generally,

ethylene production in plant tissue is promoted by an increase in temperature, and peaks at 30–35°C in many species (Field, 1985); therefore, it is suggested that ethylene could be involved in the formation of double pistils induced by high temperature.

On the other hand, the frequency of double pistils was low with the application of naphthalen acetic acid (NAA), abscisic acid (ABA), and gibberellin A_3 (GA_3) (Beppu et al., 2007b). Engin and Ünal (2008) and Engin et al. (2009) confirmed that GA_3 treatment reduced the rate of double pistils, suggesting the possibility of applying phytohormones to prevent the occurrence of this disorder.

3. Control of double pistil formation in warm regions

For the reasons mentioned above, avoiding the exposure of buds to high temperatures above 30°C while the buds are still in the sensitive period of differentiation is important to reduce pistil doubling in sweet cherry. Forcing culture and artificial shading were examined as practical solutions for this problem.

Forcing cultures accelerated flower differentiation considerably; therefore, in mid-July, when the maximum temperature began to rise rapidly following the rainy season, petal and stamen primordia had been formed in the buds under forcing conditions, but under non-forcing conditions, most of the buds were still at the stage of sepal differentiation. Forcing cultures reduced double pistil formation markedly (Beppu et al., 2001a) (Table 4).

Artificial shading in summer reduced the day air temperatures. In an extremely hot summer, when the daily maximum temperature reached 35°C for 14 days, only strong shading (78% light reduction) markedly reduced the occurrence of double pistils in the following spring. In a relatively cool summer, when the daily maximum temperature reached 35°C for only 2 days, however, even mild shading (53% light reduction) reduced pistil doubling (Beppu and Kataoka, 2000) (Fig. 5).

These findings suggest the possibility of applying forcing culture and artificial shading to reduce the occurrence of double pistils in sweet cherry grown in regions with hot summers. As another method to reduce temperatures, Southwick et al. (1991) examined over-tree sprinkler irrigation in sweet cherry grown in California. Over-tree sprinkling after harvest reduced canopy temperatures and led to a reduction in the production of abnormal fruits, including double fruit.

4. Flower development and fruit set in warm regions

Severe yield fluctuation on account of poor fruit set is a serious problem that prevents stable production of sweet cherry in the warm region of Japan. In this region, many fruit drop relatively early after bloom even with provision for cross-pollination. Thus, there may be some problems in the process of fertilization after pollination. Generally, pollen germination, ovule development, and pollen tube growth in style are limiting factors for fertilization (Thompson, 1996) and might be involved in the poor fruit set of sweet cherry grown in a warm climate.

1) Development of reproductive organs and fruit set

Pollen mother cells formed by 3 weeks before anthesis, and then began dividing and reached pollen tetrad stage 2 weeks before anthesis in Kagawa. Microspore formation was observed 10 days before anthesis (Beppu, 2000). Germination percentage of pollen collected at anthesis in Kagawa on culture medium was considerably high, up to 80% (Beppu and Kataoka, 1999b), indicating that pollen germination is not a limiting factor for fertilization in warm regions.

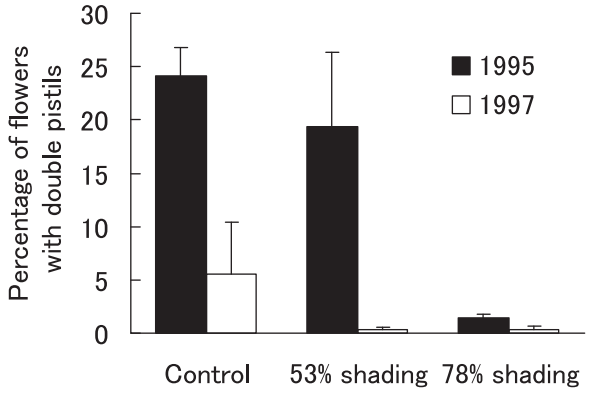

Fig. 5. Effect of shading on the occurrence of double pistils in 'Satohnishiki' sweet cherry (Beppu and Kataoka, 2000). Trees were grown under 53% and 78% levels of shade from July 23 to October 4 in 1995 and from July 16 to September 16 in 1997. Bars = SE (n = 3).

Table 4. Onset of heating and bloom, the progression of flower bud formation and the cccurrence of double pistils in 'Satohnishiki' sweet cherry under various culture conditions (Beppu et al., 2001a).

