Effect of Flowering Time on Occurrence of Reddish Pulp in the Peach

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Reddish pulp is one of the most serious disorders in peach production, in which originally white flesh undergoes pink to red discoloration with the development of a mealy texture. In order to clarify the relationship between the incidence of reddish pulp and flowering date, the occurrence of reddish pulp was investigated in ‘Benishimizu’ and ‘Shimizu Hakuto’ fruit with different flowering dates for three seasons. In both cultivars, the percentage of fruit with reddish pulp was higher in early flowering fruit than late flowering fruit in all three seasons. Analysis of pectin and boron contents in immature fruit sampled from June 23 to July 8, prior to observing the symptoms of reddish pulp, showed no statistically significant differences in pectin content between early and late flowering fruit. On the other hand, the boron content in flesh was significantly lower in early flowering fruit that bloomed on April 1 than in late flowering fruit that bloomed on April 11 in ‘Benishimizu’. In addition, a significantly higher total polyphenol content, which is one of the characteristics of reddish pulp, was found in early flowering fruit. The results indicate that the flowering date has a large impact on the incidence of reddish pulp, and the disorder is more prevalent in early than late flowering fruit. The possible relationships among the development of reddish pulp, boron deficiency, pectin content, and increase in total polyphenol content are discussed.

Key Words: boron deficiency, mealy texture, pectin content, polyphenol.

Introduction

Reddish pulp is one of the most serious disorders in peach production. It is different from red pulp staining in red-fleshed peaches (Rumainum et al., 2015) in that the flesh of an originally white-flesh cultivar undergoes pink to red discoloration. Moreover, reddish pulp is accompanied by the loss of flesh juiciness and the development of a mealy texture, which reduces fruit quality markedly (Ohnishi, 1999; Takata et al., 2005). Fruit with reddish pulp and rated 2 (half of the cross section is reddish) or higher on the indexes described by Takata et al. (2005) have low economic value (Ohnishi, 1999). The occurrence of reddish pulp is mainly observed in warm production areas in western Japan, but is spreading to production areas in the north perhaps as a result of global warming. As reddish pulp cannot be distinguished on the basis of outside appearance and the symptoms progress after harvest (Takata et al., 2006), it is difficult to prevent the contamination of fruit with reddish pulp, which decreases consumer confidence (Ohnishi, 1999). Therefore, unraveling the mechanism(s) underlying the disorder and developing techniques to reduce its incidence are required.

Compositional analysis has revealed that fruit with reddish pulp have high total polyphenol contents in their flesh prior to discoloration (Takata et al., 2005). The flesh of reddish pulp has a mealy texture and lacks juiciness. A mealy flesh is associated with changes in pectin composition, such as a decrease in total pectin content, in comparison with normal fruit (Brumell et al., 2004; Dawson et al., 1992). Boron is an important constituent of cell wall pectins, and boron deficiency has been reported to affect pectin content and composition (Brown et al., 2002; Ganie et al., 2013; Wang et al., 2015). Boron deficiency is also reported to...
cause an increase in polyphenol content (Brown et al., 2002; Ganie et al., 2013; Wang et al., 2015), a characteristic phenomenon observed in peaches with reddish pulp. A reduction in the number of roots has been observed in trees that bear a large number of fruit with reddish pulp (Takata, 2006), suggesting that limited nutrient supply to the fruit may be related to the occurrence of the disorder. It is therefore interesting to investigate the involvement of boron efficiency and pectin content in the development of reddish pulp.

Although it has been reported in trees bearing a large number of fruit with reddish pulp that fruit canopy position has little relation to the development of the disorder (Takata et al., 2006), it is generally known that in trees bearing a moderate number of fruit with reddish pulp, fruit at the lower canopy position more occasionally tend to have reddish pulp than fruit at the upper canopy position. As fruit at the lower canopy position are usually derived from flowers that bloom early on the tree, we assumed that not canopy position, but rather flowering date, would have a large impact on the occurrence of reddish pulp. This assumption was supported by the fact that the number of fruit with reddish pulp increased at the latter half of harvest time when fruit generated at the early flowering time were harvested. It was also found that the soluble solid contents (SSC) of early flowering fruit were significantly lower than those of late flowering fruit, suggesting that the former have smaller nutrient sink strength than the latter (Fukuda et al., 2012).

