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Effects of wound treatments to flower organs on fruit set, development, and quality in sweet cherry (*Prunus avium* L.)

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Key words: anthocyanin concentration, artificial removal, cultivar difference, physical damage.

Abstract: Flower organs are important elements for pollination and fruit production. Although several studies on fruit trees have shown that damage to certain flower organs affects the resultant fruit, the influences of damage to flower organs on fruit production are largely unknown for most fruit trees. Sweet cherry is one of the most commercially important fruit trees, the tender flowers of which are occasionally damaged by factors such as insects, diseases, and weather disasters. In this study, the flower organs of two different Japanese sweet cherry cultivars, 'Satohnishiki' and 'Benishuho,' were wounded at flowering time by petal removal, stamen removal, sepal removal, or peduncle wounding, and the effects on fruit set, development, and quality at harvest were examined. All wound types slightly increased the fruit drop in 'Satohnishiki,' whereas fruit drop in 'Benishuho' was promoted by petal removal and peduncle wounding, and was suppressed by sepal removal. The fruits resulting from the wounded flowers developed in a similar manner to those of control flowers in both cultivars. Fruit weight, total soluble solid concentration, and titratable acidity of the flesh juice were not affected. On the other hand, the anthocyanin concentration in fruit skins was differentially affected depending on the cultivar, increasing in 'Satohnishiki' fruits but decreasing in 'Benishuho' fruits.

1. Introduction

Flower organs are important in plant pollination, with petals and stamen attracting pollinators such as insects and bees (Proctor and Yeo, 1973; Westwood, 1978). Since pollination is generally indispensable for successful fertilization and setting, flower organs are important in fruit production as well. However, flowers are occasionally damaged by insects, diseases, and weather disasters such as strong wind and hail.

Several studies indicate that successful pollination and fruit quality are reduced in damaged flowers. In persimmon, the calyx robes play an essential role in fruit development after setting, and damage or removal of the robes results in inferior fruit growth (Nakamura, 1967; Yonemori *et al.*, 1996). It was recently reported that removal of the calyx from apple blossoms at flowering time reduced fruit size and weight at harvest (Kitahara *et al.*, 2013). However, the roles of flower organs in determining fruit set, development, and quality in most fruit trees are still largely unknown.

Sweet cherry is one of the most commercially important fruit trees. This tree has tender flowers that bloom from April to May in Japan, when hail still occurs in certain regions. In addition, flower organs such as petals and stamen, occasionally suffer damage from feeding insects. It was recently reported that artificial stamen removal (emasculation) at the balloon stage of flowering in sweet cherry trees suppressed ovule development and reduced fruit set (Hedhly *et al.*, 2009). Damage to other flower organs may also influence fruit set, development, and quality of sweet cherries.

In this study, artificial wounds were inflicted to petals, stamen, sepals, and peduncles of flowers in two Japanese cultivars of sweet cherry in order to examine the roles of these flower organs on fruit production.

2. Materials and Methods

Plant materials

Adult trees of the sweet cherry (*Prunus avium* L.) cultivars, 'Satohnishiki' and 'Benishuho,' planted at the experimental orchard of the Shonai Laboratory for Agricul-

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tural Production in Sakata City of Yamagata Prefecture were used in this study. Seventeen lateral branches were randomly selected from three trees per cultivar. A total of 150 flowers (seven to ten flowers per branch) were used for each treatment as described below.

Wound treatments

Four wound treatments were designed: (1) petal removal (all petals were removed), (2) stamen removal (all stamens were removed), (3) sepal removal (all sepals were removed), and (4) peduncle wounding (the middle part of peduncle was pierced using a sewing needle). Flowers without wounds were used as controls. Treatments were conducted either on the day of flowering or the following day ('Satohnishiki' 29 April-4 May 2013; 'Benishuho' 3-7 May 2013).