Culture condition	Onset of heating	First bloom	Percentage of flowers that had differentiated: (July 15)					Occurrence of double pisitils (%)
			Bract primordia	Flower primordia	Sepal primordia	Petal primordia	Stamen primordia	
Early forcing	Jan. 31	Mar. 6	—	—	7.1	57.1	35.7	0.8 ± 0.8[z]
Late forcing	Mar. 4	Mar. 27	7.7	15.4	7.7	46.2	23.1	0
Non-forcing	—	Apr. 25	—	66.7	33.3	—	—	10.3 ± 5.3

[z] Mean ± SE (n = 5).

The ovule had differentiated by 3 weeks before anthesis, and then nucellus tissue appeared 2 weeks before anthesis in Kagawa. The embryo sac differentiated 3 days before anthesis and developed quickly by anthesis (Beppu, 2000) (Fig. 6, Table 5). At anthesis, Eaton (1959) observed that many embryo sacs had reached the eight-nucleate stage, and then 50% and 80% degenerated 2 and 4 days after anthesis, respectively, in 'Windsor' sweet cherry grown in Ohio, where the average daily maximum temperature from bud burst to petal fall was as high as 23°C. In our observations, half of the embryo sacs of 'Satohnishiki' had reached the eight-nucleate stage at anthesis both in Kagawa and Yamagata. In Kagawa, the percentages of degenerated embryo sacs were about 30% and 80%, 2 and 4 days after anthesis, respectively, versus only 10% and 20%, respectively, in Yamagata (Beppu, 2000) (Table 5).

Regarding pollen tube elongation in pistil, Hedhly et al. (2007) observed that pollen tubes of 'Burlat' sweet cherry reached the obturator in the ovary 3 days after pollination in a warm region, Spain. In our observation, pollen tubes of 'Satohnishiki' and 'Takasago' reached the micropyle 2 to 3 days after pollination in both Kagawa and Yamagata.

This indicates that the effective pollination period in Kagawa is very short, only 1 to 2 days after anthesis, due to short embryo sac longevity; therefore, extending embryo sac longevity seems to be important for the success of fruit set in warm areas. Stigmatic receptivity also is known a possible limiting factor for fertilization. Hedhly et al. (2003) observed that stigmatic receptivity of sweet cherry grown in a warm region, Spain, began reducing 5 to 6 days after anthesis. Since the duration of stigmatic receptivity is longer than ovule longevity, stigmatic receptivity may not restrict fertilization.

Initial fruit set in Kagawa was considerably lower

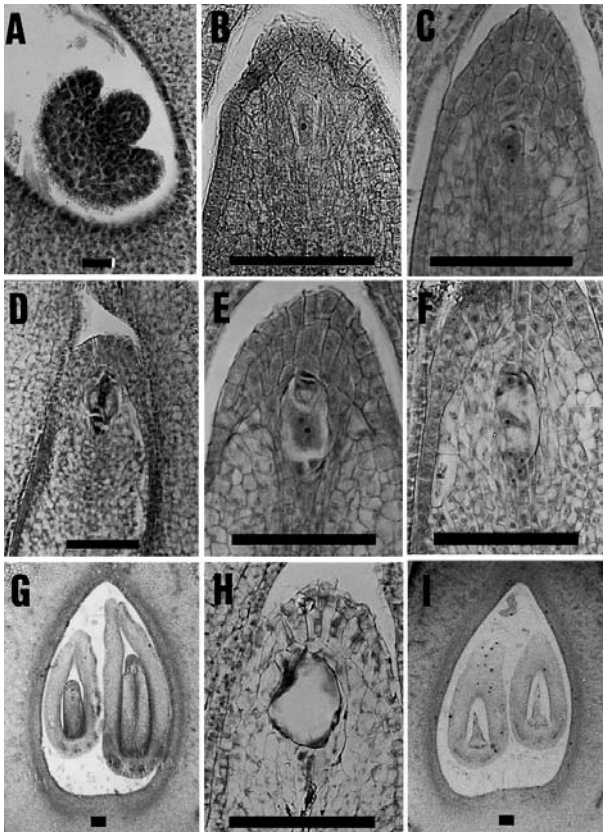

Fig. 6. Development of ovule in 'Satohnishiki' sweet cherry (Beppu, 2000). A: Differentiated nucellus. B: Embryo sac mother cell. C: Two-nucleate embryo sacs. D: Eight-nucleate embryo sac without differentiated egg cell. E: Eight-nucleate embryo sac with differentiated egg cell and unfused polar nuclei. F: Eight-nucleate embryo sac with differentiated egg cell and fused polar nuclei. G: Normal ovules with eight-nucleate embryo sac. H: Degenerated embryo sac. I: Degenerated nucellus. Bars: 100 μm.