In this study, we conducted a three-year investigation to clarify whether or not flowering date has a critical effect on the occurrence of reddish pulp. Further, we attempted to investigate the influence of flowering time on total polyphenol content, pectin content, and boron content in immature fruit when symptoms of reddish pulp did not appear.

Materials and Methods

Plant materials and sampling depending on flowering date

Three trees of the mid-ripening ‘Benishimizu’ and four to six trees of the mid-late ripening ‘Shimizu Hakuto’ grown at the orchard of the Field Science Center affiliated with the Faculty of Agriculture, Okayama University were used in this study. For three years from 2012 through 2014, the same trees were used to investigate the effect of flowering date on the incidence of reddish pulp. Throughout the flowering season, flowers in bloom were labeled at 3- to 5-day intervals and a total of 30 to 50 flowers were labeled per tree. Through this labeling process, irrespective of canopy position, early- and late-flowering fruit could be identified. Fruit thinning was carried out so that the labeled fruit were retained on the tree until harvest. At harvest, fruit were assessed for the development of reddish pulp, as described below. In 2014, 10 of 50 labeled fruit for each flowering date in ‘Benishimizu’ were collected on June 23 (end of stage 2 of fruit growth), June 30 (beginning of stage 3 of fruit growth), and July 8 (approximately 10 days before harvest), and flesh were sampled and kept at −20°C for analysis of polyphenol, pectin, and boron contents in fruit during development.

Sampling based on development of reddish pulp at harvest

Fruit of ‘Benishimizu’ were harvested at commercial maturity and the development of reddish pulp was assessed, as described below. At the same time, fruit quality, such as fruit weight, fruit width, firmness, and SSC, were measured. Firmness and SSC were measured with a fruit hardness tester (KM-1; Fujiwara Scientific, Tokyo, Japan) and a refractometer (PR-1; Atago, Tokyo, Japan), respectively. The data were collected at each level of development of reddish pulp. Flesh tissues were also sampled at each level of development of reddish pulp and stored at −20°C for analysis of polyphenol, pectin, and boron contents.

Assessment of reddish pulp

For the assessment of reddish pulp, labeled fruit in each cultivar were harvested at commercial maturity and allowed to ripen at ambient temperature for 3 days. The cheek section of each fruit was cut off and the development of reddish pulp was assessed in accordance with the indexes described by Takata et al. (2005): 0, no symptom; 1, part of the cross section is reddish; 2, half of the cross section is reddish; and 3, the whole cross section is reddish.

Measurement of polyphenol, pectin, and boron contents

Polyphenol content was measured as described by Rumainium et al. (2015) with slight modifications. Six grams of frozen flesh tissue was extracted with 80% MeOH and total polyphenol content in the extract was measured by the Folin-Denis method (Singleton and Rossi, 1965) and expressed as the catechin equivalent.

Pectin content was measured after separation into a water-soluble (WS) fraction, a sodium hexametaphosphate acid (PS) fraction, and an HCl-soluble (HS) fraction, as described in Konno et al. (1984). Fifteen grams of flesh tissue was ground with 80% ethanol. Alcohol-insoluble substances (AIS) were collected by performing three or four alcohol extractions. The three pectin fractions were continuously extracted. Firstly, distilled water was added to 100 mg of dry AIS and the suspension was incubated overnight at 25°C. Thereafter, the WS fraction was separated by filtration. Secondly, 0.4% PS was added to the residue and the suspension was incubated overnight at 25°C. Then, the PS fraction was separated by filtration. Finally, 0.5 N HCl was added to the residue and the suspension was incubated on a heating block at 85°C for two hours. Pectin content in each fraction was determined using the carbazole sulfuric
acid method (McCready and McComb, 1952) and expressed as galacturonic acid equivalent.

Boron content in flesh tissue was quantified as follows. Frozen flesh tissue was dried at 80°C and ground into fine powder. This was followed by extraction with 0.05 N HCl. Boron content in the extract was determined by the curcumin method (Dible et al., 1954).

Statistical analysis

All data were subjected to analysis of variance, and significant differences of the means were evaluated using Tukey’s multiple comparison test or Student’s t-test.

