Prevention of New Cork Spot-like Physiological Disorder in ‘Kurenainoyume’ Apples by Pre-harvest Fruit Bagging

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‘Kurenainoyume’ is a new red-fleshed apple cultivar with a gradually increasing cultivation area. However, cork spot-like physiological disorder (CSPD) of the apple skin has become a serious problem over the years. Therefore, the development of strategies to reduce and prevent CSPD is strongly desired by farmers. To this end, we investigated the effectiveness of i) spraying calcium (Ca), boron (B), or both on the tree and ii) pre-harvest fruit bagging. Ca or B solutions or both did not decrease CSPD incidence. Furthermore, no relationship was detected between CSPD and the Ca/B content of fruit and leaves, demonstrating that the occurrence of CSPD might not be due to a deficiency in these elements. Pre-harvest fruit bagging reduced the development of CSPD depending on the light permeability of the paper bags used. Moreover, CSPD development was positively correlated with sunshine duration. Therefore, to prevent CSPD, fruit should be covered with light-impermeable paper bags at least from mid-July to late-September because the fruit covered for a shorter period developed CSPD. Thus, we propose that pre-harvest fruit bagging with light impermeable paper could be a useful and practical strategy to reduce or prevent CSPD in ‘Kurenainoyume’.

Key Words: bitter pit, corky core, fruit quality, global warming, sunburn.

Introduction

The apple (Malus × domestica Borkh.) is one of the most important and widely cultivated deciduous fruit crops worldwide (Jackson, 2003). However, the occurrence of physiological disorders including bitter pit as well as sunburn, watercore, or both are serious problems. Generally, apples are susceptible to numerous physiological disorders depending on the nutritional conditions, meteorological factors, and cultivars (Ferguson et al., 1999). Cork spot-like physiological disorder (CSPD), a typical and well-known physiological disorder that develops in apple fruit skin, currently has 3 known major forms, bitter pit, corky spot, and corky core (Faust and Shear, 1968). Bitter pit is the most widespread corking disorder, and it develops before harvest and continues until storage ends (Faust and Shear, 1968). It is well known that the development of bitter pit is due to a calcium (Ca) deficiency (Yamane, 2014) and, therefore, Ca spraying effectively reduces its development (Biggs and Peck, 2015; Blanco et al., 2010; Fallahi and Eichert, 2013). Cork spot, which presents symptoms similar to those of bitter pit, is also induced by Ca deficiency and, thus, Ca spraying is a useful alleviating measure (Faust and Shear, 1968). However, unlike bitter pit, cork spot develops only during the growing period. Corky core is induced by boron (B) deficiency and develops during the growing period (Faust and Shear, 1968). Askew and Chittenden (1937) reported that borax spray increased the B supply and reduced corky core development. Thus, nutritional control such as Ca spraying is the main means of reducing or preventing apple CSPD.

In contrast, Maeda and Seito (1984) reported a new type of CSPD with different symptoms to those of the
usual bitter pit and cork spot, and exclusively observed it on the sunlit sides of the skin of some apple cultivars such as ‘Touko’, ‘Kinsei’, and ‘Red Gold’. When that article was published, the global warming phenomenon had not yet become an obvious problem for the agricultural industry, including fruit cultivation. Therefore, considering the historical context in 1984, we inferred that the involvement of temperature and light conditions in the development of this new CSPD might not have been considered as contributing factors by those researchers. However, considering the various negative effects of global warming, which have been observed in fruit cultivation recently, the possible categorization of CSPD as a sunburn symptom cannot be ruled out. Generally, sunburn syndromes are induced by both high skin temperature and solar radiation (Schrader et al., 2001). Typical sunburn symptoms are water immersion-induced browning of the fruit surface, and they clearly differ from those of CSPD. As indicated by Maeda and Seito (1984), the symptoms exhibited by ‘Kurenainoyume’ did not indicate a typical sunburn syndrome (Fig. 1), but rather resembled bitter pit and cork spot. Therefore, we exogenously applied Ca and B to the plants as our first treatment choice.

‘Kurenainoyume’ is a new red-fleshed apple cultivar which was released in 2010 (Igarashi et al., 2011). Recently, this cultivar was found to be very susceptible to CSPD (Fig. 1), although no CSPD symptoms were observed during the selection steps in its breeding. CSPD in ‘Kurenainoyume’ is characterized by dried cork-like spots on the fruit surface, and severe symptoms are accompanied by the formation of a hollow space. Since the cultivation area of ‘Kurenainoyume’ is rapidly increasing, it is essential to develop strategies to reduce or prevent CSPD. Considering the recent increase in CSPD incidence and previous reports (Maeda and Seito, 1984), we also inferred the involvement of recent global warming in the relationship between CSPD and abnormal solar radiation in summer, in addition to nutritional deficiency.