Table 5. Ovule development of 'Satohnishiki' sweet cherry in Kagawa and Yamagata in 1999 (Beppu, 2000).

Location	Days after anthesis	Stages of development					
		No megasporocyte	Megasporocyte to embryo sac cell	Two-nucleate	Four-nucleate	Eight-nucleate	Degenerated
Kagawa[z]	−4	100.0[x]	0.0	0.0	0.0	0.0	0.0
	−3	83.3	8.3	8.3	0.0	0.0	0.0
	−2	25.0	33.3	8.3	8.3	0.0	0.0
	−1	50.0	0.0	8.3	25.0	8.3	8.3
	0	18.6	0.0	8.5	13.6	49.2	10.2
	1	16.7	0.0	25.0	0.0	50.0	8.3
	2	8.6	0.0	5.2	8.6	46.6	31.0
	4	0.0	0.0	0.0	0.0	19.7	80.3
	6	0.0	0.0	0.0	0.0	0.0	100.0
Yamagata[y]	0	8.3	0.0	16.7	12.5	58.3	4.2
	2	12.5	0.0	8.3	8.3	58.3	12.5
	4	5.0	0.0	0.0	10.0	65.0	20.0

[z] Kagawa University located in Miki-cho, Kagawa Prefecture.
[y] Yamagata University located in Turuoka city, Yamagata Prefecture.
[x] Percentage of ovules with embryo sac at different stages of development.

than in Yamagata, probably due to early degeneration of the embryo sac. In this observation year, temperatures from bud burst to petal fall were similar between these regions. Flowers grown in Kagawa had smaller pistils and ovules and a lower concentration of sugar than those in Yamagata (Beppu, 2000). Sweet cherry depends mainly on reserve nutrients for flower growth (Flore, 1994); thus, the difference in embryo sac longevity between these regions might be attributed to tree nutritional conditions.

2) Yearly fluctuation

Guerrero-Prieto et al. (1985) observed that the fruit set rate of hand-pollinated 'Napoleon' sweet cherry was lower in the year with a high temperature during bloom. In our observation for 4 years in Kagawa, the higher the temperature from bud burst to petal fall, the lower the fruit set of 'Satohnishiki' (Beppu, 2000) (Fig. 7), which may suggest the involvement of temperature in the success of fruit set. In addition, we observed that embryo sacs tended to degenerate faster when the temperatures in this period were higher. In prunes also, it was reported that ovule abortion proceeded more rapidly in warmer years (Thompson and Liu, 1973), indicating that early ovule degeneration might be a reason for the low rate of fruit set in years with high temperatures.

3) Comparison among cultivars

'Takasago' is the main cultivar in Yamanashi, where temperatures are slightly higher than in Yamagata. We grew this cultivar in Kagawa, but embryo sac longevity and fruit set of 'Takasago' were similar to those of 'Satohnishiki', which is the most popular cultivar in Yamagata (Beppu, 2000).

'Benisyuhou', whose cultivated area has increased recently, sets fruit very well in the major production area, Yamagata (Nishimura, 1997; Takahashi and Arasawa, 2001). We compared flower development and fruit set between this cultivar and 'Satohnishiki' in Kagawa, and showed that in 'Benisyuhou', embryo sacs degenerated later and resulted in a considerably higher rate of fruit set even in the warm area (Beppu et al., 2008a). This trait may possibly be used for cherry cultivation in warm climates.

Hedhly et al. (2004) compared pollen performance of two cultivars that differ in their adaptation to temperature. The ratio of pollen tubes reaching the stylar base was highest at 30°C in 'Cristobalina', which is native to Spain and adapted to warmer conditions, whereas it decreased markedly above 20°C in 'Sunburst', which originated in Canada with a pedigree of cultivars from Northern Europe. If the embryo sac development of this warm-adapted cultivar is also superior at high temperatures, adequate fruit set may be obtained in warm climates. In Japan, although only cold-adapted cultivars have been introduced, the reproductive traits of warm-adapted cultivars should be utilized in the warm region of the country.

