Investigation and Prevention of Cork Spot Disorder in ‘Akizuki’ Pear (*Pyrus pyrifolia* Nakai)

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Abstract. ‘Akizuki’ (*Pyrus pyrifolia* Nakai) is a dominant Asian pear cultivar with gradually increasing cultivation area in Shandong province. However, this cultivar is found susceptible to cork spot disorder in recent years. In this study, we explored the physiological-biochemical mechanism of cork spot disorder in pear fruit, and investigated the effectiveness of spraying calcium (Ca), boron (B) solution or prohexadione calcium (P-Ca) on cork spot incidence. Cork spotted fruit had the characteristics of significantly larger fruit size with shorter fruit pedicels. Compared with normal fruit, cork spotted fruit had lower content of total soluble solids, soluble and reducing sugar, and vitamin C. In addition, cork spotted fruit accumulated much higher levels of N and Mg, and lower levels of K and P. However, Ca deficiency was not observed in cork spotted fruit, on the contrary, we determined high concentrations of Ca and free Ca²⁺ in disordered fruit. At the same time, the ratios of K/Ca, Mg/Ca, and (K+Mg)/Ca were significantly lower in cork spotted fruit as compared with normal fruit. Among all treatments, spraying with 3500 times dilution of P-Ca at 15-day intervals from 30 to 90 days after full bloom showed promise for reducing cork spot incidence in ‘Akizuki’ pear without affecting fruit quality attributes. This research herein reveals the physiological-biochemical characteristic of cork spot disorder, and implicates P-Ca as a potential tool to reduce cork spot incidence in Asian pear cultivar Akizuki.

‘Akizuki’ (*Pyrus pyrifolia* Nakai), a Japanese pear, plays an important role in pear production in China because of its good quality characteristics such as large fruit, pretty shape, delicate pulp, and high soluble solid content. However, with the cultivation area increasing, a physiological disorder that appears to be cork spot in this cultivar has occurred in several pear orchards in Shandong province of China, and become a serious problem over the years.

Cork spot, bitter pit, and corky core, the most typical physiological disorders that develop in apple fruit skin, are commonly called cork spot-like physiological disorder (CSPD) (Faust and Shear, 1968; Ferguson and Watkins, 2011). In pear fruits, cork spot is also the most common physiological disorder except for hard end, corky core, and water core (Inomata et al., 1993; Lu et al., 2014). Except for ‘Akizuki’, this disorder is also frequently found in other pear varieties such as ‘Anjou’, ‘Alexander Lucas’, and ‘Oushuu’ pear (Hayama et al., 2017; Raese and Drake, 1993; Richardson and Lombard, 1979; Tomala and Trzak, 1994). Cork spot-

like disorder involves the suberization of cells in pear flesh (Tamura, 2017). A recent report indicated that cork spot symptoms were observed more markedly in the late maturing fruit in ‘Akizuki’ pear (Hayama et al., 2017). In addition, the severity of the cork spot disorder showed an association with the fruit fresh weights: more cork spots appeared on larger fruit (Hayama et al., 2017). Moreover, more cork spots were observed in the middle of the fruit, with few at the stem end (Hayama et al., 2017).

Many physiological disorders are closely related to Ca deficiency in fruit, and control of these physiological disorders requires maintenance of adequate levels of Ca (Dong et al., 2015; Lee et al., 2007; Miqueloto et al., 2014; Raese, 1988; Raese and Drake 1995). Cork spot presented symptoms similar to those of bitter pit, was induced by Ca deficiency, and, thus, Ca spraying was a useful alleviating measure (Faust and Shear, 1969; Raese and Drake, 1993, 1995, 2006; Richardson and Lombard, 1979). Besides Ca, the levels of other mineral nutrients could also influence fruit susceptibility to calcium deficiency disorders (Lee et al., 2007). For example, K and Mg can antagonize the uptake or the function of Ca at the membrane or compete with Ca at active site on membranes (Schönherr and Bukovac, 1973; Yermiyahu et al., 1994). High levels of N in the fruit have been reported to aggravate the development of Ca deficiency symptoms in apple (Bangerth, 1974; Shear, 1971). However, there are also some conflicts, for example, Mason and Welsh (1970) showed that fruits