All flowers used in this study were artificially pollinated with the mixed pollen of 'Rockport Bigarreau,' 'Napoleon,' and 'Jabouley' sweet cherries immediately after wounding. Pollen source flowers were collected from the experimental orchard of the Faculty of Agriculture, Yamagata University in Tsuruoka City of Yamagata Prefecture. Fruit thinning was performed on 4 June (37 days after full bloom) in 'Satohnishiki' and on 18 June (46 days after full bloom) in 'Benishuho', according to the customary practices in Yamagata Prefecture, with minor modification. The fruits from the wounded flowers were retained, while all other fruits from the same branch were thinned so that one branch bore two or three pieces of fruit per bouquet spur.

Fruit drop and fruit development

Fruit drops were counted twice per week from flowering to harvest. The fruit drop rate was calculated as the number of fruits remaining compared to the number of wounded flowers.

Fruit development was observed by measuring fruit diameter twice per week from 29 May when fruit set was first established.

Measurements of fruit quality

All fruits were harvested at the optimum time according to each cultivar. The diameter and weight of the fruits, total concentration of soluble solids, titratable acidity of the flesh juice, and anthocyanin concentration of the fruit skin were examined.

The total soluble solids concentration was determined using a hand refractometer (ATC-1, ATAGO Co. Ltd., Tokyo). Titratable acidity was determined by titrating the juice with 0.1 N NaOH. The results are expressed as equivalents of malic acid.

Fruit skin discs were removed using a cork borer. Anthocyanin was extracted from the discs by incubation with 1% HCl-MeOH for 24 h under refrigeration. The anthocyanin concentration was determined based on the absorbance at 530 nm (UV-150-01 spectrophotometer, Shimadzu Co. Ltd., Kyoto) and calculated as equivalents of cyanidin-3-glucoside.

3. Results and Discussion

Fruit drop

Figures 1 and 2 show the time-course of fruit drop and cumulative fruit drop rate in the 'Satohnishiki' and 'Benishuho' cultivars, respectively. Compared to control fruits, wounding promoted fruit drop in both cultivars, except for sepal removal in 'Benishuho' which slightly depressed the fruit drop. The wound-induced fruit drop rate was higher in 'Benishuho' than in 'Satohnishiki'. Although fruit drops were normally heavier in 'Satohnishiki' than in 'Benishuho' (Takahashi and Arasawa, 2001), the latter was more sensitive to wounding, and thus led to larger fruit drops.

Two peaks in fruit drop were observed (Figs. 1 and 2). The first large peak was caused by fertilization failure, whereas the second smaller peak was caused by competition for nutrients among the developing fruits (Westwood, 1978; Fukai, 1995). Peduncle wounding commonly promoted fruit drop in both cultivars, but the peak within which the drops occurred differed between the two cultivars. More fruits dropped during the second peak in