5. Factors in flower development and fruit set

1) Temperature from bud burst to flowering

The observations under natural growing conditions indicated the involvement of temperature in flower development and fruit set of sweet cherry, as described above. To demonstrate this and to clarify the critical temperature influencing fruit set, we conducted experiments under controlled temperatures with sunlit growth chambers. High temperature treatment above 20°C from one month before anthesis to petal fall reduced flower size and markedly decreased the fruit set rate (Beppu et al., 1997) (Fig. 8). Mahmood et al. (2000) also observed that high temperature treatment (25°C) after chilling reduced the flower size and fruit set rate compared to low (9°C) and intermediate (19°C)

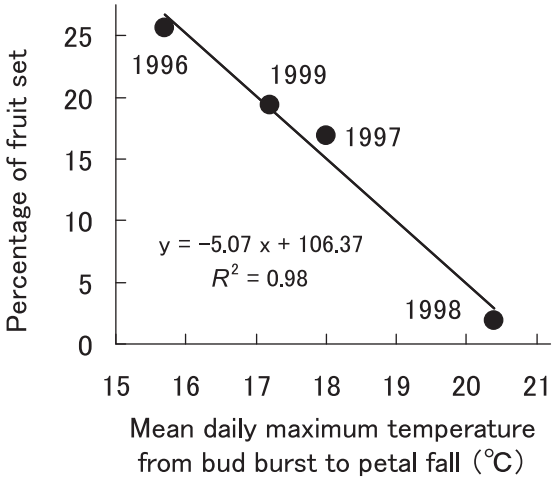

Fig. 7. Relationship between temperature from bud burst to petal fall and fruit set in 'Satohnishiki' sweet cherry grown in the research field of Kagawa University between 1996 and 1999 (Beppu, 2000).

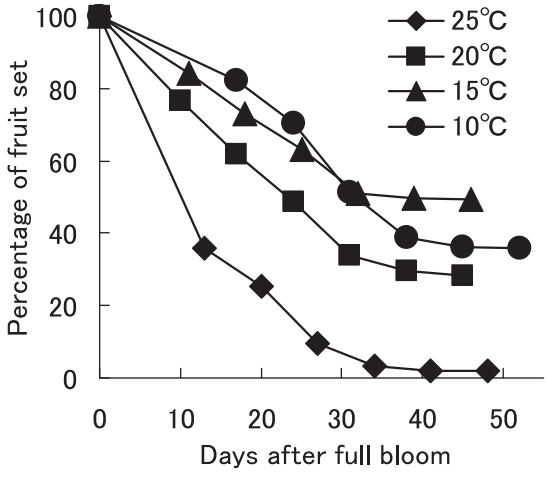

Fig. 8. Effect of day temperature on fruit set of 'Satohnishiki' sweet cherry. Trees were grown at each temperature in growth chambers/field temperature (day/night) from March 16 to petal fall (Beppu et al., 1997).

temperatures. These results suggest that high temperatures above 20°C reduce fruit set in sweet cherry. In the southwestern part of Japan, air temperature rises rapidly in early spring, and the daily maximum temperature often reaches 20°C from bud burst to petal fall; therefore, the high temperature at this time may be a cause of poor fruit set in this region.

In our experiment, temperatures hardly affected the elongation of pollen tubes in pistils (Beppu et al., 1997), whereas Hedhly et al. (2007) showed that warm treatment accelerated pollen tube growth. Considering that the appropriate temperature for pollen germination on culture media is relatively high, 25°C (Beppu and Kataoka, 1999b), the low fruit set rate at high temperatures may not due to the pollen elongation rate in the style.

On the other hand, the rate of ovules with a degenerated embryo sac or nucellus increased considerably after anthesis at high temperatures (Beppu et al., 1997, 2001b, 2005) (Table 6). Postweiler et al. (1985) also reported that high temperature forced ovule senescence on detached shoots incubated in a growth chamber, based on fluorescence-microscopic observations. These results reveal that the rapid degeneration of the embryo sac and nucellus is a major reason for the reduction in fruit set when the developing buds are exposed to high temperatures.