In this study, we carried out experiments to elucidate the factors contributing to CSPD incidence and develop strategies to reduce or prevent CSPD in ‘Kurenainoyume’. First, we evaluated the effects of Ca and B spraying on CSPD development. Second, we investigated the effect of pre-harvest fruit bagging treatment using 3 types of paper bags with different light permeability to confirm the effect of sunlight intensities on CSPD. Finally, we investigated the best periods for pre-harvest fruit bagging to efficiently prevent CSPD.

Materials and Methods

**Effect of Ca and B treatment on CSPD development (Exp. 1)**

Experiments were conducted using 32-year-old (7 years after top-grafting onto ‘Jonathan’) ‘Kurenainoyume’ apple trees grafted onto Malus prunifolia trees grown on an experimental farm of Hirosaki University, known as Fujisaki farm (40°39'25''N, 140°29'9''E) in 2010. Five trees trained to a flat open center form (5.0 m × 3.5 m planting) with 4 primary scaffolds were used for the experiment.

Ca, B, Ca + B, or water (control) were used in the first experiment. Each treatment was allocated to a scaffold on the trees; 5 scaffolds were used for one treatment. All fruit harvested from 5 scaffolds were mixed together and used for the fruit quality analysis. For the Ca treatment, 34% calcium oxide (CaO) powder (Bycalty©; Koei Chemical, Nagoya, Japan) diluted 1000 times with water was sprayed on the scaffolds. For the B treatment, 54% diboron dioxide (B$_2$O$_3$) powder (PlusB©; Koei Chemical) diluted to 1000 times with water was sprayed on the scaffolds. For the Ca + B treatment, a mixture of 34% CaO powder and 54% B$_2$O$_3$ powder diluted 1000 times with water was sprayed. For the control treatment, only water was used. All the treatments were applied 5 times (May 29, June 8 and 18, and July 2 and 13, 2010), and the same solution volumes (350, 350, 420, 500, and 500 L/1000 m$^2$) were applied on each treatment day using a power sprayer according to the instruction manual. The trees received annual routine horticultural care, and the fruit and leaves were harvested on October 28, 2010.

Fifteen fruit were randomly harvested from each primary scaffold, and the fruit quality was examined (n = 75; 15 fruit each were randomly harvested from 5 trees). The fresh weight of each fruit was measured using a digital scale (EB-3200D; Shimadzu, Kyoto, Japan). Fruit length and diameter were measured using digital calipers (DIGIPA; Mitutoyo, Kanagawa, Japan). After the skin was removed, the flesh firmness was measured at 2 points on the equatorial area of the fruit using a penetrometer with an 11.1 mm tip (FT327; Facchinì srl, Alfonse, Italy). The total soluble solid content of the juice was determined using a digital refractometer (N-1; Atago, Tokyo, Japan). The total titratable acidity was measured using titration with 0.1N sodium hydroxide (NaOH) and calculated as a malic content of the juice was determined using a digital refractometer (N-1; Atago, Tokyo, Japan). The total titratable acidity was measured using titration with 0.1N sodium hydroxide (NaOH) and calculated as a malic
acrylic equivalent. The incidence of CSPD was indexed into 4 categories depending on the number of spots observed (0, 1, 2, and 3: no, 1–2, 3–5, and > 6 spots, respectively). Fruit indexed at grades higher than 2 were defined as disordered, and their frequency was calculated as the number of disordered fruit divided by the total number of fruit.

To determine the Ca and B content, 2 fruit showing CSPD incidence and all the spur leaves around the sampled fruit were harvested from each primary scaffold (n = 10). The fruit flesh, skin, and leaves were oven-dried at 78°C for 72 h, ground lightly into powder, and the samples were digested using the sulfuric acid (H$_2$SO$_4$)–hydrogen peroxide (H$_2$O$_2$) Kjeldahl digestion method (Ohyama et al., 1991). The Ca and B content was measured using an inductively-coupled plasma (ICP) emission spectrometer (SPS1500; Seiko, Chiba, Japan).

Comparison of Ca content in fruit flesh, skin, and leaves between Fujisaki and Kazuno orchards (Exp. 2)

The Ca contents in the fruit flesh, skin, and leaves were compared between fruit from 2 orchards (Fujisaki and Kazuno). The former orchard was the same as that used in Exp. 1, where CSPD is commonly observed. The latter orchard, Kazuno, is located in Akita Prefecture (40°13’1”N, 140°48’41”E) where CSPD rarely occurs. From the Kazuno farm, 10 healthy fruit without CSPD or leaves were randomly harvested from 30-year-old (10 years after top-grafting on ‘Fuji’) ‘Kurenainoyume’ apple trees grafted on M. prunifolia rootstocks on October 25 in 2010. The Ca content was determined using the methods already described. Weather data in Kazuno was obtained from the Japan Meteorological Agency, Kazuno measurement station (40°12’9”N, 140°47’2”E).