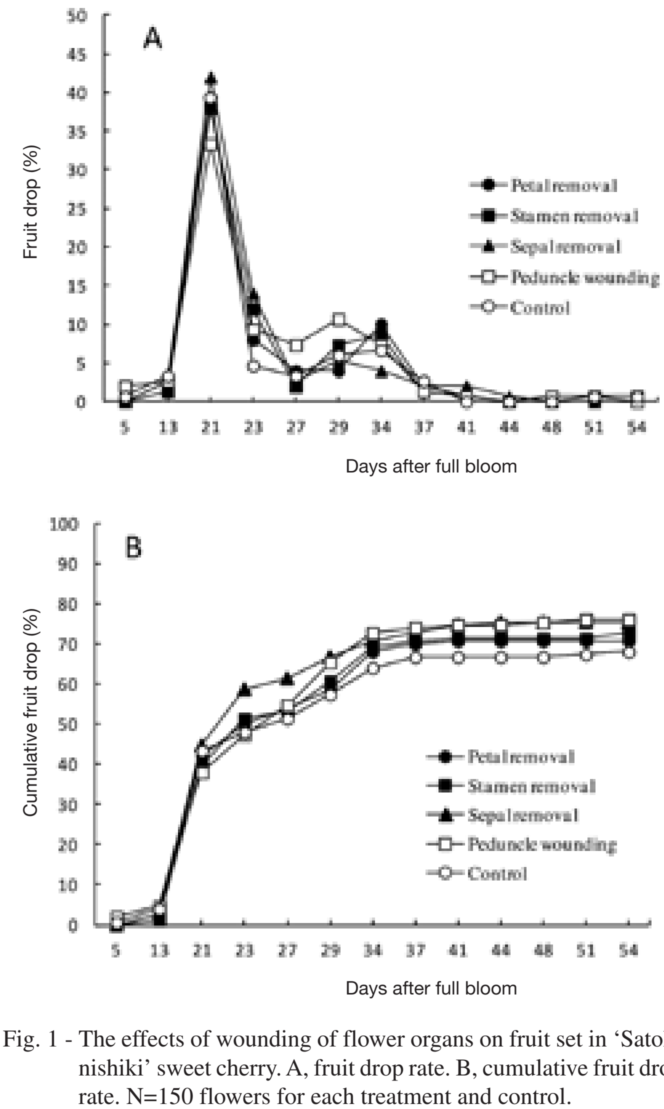

Fig. 1 - The effects of wounding of flower organs on fruit set in 'Satohnishiki' sweet cherry. A, fruit drop rate. B, cumulative fruit drop rate. N=150 flowers for each treatment and control.

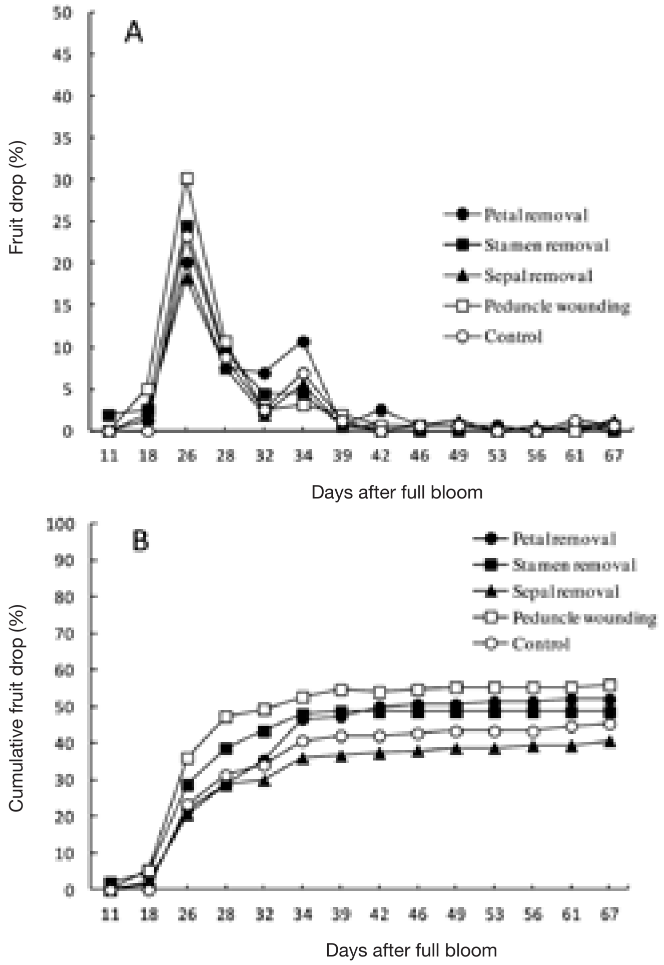

Fig. 2 - The effects of wounding of flower organs on fruit set in 'Benishuho' sweet cherry. A, fruit drop rate. B, cumulative fruit drop rate. N=150 flowers for each treatment and control.

'Satohnishiki,' while more fruits dropped during the first peak in 'Benishuho'. It is possible that peduncle wounding restricts the translocation of photosynthates from the leaves to the young fruits in 'Satohnishiki,' whereas it might also disturb fertilization in 'Benishuho'.