In forcing culture in a greenhouse, which has been extended in major production areas recently, failure of fruit set has become a serious problem. Especially when temperatures in the greenhouse are 25°C or higher, fruit set rates become very low even after artificial pollination (Endo, 1993). This also might be attributed to early ovule degeneration caused by high temperatures. Hedhly et al. (2007) demonstrated that warm treatment by covering the trees in the field with a polyethylene cage, which increased the maximum temperature by 5–7°C, accelerated ovule degeneration and markedly reduced fruit set.

2) Gibberellin

The development of reproductive organs, such as the embryo sac and pollen, is often influenced by the application of plant hormones, especially gibberellin (GA), which suppresses the development of the embryo sac and shortens its longevity in grapes (Komatsu, 1987; Okamoto and Omori, 1991; Sugiura, 1969; Takagi, 1980). In sweet cherry, Stösser and Anvari (1982) reported that GA enhanced ovule senescence, based on fluorescence-microscopic observations. In our experiment, the application of GA_3 to bursting buds considerably increased the percentage of ovules with a degenerated embryo sac or nucellus by 2 days after anthesis and resulted in reduced fruit set (Beppu et al., 2001b, 2005) (Table 7). These results suggest that GA regulates ovule development in sweet cherry flowers.

On the other hand, we compared endogenous GA levels of flowers grown under different temperatures. The flowers developing at high temperatures, whose ovules degenerated rapidly, showed a higher endogenous GA level than those at low temperatures (Beppu et al., 2001b, 2005) (Table 6). This increased level of endogenous GA under high temperature may induce early ovule degeneration.

Table 6. Effect of temperature on ovule development and content of GA-like substances in 'Satohnishiki' sweet cherry (Beppu et al., 2001b).

Temperature (°C)	Stages of development 2 days after anthesis					GA (GA_3 equiv.) concentration ($pg \cdot g\ FW^{-1}$)
	Embryo sac mother cell	Two-nucleate	Four-nucleate	Eight-nucleate	Degenerated	
15	0.0[z]	5.3	14.7	41.3	38.7	427
25	0.0	0.0	2.5	30.7	66.8	744
Significance[y]	NS	NS	NS	NS	*	*

[z] Percentage of ovules with embryo sac at different stages of development.
[y] NS, *: Nonsignificant or significant at $P < 0.05$ by ANOVA (n = 5).

Table 7. Effects of GA_3 treatment on ovule development and fruit set in 'Satohnishiki' sweet cherry (Beppu et al., 2001b).

Treatment (ppm)[z]	Stage of development 2 days after anthesis					Fruit set (%)	
	Embryo sac mother cell	Two-nucleate	Four-nucleate	Eight-nucleate	Degenerated	Initial	Final
Control	1.5[y]	0.0	16.3	59.8	22.4	42.8	16.9
GA_3 (10)	4.5	2.4	2.4	34.8	55.8	15.9	12.2
GA_3 (100)	6.0	6.0	6.3	27.8	53.9	18.2	8.9
Significance[x]	NS	NS	NS	*	*	*	NS

[z] Aqueous solutions of GA_3 were applied to spur buds by a hand sprayer on April 8, 1997.
[y] Percentage of ovules with embryo sac at different stages of development.
[x] NS, *: Nonsignificant or significant at $P < 0.05$ by ANOVA (n = 4).

3) Tree assimilate accumulation

In sweet cherry, flowering occurs before the leaves begin working as a photoassimilate source; therefore, flower development strongly related to fruit set must rely upon carbohydrate reserves accumulated in the previous year. Defoliation in early autumn, which reduces the carbohydrate reserve of the tree, increased the percentage of flower buds failing to burst and abnormal flowers without styles (Beppu et al., 2003a). Similar effects were observed in sour cherry (Howell and Stackhouse, 1973; Kennard, 1949). Furthermore, defoliation shortened the ovule longevity and consequently reduced fruit set (Beppu et al., 2003a). Thus, to ensure adequate fruit set of sweet cherry, the tree needs to produce carbohydrate reserves sufficient for flower development in the following spring by high photosynthetic activity and by leaf maintenance through summer and autumn.