with bitter pit or cork spot had higher Mg concentration than normal fruits, whereas Woodbridge (1971) found no significant differences between them. Therefore, the nutrient concentration ratios such as K/Ca, Mg/Ca, and (K+Mg)/Mg are more meaningful to predict Ca deficiency disorders than total fruit Ca concentration alone (Dris et al., 1998; Freitas et al., 2010; Lanauskas and Kviklienė, 2006; Schumacher and Fankhauser, 1970). Although the association between cork spot and mineral concentrations in European pear has been frequently reported, little has been done on Asian pear cultivars.

Prohexadione calcium (P-Ca) inhibits the biosynthesis of gibberellin (Evans et al., 1999; Rademacher et al., 2004). Compared with other three triazole growth inhibitors, P-Ca has low toxicity and limited persistence in the environment, being metabolized or decomposed 6 to 7 weeks after application (Evans et al., 1999; Owens and Stover, 1999; Rademacher et al., 2004). Previous studies have demonstrated significant control of shoot growth by P-Ca on a range of pear varieties (Asín et al., 2007; Costa et al., 2001; Einhorn et al., 2014; Elfving et al., 2003; Hawerth et al., 2012), and P-Ca has little effect on fruit quality attributes in pear (Costa et al., 2004; Einhorn et al., 2014; Elfving et al., 2003). Moreover, P-Ca was reported to induce resistance to apple scab and fire blight (Costa et al., 2001; Römmelt et al., 1999). However, whether P-Ca has potential to reduce cork spot incidence in ‘Akizuki’ pear has not been studied.

The goals of our study were to elucidate the difference of fruit characteristics and content of mineral elements between cork spotted and normal fruits in ‘Akizuki’ pear, and to determine an effective spray material for reducing the incidence of cork spot without affecting fruit quality attributes.

Materials and Methods

Plant materials. Six-year-old ‘Akizuki’ pear trees grown on *Pyrus betulaefolia* Bunge pear rootstocks were selected for the experiment. The orchard, trained to a trellis cultivation system, with spacing of 4 × 3 m, was located in Qingdao City, Shandong Province, People’s Republic of China. Cork spotted and normal fruits in this same orchard were collected at maturity, 140 d after full bloom (DAFB) in 2014–16. Whole fruit flesh tissues were divided into three parts (the calyx end, the middle part, and the stem end) with the core and seeds excluded, and then were sliced and immediately frozen in liquid nitrogen and maintained at –70 °C for further study.

Performance of exogenous Ca, B, and P-Ca treatments. Ca, B, P-Ca (Kumiai Chemical Industry Co., Ltd, Tokyo, Japan), or unsprayed control were used in this experiment. Each treatment was randomly allocated to three trees selected by uniformity and size (canopy volume), making five replications for each. All treatments were conducted every 15 d, with a total of five replications from

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beginning at 30 DAFB. When spraying, H_3BO_3 and $Ca(NO_3)_2$ were diluted 600 times with water, and P-Ca was diluted 1500, 2500, and 3500 times with water, respectively. Fruit samples from the treated and control trees were harvested at commercial maturity (140 DAFB) and subsequently transported to the laboratory within a period of 2 h, and then used for fruit quality evaluation. Effects on cork spot incidence were assessed on fruits, and data were expressed as percentage of the number of disordered fruit divided by the total number of fruit.

Fruit quality analysis. Fruit firmness was determined by removing fruit skin on four sides of each fruit, using a texture analyzer (CT3; Brookfield, Middleboro, MA) with a 2-mm-diameter probe, 10-mm penetration depth, and $0.5\text{-mm}\cdot\text{s}^{-1}$ penetration rate, expressed in $\text{kg}\cdot\text{cm}^{-2}$.

Average fruit weight was determined by sampling and individually weighing 200 fruits randomly chosen from all trees per treatment. The vertical and horizontal diameter of fruit, the length and diameter of fruit pedicel, and the calyx concave depth were measured by vernier caliper.