Results and Discussion

Effects of flowering date on incidence of reddish pulp

The incidence and severity of reddish pulp were closely related to the flowering date in both ‘Benishimizu’ and ‘Shimizu Hakuto’ in all the three seasons investigated (Fig. 1). In ‘Benishimizu’, the seasonal variation of the percentage of fruit with reddish pulp was small. Around 60% of early flowering fruit developed reddish pulp. On the other hand, less than 40% of late flowering fruit developed reddish pulp in 2012 and 2013, and this value was 20% lower than that of early flowering fruit. In addition, rates of R2 or R3 fruit were slightly higher than in late flowering fruit. In 2014, no marked reduction in the percentage of fruit with reddish pulp was observed in late flowering fruit because of the presence of a large number of R1 fruit with reddish pulp in late flowering fruit. R2 or R3 fruit accounted for 24% of fruit that bloomed on April 1, and 6% of fruit that bloomed on April 11. The severity of reddish pulp, which was assessed on the basis of the area of discolored tissues, was significantly reduced in late flowering fruit in all the three seasons. Even in 2014, when the incidence of reddish pulp was not significantly dependent on flowering date, the severity score was more than 0.9 in early flowering fruit and less than 0.55 in late flowering fruit (Fig. 1). In ‘Shimizu Hakuto’, the seasonal variation in the development of reddish pulp was quite large. Irrespective of seasonal variation, however, the percentage of fruit with reddish pulp in late flowering fruit was much lower than that in early flowering fruit; it was 50% lower than that in early flowering fruit. The severity of reddish pulp was also reduced significantly in late flowering fruit in every season.

It is generally known that flowers at the lower part of the tree canopy tend to bloom earlier than those at the upper part. In this study, we labeled fruit without bias of labeling day at symmetric canopy positions so that the influence of canopy position could be suppressed. Harvest dates of labeled fruit were similar irrespective of flowering date. Furthermore, the effect of canopy position on the development of reddish pulp was not recognized in this study (data not shown). It was assumed that flowering date affected the development of reddish pulp independent of canopy position.

In this study, a seasonal variation in the incidence of
reddish pulp was observed, particularly in ‘Shimizu Hakuto’. It was assumed that this variation may be a result of differences in temperature during fruit development. In 2013, when severe reddish pulp was noted in ‘Shimizu Hakuto’, the maximum and average temperatures were high compared with those in 2012 and 2014 in the middle of June and the first half of July (data not shown). It has been reported that exposing fruit to high average temperature (more than 25°C) around 2 or 3 weeks before harvest increased the occurrence of reddish pulp (Morinaga et al., 2015). It seems that ‘Shimizu Hakuto’ is susceptible to high temperature, developing a reddish pulp. It is noted that even in ‘Shimizu Hakuto’ exposed to high temperature in 2013, late flowering fruit had much lower occurrence of reddish pulp than early flowering fruit, indicating that this concept is applicable regardless of seasonal variation.

**Polyphenol, pectin, and boron contents in fruit with and without reddish pulp**

To further understand the mechanism(s) underlying the development of reddish pulp and the mealy texture, we first compared fruit quality, polyphenol content, pectin content, and boron content in fruit that showed different indexes of reddish pulp. Except for firmness, there was no significant difference in fruit quality, such as fruit weight, fruit width, and SSC, between normal fruit and fruit with reddish pulp (Table 1). Flesh firmness was low in R2 and R3 fruit with severe reddish pulp. Total polyphenol content increased as the severity of reddish pulp increased (Fig. 2A). Pectin content decreased with the development of reddish pulp, particularly in the HS fraction (Fig. 2B). Boron content also decreased with the development of reddish pulp, and a significantly low boron content was measured in fruit with the severest reddish pulp (Fig. 2C).

The low firmness and the increased polyphenol content in fruit with reddish pulp were consistent with previous reports (Takata et al., 2005) and seemed to be characteristic of reddish pulp. The accumulation of anthocyanin in red-fleshed cultivars has been reported (Rumainum et al., 2015). As anthocyanin is a type of polyphenol, the high polyphenol content in reddish pulp is reasonable. As expected from the low flesh firmness, pectin contents in HS fractions and boron contents decreased with the development of reddish pulp. Pectin solubilization in HS fractions coupled with fruit softening has been reported (Taira et al., 1997). Most boron ions in the cell are localized by forming complexes with pectin (Hu and Brown, 1994), and boron deficiency is reported to result in anatomical and physical changes. At this point in time, we cannot clarify the mechanism(s) of the decrease in pectin and/or boron contents and the mealy texture in reddish pulp; however, we recognize the need to take boron deficiency into consideration when we further investigate the mechanism(s) of the development of reddish pulp. Brummell et al. (2004) demonstrated that in mealy fruit exposed to chilling injury, the solubility of high molecular weight pectins remained low. In this study, a decrease in pectin

![Fig. 2](image-url)
content in the fraction containing high molecular weight pectins (HS fraction) was observed. Although the fractionation steps used in this study are different from those in Brummell et al. (2004), it is assumed that the mealy tissue in reddish pulp has different characteristics from that in chilling injury fruit.