In warm areas in Japan, however, summer day temperatures continuously exceed 30°C and concomitantly, soil conditions often become excessively dry due to scant rainfall. Under these conditions, the photosynthetic activity of sweet cherry basically adapted to a cool climate seems to be adversely affected. Our experiment demonstrated that both high temperatures and dry soil conditions reduced the leaf photosynthetic rate and accelerated leaf abscission in 'Satohnishiki' sweet cherry, and resulted in decreasing carbohydrate accumulation (Beppu et al., 2003b) (Table 8). Roper and Kennedy (1986) also observed reduction of the leaf photosynthetic rate due to high temperatures above 30°C in research on photosynthetic characteristics in 'Bing' sweet cherry; however, we showed that even at high temperatures, if the trees are irrigated sufficiently, the photosynthetic rate is relatively high and nonstructural carbohydrate concentration is almost the same level as that at low temperatures (Beppu et al., 2003b).

6. Improvement of fruit set in warm regions

1) Inhibition of gibberellin synthesis

As mentioned above, the increased level of endogenous GA under high temperatures may induce early ovule degeneration in sweet cherry, which results in low fruit set; therefore, the reduction of the endogenous GA level in flowers by applying GA inhibitors may improve fruit set in warm areas. We demonstrated that the application of paclobutrazol (PBZ), an inhibitor of GA biosynthesis, to bursting buds prolonged embryo sac longevity and hence increased fruit set (Beppu et al., 2001b) (Table 9). Ogata et al. (1991) and Takahashi et al. (1995) also confirmed the effectiveness of PBZ on the fruit set of sweet cherry; however, Looney and McKellar (1987), Webster (1990), and Webster et al. (1986) found no effect of PBZ on fruit set. These differences might be related to the timings and concentrations of the treatments, the cultivars, and

Table 8. Effects of temperature and soil moisture condition on midday leaf photosynthesis, leaf abscission, dry weight of a tree, and starch content of each part of the tree in 'Satohnishiki' sweet cherry (Beppu et al., 2003b).

Treatment[z]		Photosynthetic rate[y] ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Percentage of abscised leaves on Oct. 11	Tree dry weight (g)	Starch content of each part (% DW)				
Temp.	Soil moisture condition				Shoots	Trunk	Root stump	Root	
								$\varphi \geqq 2$ mm	$\varphi < 2$ mm
High	Dry	7.5	75.8	237.2	2.19	2.10	8.95	9.66	4.39
	Moist	15.1	56.1	263.5	2.55	2.67	10.95	11.98	6.95
Low	Dry	9.4	63.1	287.8	1.81	2.94	12.15	14.18	6.91
	Moist	16.9	41.9	403.0	3.09	2.87	12.52	12.69	5.97
Significance[x]		*	*	*	NS	NS	*	NS	*

[z] Trees were grown under moist (soil moisture tension < 6 kPa) and dry (SMT < 40 kPa) conditions in growth chambers controlled at 25°C/15°C (day/night) and 35°C/25°C from July 1 to September 15.
[y] Data were shown as an average during treatment.
[x] NS, *: Nonsignificant or significant at $P < 0.05$ by ANOVA (n = 3).

Table 9. Effect of paclobutrazol (PBZ) on ovule development and fruit set in 'Satohnishiki' sweet cherry (Beppu et al., 2001b).

Treatment[z]	Stage of development 4 days after anthesis			Final fruit set (%)
	Embryo sac mother cell to four-nucleate	Eight-nucleate	Degenerated	
Control	0.0[y]	9.8	90.2	1.9
PBZ	0.0	30.4	69.6	11.2
Significance[x]	NS	*	*	*

[z] A 500 ppm paclobutrazol solution was applied to spur buds by a hand sprayer on March 31, 1998.
[y] Percentage of ovules with embryo sac at different stages of development.
[x] NS, *: Nonsignificant or significant at $P < 0.05$ by ANOVA (n = 3).

the temperatures at flowering time. In our study, since the temperature from bud burst to full bloom was unusually high, which markedly reduced fruit set under field conditions, the effect of PBZ might have been more apparent. As for the effect of growth regulators, Thurzó et al. (2008) found that the application of NPPA (N-phenyl-phthalamic acid), which is a bioregulator with a synergistic effect with auxin, improved the fruit set of sweet cherry. Effects of other growth regulators on fruit set need to be examined.

It was reported that the endogenous GA level in above-ground parts decreased when the root temperature was reduced in satsuma mandarins (Poerwanto and Inoue, 1990) and grapes (Kubota et al., 1986). Using this phenomenon, the poor flower bearing of satsuma mandarin due to high temperatures in early forcing culture was improved by cooling only the root zone (Poerwanto et al., 1989). We applied this method to sweet cherry grown in the warm area. Root zone cooling prolonged ovule longevity and consequently increased fruit set (Beppu et al., 2008b).