To measure the total soluble solids, the sample of the stem end, the middle part, and the calyx end of fruit was juiced, respectively, placed on the prism of a digital handheld Abbe-type refractometer and expressed as °Brix. Titratable acidity of different fruit parts was measured using titration with 0.1 N sodium hydroxide (NaOH) and calculated as a malic acid equivalent. The content of vitamin C, soluble sugar and starch, and reducing sugars was determined by colorimetry by molybdenum blue method, anthrone reagent method, and a 3, 5-dinitrosalicylic acid colorimetric method, respectively.

Measurement of the mineral nutrient content. Fruit flesh tissues were dried in an oven at 105 °C for 30 min, and then at 75 °C until a constant weight was achieved; 0.5 g of flesh tissues was mixed with 2 mL perchloric and 10 mL nitric acids. After digestion and dissolution, the total contents of Ca, Mg, K, Fe, Zn, and P were analyzed by using an ICP-OES optima 8000 (PerkinElmer Inc., Waltham, MA).

For the determination of total N content, 1 g of dried flesh samples was mixed with 0.3 g CuSO_4 , 3 g anhydrous Na_2SO_4 , and 10 to 12 mL concentrated H_2SO_4 . After digestion and dissolution, the N content was assayed using a Kjeldahl nitrogen apparatus (K9860; Hanon instruments Co., Ltd., Jinan, China).

Observation of Ca^{2+} localization. The localization of free Ca^{2+} was observed by fluorescence imaging as previously described by Qu et al. (2012), with some modifications. Thin slices of flesh were collected from the same region under the peel tissue of normal and cork spot fruit by using a razor blade. The flesh tissues were initially washed twice with HEPES buffer solution, which were loaded with fluo-4/AM supplemented with 25% Pluronic F-127 and then subsequently washed three times with HEPES buffer solution. After maintaining the tissue in the dark

Fig. 1. Symptoms of 'Akizuki' pear fruit with cork spot. (A) Fruit with light cork spot. (B) Longitudinal section of fruit with light cork spot. (C) Fruit with severe cork spot. (D) Longitudinal section of fruit with severe cork spot. (E, F) Endogenous type or subcutaneous of suberized tissue in pear fruit flesh, respectively.

at 37 °C for 1 h, we visualized fluo-4 fluorescence (488-nm excitation laser light and 516-nm long-pass emission filter) using a laser scanning confocal microscope (TCS SP5 II; Leica, Wetzlar, Germany). The fluorescence results were analyzed using Image-Pro Plus software (Media Cybernetics, Rockville, MD).

Statistical analysis. The statistical analysis was performed using SPSS 17.0 software (IBM-SPSS, Armonk, NY). Data expressed as percentage were transformed by arcsine [square root ($n + 1$)] analysis. Sample means

were compared using analysis of variance. Mean separation was determined by Duncan's multiple range test, and significance was tested at 5% or 1%. Figures were composed using Microsoft Excel software (Redmond, WA).

Results and Discussion

Symptoms of cork spotted pear fruit. The symptoms of cork spot were observed more markedly in the late maturing fruit in 'Akizuki' pear, which is consistent with

previous reports (Hayama et al., 2017; Mason and Welsh, 1970). At harvest stage, one or more small dark green or brown round dimpled spots of 0.3 to 1.0 cm in diameter were observed on the skin of disordered fruits, and below them was the affected brown spot (Fig. 1A and B). Much larger bumpy surface with necrotic flesh tissue under the affected area were observed with severe cork spotted fruits (Fig. 1C and D). Consistent with the report of Hayama et al. (2017), more cork spots were observed in the middle of the fruit (Fig. 1B and D). The brown spot could be anywhere between the skin and the core, but in most cases, it was close to the surface of the fruit just beneath the skin, which was similar to the report of Faust and Shear (1968). A depression developed above the internal spots as the fruit enlarged, due to the reduced growth in the affected tissues. With longitudinal cutting of the fruit, a localized desiccated tissue resembling cork was observed in the flesh of the fruit, which was brown and necrotic with dry and water-deficiency in vascular bundle tissue (Fig. 1E and F).