Wounding at flowering did not severely influence fruit set in this study. On the other hand, removal of the stamen at the balloon stage was reported to reduce fruit set in 'Vignola' and 'Sunburst' sweet cherry trees (Hedhly *et al.*, 2009). In addition, the removal of sepals at the pink bud stage in 'Cox's Orange Pippin' apples was reported to reduce fruit set to approximately 85% of normal (Vemmos and Goldwin, 1994). Taken together, these results suggest that wound stresses at immature stages of flower development are more detrimental to fruit set than wounds occurring at flowering. Further studies are necessary to determine whether wound stresses on flower organs at earlier stages of flower development influence fruit set in sweet cherry trees.

Fruit development

Figures 3 and 4 show the changes in diameter of 'Satohnishiki' and 'Benishuho' fruits, respectively. The growth patterns revealed double sigmoidal curves in both cultivars. Fruits from wounded flowers grew in a manner similar to those from non-wounded flowers in both cultivars. This result indicates that fruits from wounded flowers can continue to grow in a similar manner to normal ones.

Fruit quality

Fruit weight, total soluble solids concentration, and titratable acidity of the flesh juice at harvest were not af-

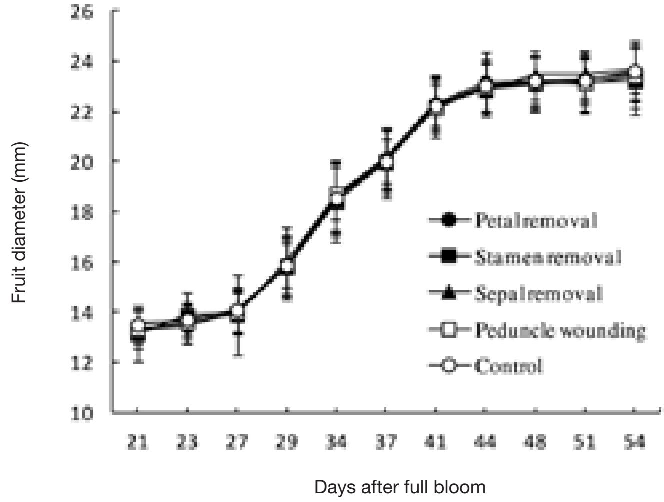

Fig. 3 - The effects of wounding of flower organs on fruit development in 'Satohnishiki' sweet cherry. Vertical bars represent ±SD (Petal removal, n = 48; Stamen removal, n = 43; Sepal removal, n = 41; Peduncle wounding, n = 43; Control, n = 54).

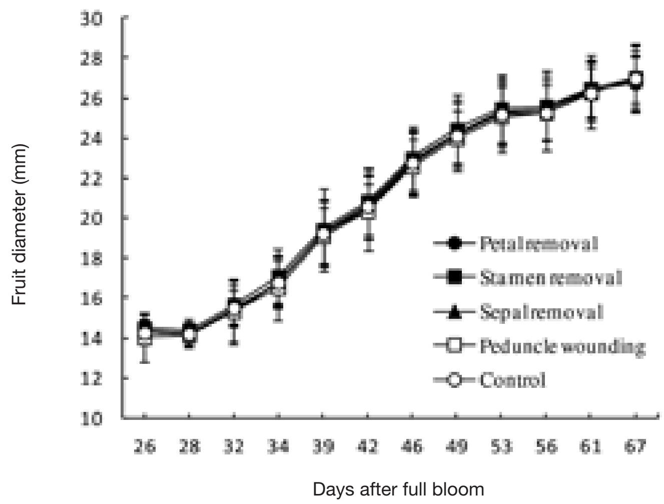

Fig. 4 - The effects of wounding of flower organs on fruit development in 'Benishuho' sweet cherry. Vertical bars represents ±SD (Petal removal, n = 72; Stamen removal, n = 83; Sepal removal, n = 93; Peduncle wounding, n = 72; Control, n = 82).

fected by wounding in 'Satohnishiki' (Table 1). None of these parameters were affected in 'Benishuho' either, except that stamen removal significantly increased the total soluble solids concentration (Table 2). On the other hand, anthocyanin concentration in the fruit skin was affected in both cultivars in different ways. In 'Satohnishiki', all wounds increased fruit skin anthocyanin concentration, with peduncle wounding causing a significant increase in anthocyanin concentration. Conversely, in 'Benishuho', all wound types decreased anthocyanin concentration, among which stamen and sepal removal significantly decreased anthocyanin concentration.