2) Artificial shading in summer

As described above, excessively high temperatures in summer reduce the photosynthetic rate and cause early defoliation in warm regions, resulting in decreased carbohydrate reserve utilized for flower development in the following spring. As a practical method for reducing the stress from high temperatures and high solar radiation, artificial shading in summer was examined. Shading (53% light reduction) in summer slightly increased the daily leaf photosynthetic rate compared to the control (Beppu and Kataoka, 2005). In 'Bing' and 'Mazzard' sweet cherry, the light saturation for photosynthesis is as low as 400–700 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFDs (DeJong, 1983; Roper and Kennedy, 1986); thus, the level of light transmission in our experiment seemed to reach the light saturation point. The reduction of the respiration rate, probably due to decreased leaf temperatures under shading, might increase the apparent photosynthetic rate. Shading reduced the occurrence of leaf burn and delayed leaf abscission. As a result, reserve carbohydrate concentration of the tree under shaded conditions was slightly higher than that under unshaded conditions (Table 10). Similarly, Centritto et al. (2000) showed that mild shading increased the tree dry weight of sweet cherry grown under high temperature conditions. In our experiment, shading prolonged embryo sac longevity in the following spring, and hence increased fruit set. These findings suggest the possibility of applying artificial shading in summer to increase assimilate accumulation and fruit set in the following spring in sweet cherry grown in regions with hot summers (Beppu and Kataoka, 2005).

3) Foliar boron application

Temperature increase and soil drying after the rainy season in the warm region may reduce the absorption of boron, which is thought to be important for the development of reproductive organs, and resulted in the reduction of fruit set in the following spring. On the other hand, it has been reported that foliar spraying of boron in autumn improves fruit set in the following spring in other *Prunus* fruit tree species, such as prunes (Callan et al., 1978; Chaplin et al., 1977; Hanson and Breen, 1985), almond (Nyomora and Brown, 1997), and sour cherry (Hanson, 1991). In our experiment with sweet cherry, foliar spraying of boron in autumn increased boron concentration in the flowers in the following spring, meaning that absorbed boron from leaves moved to the flower buds. Boron treatment prolonged ovule longevity and accelerated the elongation of pollen tubes in pistils (Beppu et al., 2007a). As for the effect of boron on pollen activity, we reported that the addition of boron to culture media markedly increased both the pollen germination percentage and pollen tube length (Beppu and Kataoka, 1999b). As a result, the fruit set rate of boron-treated trees was increased (Beppu et al., 2007a). These results suggest the potential of the foliar spraying of boron in fall to increase fruit set in the following spring in sweet cherry grown in warm regions.

Future studies

From the above studies, we clarified the involvement of temperature, phytohormone, and tree nutrition in double pistil formation and poor fruit set, which are severe problems in sweet cherry cultivation in the warm region. In addition, we showed the possibility of their regulation by preventing temperature increases using shading and forcing cultures and by applying several chemicals. In the future, the timing and extent of these

Table 10. Effects of shading on leaf temperature, photosynthesis and abscission and tree reserve carbohydrate in 'Satohnishiki' sweet cherry (Beppu and Kataoka, 2005).

Treatment[z]	Leaf temperature[y] (°C)	Photosynthetic rate[y] ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Leaf abscission on Sep. 11 (%)	Tree carbohydrate level (% DW)	
				Starch	Sugar
Control	39.8 ± 0.4[x]	4.2 ± 0.5	33.2 ± 4.8	5.3 ± 0.4	2.8 ± 0.2
Shading	35.5 ± 0.1	5.3 ± 0.3	20.4 ± 4.5	6.8 ± 0.2	3.5 ± 0.2

[z] Trees were grown under a 53% level of shade from July 29 to September 11, 1996.
[y] Leaf temperature and photosynthetic rate were measured at noon on August 16.
[x] Mean ± SE (n = 3).

treatments need to be examined in detail, and cultivars and rootstocks adapted to warm conditions should be selected and bred to establish technologies for the stable production of sweet cherry in this region. Additionally, although most cultivars of sweet cherry are self-incompatible, to further ensure fruit set in warm climates, the trait of self-compatibility, which has been used in breeding programs worldwide recently (Choi and Andersen, 2001; Ishiguro et al., 2007; Yamane and Tao, 2009), is expected to be introduced to warm-adapted cultivars.

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