**Effects of flowering date on polyphenol, pectin, and boron contents in immature fruit prior to development of reddish pulp**

We found that reddish pulp occurs more frequently and severely in early flowering fruit than late flowering fruit. To investigate the relationship between flowering date and cellular components related to the development of reddish pulp, we analyzed the polyphenol content, pectin content, and boron content in ‘Benishimizu’ fruit with different flowering dates: early flowering on April 1 and late flowering on April 11. The analysis was performed in immature fruit prior to the development of reddish pulp so that we could study the effects of flowering date on these components before the development of reddish pulp.

Flesh polyphenol contents measured on June 23 and June 30 were significantly higher in early flowering fruit than late flowering fruit (Fig. 3). Polyphenol content in flesh is known to decline rapidly at stage 3 of fruit growth as fruit expands. In early flowering fruit, this decline in polyphenol content seems to slow down. As described above, a high polyphenol content is one of the characteristics of reddish pulp; Takata et al. (2005) reported a high polyphenol content in immature fruit from trees with a high incidence of reddish pulp. The high polyphenol content in early flowering fruit observed in this study supports the idea that reddish pulp is dependent on flowering date.

No significant difference in pectin content was observed in immature fruit sampled on June 23, June 30, and July 8 (Fig. 4 upper). Boron content in flesh showed a significant difference between early flowering fruit and late flowering fruit, and early flowering fruit that displayed severe reddish pulp contained less boron than late flowering fruit on every sampling date (Fig. 4 lower). Total phenolic content against boron contents in individual fruits are plotted in Figure 5. In late flowering fruit (April 11), no clear relationships could be detected ($r = -0.37$). On the other hand, in early flowering fruit (April 1), inverse relationships could indeed be detected; however, this was with a relatively low correlation coefficient ($r = -0.43$). In all of the data regardless of flowering time, a quadratic curve was significantly fitted ($r = -0.71^{**}$). It is assumed that boron contents over certain threshold (about 10 μg·g⁻¹FW) values...
would not affect total phenol contents. As boron content in early flowering fruit was approximately 80% of that in late flowering fruit, boron deficiency in early flowering fruit may not be so severe; however, considering that fruit with and without reddish pulp were mixed in both early and late flowering samples in this experiment, the significant difference detected in this study may indicate an important relationship with flowering date dependence and the incidence of reddish pulp. Low boron contents in early flowering fruit were detected in immature fruit at the end of stage 2, suggesting that the sink strength for this nutrient has been reduced in early flowering fruit one month prior to the development of reddish pulp. As described above, boron plays an important role in the integrity of cell wall structure (Hu and Brown, 1994). Boron content is also related to polyphenol production, and boron deficiency causes an increase in polyphenol content (Brown et al., 2002; Ganie et al., 2013; Wang et al., 2015). Boron deficiency could be related to polyphenol accumulation and the mealy texture of fruit with reddish pulp. The prerequisite for significant boron mobility is reported to be the presence of sugar alcohols, such as sorbitol, in Prunus species (Brown and Hu, 1996). Fukuda et al. (2012) showed that early flowering fruit enlarged more slowly and sugar content tended to be lower than late flowering fruit. It was proposed that flowering time within a tree could affect sink strength for various nutrients, including boron, which is necessary for healthy fruit development.

The results of this study may be used to establish techniques for reducing the incidence of reddish pulp in peach production. After natural fruit set was completed in the beginning of May, fruit thinning was carried out to reduce the fruit number to two or three times of the expected fruit number at harvest (Ariyoshi, 2016; Costa and Vizzotto, 2000). Late flowering fruit existed at the basal part of bearing shoots, or short bearing shoots were attached to the basal part of the bearing mother shoot within a lateral shoot. By paying attention to the flowering date, retaining smaller fruit at the position described above may alleviate the risk of reddish pulp.

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Literature Cited