These results indicate that the fruit quality of these sweet cherry cultivars at harvest was minimally affected by flower wounding, with the exception of anthocyanin accumulation in the fruit skin. Interestingly, the effect on the anthocyanin concentration observed in 'Benishuho' appeared to be quite opposite to that in 'Satohnishiki'. The reason for this difference is unclear. Thus, such cultivar differences should be examined further, and the responses of other cultivars should be identified in the future.

Anthocyanin accumulation in fruit skin is controlled by both environmental and physiological factors (Westwood, 1978). For example, L-phenylalanine ammonia-lyase (PAL) is a key enzyme in anthocyanin biosynthesis in various kinds of fruits (Kataoka *et al.*, 1983; Farger and Chalmers, 1997). Increases in ethylene production induced by wounding of plant organs were reported to increase PAL activity and promote anthocyanin synthesis (Kataoka *et al.*, 1983; Arakawa, 1990). Further studies are required to clarify the effects of wounding on ethylene production and PAL activity, which is possibly related to anthocyanin accumulation in the fruit skin of sweet cherries.

4. Conclusions

The wounding of flower organs at the time of flowering slightly suppressed fruit set, but did not strongly affect fruit development or fruit quality at harvest in two Japanese cultivars of sweet cherry. On the other hand, anthocyanin concentrations increased in the skins of wounded 'Satohnishiki' fruits, but declined in wounded 'Benishuho' fruits. In the future, the effects of wound stresses at earlier stages of flower bud development and flowering need to be examined and it would be interesting to explore cultivar differences in response to such stresses.

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Table 1 - The effects of the wounding of flower organs on fruit quality at harvest in 'Satohnishiki' sweet cherry

Treatment	Number of fruits	Fruit weight (g)	Total soluble solids (° Brix)	Titratable acidity[z] (g/100 ml juice)	Anthocanin in fruit skin [y] (μg/cm^2)
Petal removal	48	6.97 a	20.2 a	0.75 a	24.6 ab
Stamen removal	43	6.80 a	20.4 a	0.83 b	24.4 ab
Sepal removal	41	7.04 a	20.1 a	0.77 ab	25.5 ab
Peduncle wounding	43	6.88 a	20.5 a	0.83 b	29.9 a
Control	54	6.88 a	19.9 a	0.81 ab	21.2 b

[z] as malic acid.
[y] as cyanidin-3-glucoside.
Letters following the means indicate statistical significance by Tukey's test, $p < 0.05$.

Table 2 - The effects of the wounding of flower organs on fruit quality at harvest in 'Benishuho' sweet cherry

Treatment	Number of fruits	Fruit weight (g)	Total soluble solids (° Brix)	Titratable acidity[z] (g/100 ml juice)	Anthocianin in fruit skin [y] (μg/cm^2)
Petal removal	72	9.81 a	17.2 ab	0.59 a	22.8 ab
Stamen removal	83	9.87 a	17.6 a	0.69 a	19.6 bc
Sepal removal	93	9.83 a	17.3 ab	0.62 a	17.7 c
Peduncle wounding	72	9.62 a	17.2 ab	0.62 a	22.0 abc
Control	82	9.62 a	16.9 b	0.57 a	26.4 a

[z] as malic acid.
[y] as cyanidin-3-glucoside.
Letters following the means indicate statistical significance by Tukey's test, $p < 0.05$.

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