Comparison of fruit quality attributes between normal and cork spotted fruit. The average fruit weight of disordered fruit was significantly higher than that of normal fruit (Table 1), which is similar to the report of Hayama et al. (2017). The diameter of fruit pedicels and the calyx concave depth in disordered fruit were also significantly greater than that of normal ones (Table 1); however, total soluble solids in each part of disordered fruit was significantly lower than that in the same part of normal ones (Table 2). In addition, there was no significant difference in titratable acidity and fruit firmness with the exception of that in the calyx end (Table 2).

We also determined the contents of vitamin C and carbohydrates, including soluble sugar, reducing sugar, and starch of different fruits. From the calyx end to the stem end, the content of soluble and reducing sugar, and vitamin C in normal fruit were significantly higher than those of disordered fruit (Fig. 2). Inconsistent with the report of Li et al. (1999), the starch content in our research was much higher in cork spotted fruits than normal fruit, but the difference was not significant. All the results presented previously indicated that cork spotted pear fruit had larger fruit size and decreased the contents of total soluble solids, soluble and reducing sugar, and vitamin C, which resulted in fruit quality deterioration.

Analysis of mineral nutrient content in normal and cork spotted pear fruit. Cork spot has been linked to fruit nutritional imbalance (Facteau et al., 2010; Richardson and Al-Ani, 1982; Tomala and Trzak, 1994). Mineral nutrient contents in the stem end, middle part, and calyx end of normal and disordered pear fruit were determined in this research. At the same time, the ratios of K/Ca, Mg/Ca, and (K+Mg)/Ca were also evaluated. Compared with normal fruit, 'Akizuki' pear with cork spot showed much higher N, significantly

Table 1. Fruit characteristic of cork spotted and normal 'Akizuki' fruits.

Treatments	Avg fruit wt (g)	Fruit shape index	Length of fruit pedicels (cm)	Diam of fruit pedicels (mm)	The calyx concave depth (cm)
Normal fruit	453.76 ± 27.66 b	0.88 ± 0.03 b	2.23 ± 0.47 a	3.40 ± 0.27 b	0.90 ± 0.18 b
Cork spotted fruit	517.49 ± 36.77 a	0.89 ± 0.03 a	1.42 ± 0.23 b	3.92 ± 0.28 a	0.99 ± 0.18 a

Means followed by same letter(s) within a column are not significantly different by Duncan's multiple range test ($P < 0.05$).

Table 2. Fruit quality of cork spotted and normal 'Akizuki' fruits.

Fruit type	Fruit parts	Total soluble solids (°Brix)	Titrate acidity (%)	Flesh firmness (kg·cm ⁻²)
Normal fruit	Stem end	13.52 ± 0.31 ab	0.89 ± 0.02 a	8.56 ± 0.94 b
	Middle	13.91 ± 0.40 a	0.89 ± 0.01 a	9.17 ± 0.75 b
	Calyx end	13.47 ± 0.28 ab	0.81 ± 0.12 a	9.26 ± 0.96 b
Cork spotted fruit	Stem end	12.71 ± 0.63 c	0.89 ± 0.02 a	9.17 ± 0.57 b
	Middle	13.29 ± 0.47 b	0.90 ± 0.01 a	9.06 ± 0.95 b
	Calyx end	12.62 ± 0.58 c	0.89 ± 0.01 a	12.2 ± 1.15 a

Means followed by same letter(s) within a column are not significantly different by Duncan's multiple range test ($P < 0.05$).

Fig. 2. Comparison of carbohydrate content of fruit flesh between cork spotted and normal 'Akizuki' pear. Error bars with different lowercase letters represent a statistical difference by Duncan's multiple range test ($P < 0.05$).

higher Mg, and lower P content (Table 3). The results herein were not quite consistent with previous results, which showed significantly higher Mg and P in the spotted fruit (Faust and Shear, 1969; Tomala and Trzak, 1994). This conflict with P was probably due to the different cultivar or the cultivation conditions. The K content in the stem end and the middle part of normal fruit was significantly higher than those showing cork spot, which was in agreement with Al-Ani (1978), who found that low K in the fruit may be related to the incidence of cork spot. Previous studies suggested that a high level of N reduces the movement of Ca toward the fruit (Ho et al., 1999) and triggers rapid fruit and cell expansion, which can potentially result in further dilution of the limited Ca content that moves into the fruit (Bar-Tal et al., 2001; Saure, 2001), and thus results in the occurrence of cork spot (Raese and Staiff, 1990). In our study, high levels of N correlated with cork spot in 'Akizuki' pear, but did not lead to Ca

deficiency. Zn and Fe content seemed irrelevant to this disorder.

In some research, adequate Ca in fruit is necessary for controlling cork spot disorder in pears (Raese, 1989; Raese and Drake, 1995; Tomala and Trzak, 1994), but there is a report that indicated that cork spotted pear fruit had higher Ca concentrations than normal fruit (Mason and Welsh, 1970). However, our results showed that Ca concentration in all three parts of disordered 'Akizuki' pear fruit was much higher than those of normal fruit, especially for the middle and calyx end where the differences of Ca content were significant, suggesting that the occurrence of cork spot in this cultivar might not be due to a deficiency of total Ca (Fig. 3). Mg^{2+} and K^{+} could compete with Ca^{2+} for binding sites at the plasma membrane; therefore, high levels of them reduced Ca absorption (Schönherr and Bukovac, 1973; Yermiyahu et al., 1994) and increased the susceptibility to Ca deficiency disorders (Askew et al., 1960).

Table 3. Total mineral element content of cork spotted and normal 'Akizuki' fruits from the calyx to stem end.

Fruit type	Fruit parts	N (%)	P (mg/kg)	K (mg/kg)	Mg (mg/kg)	Zn (mg/kg)	Fe (mg/kg)
Normal fruit	Stem end	4.00 ± 1.35 c	1,654.51 ± 24.28 a	11,917.83 ± 341.26 ab	380.26 ± 9.32 e	10.76 ± 3.68 a	56.36 ± 15.83 a
	Middle	4.13 ± 0.45 c	1,724.39 ± 148.26 a	12,509.13 ± 342.12 a	371.56 ± 8.55 e	7.67 ± 1.17 ab	52.99 ± 7.65 b
	Calyx end	3.87 ± 0.31 c	1,327.41 ± 53.46 b	11,685.07 ± 494.39 ab	469.10 ± 30.47 d	7.75 ± 2.16 ab	33.94 ± 13.55 b
Cork spotted fruit	Stem end	5.10 ± 0.89 bc	637.18 ± 12.74 d	9,300.63 ± 71.00 c	601.26 ± 41.39 c	4.47 ± 0.58 b	45.78 ± 15.26 ab
	Middle	7.57 ± 0.95 a	691.33 ± 86.29 cd	11,239.73 ± 1,404.53 b	695.67 ± 86.66 b	6.74 ± 0.29 b	28.36 ± 8.42b
	Calyx end	6.07 ± 0.57 b	817.66 ± 44.63 c	12,669.00 ± 318.06 a	815.34 ± 1.97 a	6.12 ± 1.15 b	27.41 ± 3.42 b

Different letters in the same column indicate significant differences by Duncan's multiple range test ($P < 0.05$).

Fig. 3. The ratio of K/Ca, Mg/Ca, (K+Mg)/Ca, and Ca content in normal and cork spotted 'Akizuki' pear fruit. Error bars indicate SE from three replicates. Error bars with different letters represent a statistical difference by Duncan's multiple range test ($P < 0.05$).

Fig. 4. Localization of free Ca^{2+} fluorescence signals in the flesh cells of normal and cork spotted 'Akizuki' pear. (A–C) Free Ca^{2+} fluorescence signals in normal fruits by Argon ion laser excitation (A), light field (B), and merge field (C). (D–F) Free Ca^{2+} fluorescence signals in cork spotted fruits by Argon ion laser excitation (D), light field (E), and merge field (F). Scale bar is 250 μm for (A–C), and 100 μm for (D–F), respectively.

Therefore, it was more precise to use the ratios of N/Ca, K/Ca, and Mg/Ca to predict Ca deficiency disorders than fruit total Ca concentration alone (Dris et al., 1998; Freitas et al., 2010; Lanauskas and Kviklienė, 2006). In our study, cork spot in 'Akizuki' pear was correlated with lower ratios of K/Ca, (K+Mg)/Ca, and Mg/Ca (Fig. 3). In addition, the most susceptible fruit part to cork spot (the middle part) not only accumulated significantly higher levels of Mg but also Ca, whereas it had significantly lower K content. These conflicts may have resulted from the fruit development difference between Asian and European pears, because 'Akizuki' and 'Anjou' pears belong to Asian and European pear cultivars, respectively.

Analysis of the free Ca^{2+} localization in the flesh of cork spotted and normal pear fruit. At harvest, the free Ca^{2+} localization was detected in the flesh cells loaded with fluo-4/AM. High levels of free Ca^{2+} were observed in the cell wall and intercellular space of cork spotted fruit flesh; however, lower free Ca^{2+} spread over all of the flesh cells of normal fruit (Fig. 4). This cellular distribution difference of free Ca^{2+} between cork spotted and normal fruit may be a reason for cork spot. In addition, the free Ca^{2+} in the cork spotted fruit was much greater than normal fruit, which was in accordance with the results of total Ca content (Fig. 3), and partially consistent with the results of Wang et al. (2018), who suggested that the free Ca^{2+} localization in the flesh cells of hard end fruit was greater than that of normal fruit at harvest (120 DAFB), whereas it showed an opposite tendency during 'Whangkeumbae' pear fruit development (75–105 DAFB) (Wang et al., 2018).

Effect of exogenous treatments on the incidence of cork spot in 'Akizuki' pear. CSPD symptoms are reported to be induced by Ca or B deficiency, and the exogenous application of supplemental sprays containing these mineral nutrients improves the symptoms (Faust and Shear, 1968; Raese and Drake, 1995; Richardson and Lombard, 1979; Vang-Petersen, 1980). To prevent the incidence of cork spot in 'Akizuki' pear, five sprays at 30, 45, 60, 75, and 90 DAFB with different treatments [H_3BO_3 , $\text{Ca}(\text{NO}_3)_2$, and P-Ca] were adopted in this research. As expected, Ca, B, and P-Ca treatments helped in controlling the cork spot in fruits from both axillary and spur bud (Fig. 5), which agreed with previous reports (Faust and Shear, 1968; Raese and Drake, 1993, 1995; Richardson and Lombard, 1979). However, the research of Matsumoto et al. (2018) indicated that

none of Ca or B solutions decreased the CSPD incidence in 'Kurenainoyume' apple. In our research, we also observed higher levels of Ca and free Ca^{2+} in cork spotted fruit, indicating that deficiency of total Ca was not the major cause of this disorder in 'Akizuki' pear. But one consideration is why cork spot incidence could be inhibited by exogenous Ca solutions, because this disorder was not caused by lack of Ca? It may be more meaningful to consider factors such as the existing state of Ca (soluble or insoluble) or the balance between Ca and other elements besides the hereditary factor, soil pH, cultivation conditions, tree vigor, and the climate condition, which will affect the incidence of cork spot (Richardson and Lombard, 1979; Tamura, 2017).

There were significant differences in cork spot incidence of fruits from axillary bud between treatments and control, with the exception of H_3BO_3 (Fig. 5). The inhibitory effect of $\text{Ca}(\text{NO}_3)_2$ on cork spot was better than that of H_3BO_3 , but there was no significant difference between them. Among the exogenous treatments, the most effective one was P-Ca sprayed with 3500 times dilution, which reduced the incidence of cork spot from 16.39% to 8% and from 15.87% to 6% in fruits from axillary and spur bud, respectively (Fig. 5). As is known, P-Ca has been widely used in the control of vegetative growth of several pear cultivars (Asín et al., 2007; Hawerth et al., 2012; Pasa and Einhorn, 2014). 'Akizuki' pear shows a strong growth habit, from which new shoots stop growth 20 d later than other Asian pear cultivars, which may be correlated with its susceptibility to cork spot. Application of P-Ca alleviates the nutrition competition between fruit development and new shoot growth, which may have resulted in the prevention of cork spot incidence. In a word, P-Ca efficiently helped to reduce cork spot incidence in 'Akizuki' pear, thus providing a new control approach for cork spot in pear.

Effect of exogenous treatments on fruit quality in 'Akizuki' pear. To further test the effects of exogenous treatments on fruit quality in 'Akizuki' pear, we investigated the fruit characteristics of treated fruit and unsprayed control. The results showed that all exogenous treatments had no negative effects on fruit quality attributes, especially for fruit size (Table 4). Fruit quality attributes were unaffected by P-Ca, similar to that found for 'Anjou' (Einhorn et al., 2014), 'Bartlett' (Elfving, et al., 2003), 'Abate Fetel' (Costa et al., 2004), and 'Le Conte' (Carra et al., 2016); however, it was not in agreement with the results in which P-Ca sprayed fruits were much larger than control fruits (Costa et al., 2001), or reports in which P-Ca has been reported to be associated with reduction of fruit size (Smit et al., 2005; Sugar et al., 2004). This may be due to the application time and concentration of P-Ca.

Furthermore, exogenous treatments helped to improve the development of fruit pedicels and the calyx concave: $\text{Ca}(\text{NO}_3)_2$ significantly

Fig. 5. Effect of exogenous treatments on cork spot incidence of fruit from axillary and spur bud in 'Akizuki' pear. Error bars with different letters represent a statistical difference by Duncan's multiple range test ($P < 0.05$).

Table 4. Effects of exogenous treatments on fruit characters of 'Akizuki' pear.

Treatments	Fruit wt (g)	Fruit shape index	Length of fruit pedicels (cm)	Diam of fruit pedicels (mm)	The calyx concave depth (cm)
Control	453.76 ± 26.01 A	0.88 ± 0.03 A	2.23 ± 0.45 A	3.40 ± 0.26 BC	0.90 ± 0.18 AB
H_3BO_3	450.67 ± 22.69 A	0.86 ± 0.05 A	2.19 ± 0.50 A	3.80 ± 0.45 AB	0.74 ± 0.25 B
$\text{Ca}(\text{NO}_3)_2$	479.99 ± 62.57 A	0.86 ± 0.04 A	1.56 ± 0.44 B	3.91 ± 0.39 A	1.05 ± 0.32 A
P-Ca 1500x	489.62 ± 38.58 A	0.85 ± 0.04 A	2.20 ± 0.44 A	3.87 ± 0.42 A	0.66 ± 0.16 B
P-Ca 2500x	484.71 ± 50.94 A	0.86 ± 0.04 A	2.81 ± 0.47 A	3.00 ± 0.17 C	0.62 ± 0.24 B
P-Ca 3500x	488.64 ± 56.78 A	0.85 ± 0.04 A	2.46 ± 0.64 A	3.56 ± 0.31 AB	0.59 ± 0.26 B

Different letters in the same column indicate significant differences by Duncan's multiple range test ($P < 0.01$).

Table 5. Effects of exogenous treatments on fruit quality.

Treatments	Different parts of fruit	Flesh firmness ($\text{kg}\cdot\text{cm}^{-2}$)	Total soluble solids ($^{\circ}\text{Brix}$)	Titrateable acidity (%)
Control	Stem end	8.56 ± 0.94 CDEFG	13.52 ± 0.31 CDEF	0.89 ± 0.02 A
	Middle	9.17 ± 0.75 BCD	13.91 ± 0.40 CDE	0.89 ± 0.01 A
	Calyx end	9.26 ± 0.96 ABCD	13.47 ± 0.28 DEF	0.81 ± 0.12 AB
H_3BO_3	Stem end	8.03 ± 1.00 DEFG	12.73 ± 0.83 F	0.45 ± 0.01 D
	Middle	8.46 ± 0.83 CDEFG	13.53 ± 1.03 CDEF	0.53 ± 0.13 CD
	Calyx end	8.90 ± 1.10 BCDE	12.92 ± 0.75 EF	0.67 ± 0.24 BC
$\text{Ca}(\text{NO}_3)_2$	Stem end	10.55 ± 1.00 A	14.31 ± 0.55 CD	0.45 ± 0.02 D
	Middle	10.03 ± 0.83 AB	14.41 ± 0.88 CD	0.45 ± 0.00 D
	Calyx end	9.96 ± 1.10 AB	14.66 ± 0.77 CD	0.44 ± 0.02 D
P-Ca 1500x	Stem end	9.35 ± 0.93 ABCD	13.43 ± 1.17 DEF	0.89 ± 0.02 A
	Middle	9.53 ± 1.28 ABC	14.02 ± 1.08 CDE	0.88 ± 0.00 A
	Calyx end	10.01 ± 0.94 AB	14.11 ± 0.83 CD	0.89 ± 0.01 A
P-Ca 2500x	Stem end	7.60 ± 0.79 EFG	15.48 ± 0.46 AB	0.88 ± 0.01 A
	Middle	7.49 ± 0.82 FG	15.70 ± 0.46 AB	0.89 ± 0.00 A
	Calyx end	7.35 ± 0.90 G	16.12 ± 0.56 A	0.88 ± 0.03 A
P-Ca 3500x	Stem end	9.44 ± 0.92 ABC	13.42 ± 0.97 DEF	0.90 ± 0.01 A
	Middle	8.70 ± 0.94 BCDEF	13.66 ± 1.12 CDEF	0.90 ± 0.02 A
	Calyx end	8.58 ± 1.09 CDEFG	13.91 ± 1.25 CDE	0.89 ± 0.01 A

Different letters in the same column indicate significant differences using Duncan's multiple range test at the 1% level.

increased the diameter and shortened the fruit pedicels, H_3BO_3 promoted the diameter increment of fruit pedicels, and P-Ca at different rates decreased the calyx concave depth (Table 5). Moreover, spraying of $\text{Ca}(\text{NO}_3)_2$ and H_3BO_3 solutions significantly reduced the titrateable acidity, resulting in improved fruit flavor. For soluble solids, P-Ca with 1500 or 3500 times dilution did not differ from control (Table 5), which agrees with those found for 'Rosemarie', 'Forelle', 'Packham's Triumph', and 'Le Conte' pear (Carra et al., 2016; Smit et al., 2005). P-Ca with 2500 times dilution

significantly decreased fruit firmness and improved total soluble solids in the middle and the calyx of fruit (Table 5). To sum up, five sprays of P-Ca with 3500 times dilution was promise for inhibiting the incidence of cork spot without affecting fruit quality attributes, and could be used widely in 'Akizuki' pear cultivation.

Conclusions

Compared with normal 'Akizuki' pear fruit, cork spotted fruit had significantly larger

fruit size with shorter fruit pedicels. Furthermore, higher levels of N, Ca, and Mg, and lower ratios of K/Ca, Mg/Ca, and (K+Mg)/Ca were correlated with cork spot in 'Akizuki' pear fruit. Moreover, localization of free Ca^{2+} mainly in the cell wall and intercellular space may be strongly correlated with cork spot. Five sprays of P-Ca with 3500 times dilution at 15-d intervals from 30 to 90 DAFB showed promise for reducing cork spot incidence in 'Akizuki' pear without affecting fruit quality attributes. However, the mechanism of cork spot is complex and there are still many unclear details; therefore, further studies are needed focus on the relationship between cork spot and influence factors such as cultivar, climatic and soil conditions, and cultural practices including orchard fertilizer practices, excessive pruning, and poor rootstocks.

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