Effect of pre-harvest fruit bagging treatment on CSPD development (Exp. 3)

The experiment was conducted from 2011 to 2012 at the Fujisaki farm using 5 of the trees used in Exp. 1. In 2011, approximately 20 fruitlets from each tree were randomly covered with light impermeable double-layered paper bags (2-layer bag, 194 × 162 mm; Masudaya Co., Aomori, Japan; Fig. 2) 33 days after full bloom (DAFB). All the paper bags were removed at 124 DAFB. The control fruit was not covered. Ten fruit from both the covered and uncovered treatment groups were harvested periodically from 33 to 175 DAFB, and the incidence of CSPD was recorded on each sampling date.

In 2012, 3 types of bags with different light permeabilities were used to clarify the effect of solar radiation intensities (Fig. 2). Approximately 10 fruitlets from each tree were randomly covered with a 2-layer bag, a light-permeable single-layered white waxed paper bag (1-layer white bag, 179 × 140 mm; JA Zen-noh Tottori, Tottori, Japan; Fig. 2), or a light-permeable single-layered red waxed paper bag (1-layer red bag, 195 × 173 mm; Masudaya Co.; Fig. 2) at 42 DAFB and the paper bags were removed at 129 DAFB. The control fruit were not covered. Ten fruit from each treatment were harvested periodically from 42 to 170 DAFB, and the incidence of CSPD was recorded on each sampling date. The fruit quality was examined using the methods described in Exp. 1.

The atmospheric temperatures of the inside and outside of the paper bags were measured using 2-channel card loggers (MR5320; Chino, Tokyo, Japan). The temperatures were recorded hourly, and the temperature between 8:00 and 17:00 from July 1 to August 31 were used to analyze the average and maximum temperatures. Measurements were conducted on the fruit of 3 different trees (n = 3) which did not receive strong sunlight directly.

Effect of meteorological factors on CSPD development

Weather data from the nearest meteorological measurement point of the Japanese government (Japan Meteorological Agency, Hirosaki Measurement Station: 40°36’7”N, 140°27’3”E) were used for this analysis. The data on total sunshine duration (h), average daily average temperature, maximum temperature (°C), and the number of days the temperature was > 30°C (days) were used for the correlation analysis of CSPD incidence. The CSPD incidence was obtained from the control fruit data of our experiments (n = 75, 10, 10, 12, 15, and 30 on 2010, 2011, 2012, 2013, 2014, and 2015, respectively). The weather data of 2 summer months, July and August, were used for the analysis. The analysis...
was conducted using 6-year data from 2010 to 2015.

Effect of shading treatment on CSPD development (Exp. 4)

This experiment was conducted in 2015 at the Fujisaki farm using 8-year-old ‘Kurenainoyume’ apple trees grafted onto *M. prunifolia*. Four trees trained to a central leader form (2.5 m × 3.5 m planting) were used. Two trees were covered with black colored cheesecloth (light permeability was 25%, Dio Chemicals, Ltd., Tokyo, Japan) using a steel trellis of 8 m (length) × 4 m (width) × 5 m (height). The test trees were covered with the cheesecloth from July 15 to August 31, and the control trees were not covered. Thirty fruit per each treatment, which were randomly harvested from 2 trees, were used for the fruit quality tests and CSPD incidence according to the methods described in Exp. 1 (n = 30).

Effect of fruit covering and uncovering timing on CSPD development (Exp. 5)

In 2013 and 2014, we used the same trees as those used in Exp. 3 in 2012. Approximately 20 fruitlets per treatment were randomly selected from 5 trees, and we investigated the effect of covering or covering removal timing of the fruit bag. In this experiment, we used a 2-layer bag (Fig. 2). To determine the effect of timing of fruit bag removal, we covered the fruit at 50 DAFB and removed the covering on 80, 95, 110, 125, or 140 DAFB. To determine the effect of the timing of fruit bag covering, we covered the fruit at 65, 80, 95, 110, or 125 DAFB, and removed the coverings at 140 DAFB. We created another 2 treatments in which the fruit were covered between 80 and 110 DAFB and 95 and 125 DAFB. All the fruit were harvested at 173 DAFB, and the fruit quality was examined using the methods described in Exp. 1 (n = 15).

Statistical analysis

The data were analyzed using the Tukey-Kramer honest significant difference (HSD) test or Chi-squared test using the JMP IN (SAS Institute, Cary, NC, USA) or js-STAR (KISNET, Kasiwazaki, Japan), software, respectively, and significant differences among the treatments were determined. Unless otherwise stated, differences were considered statistically significant at $P < 0.05$.

Results

Effect of Ca and B treatment on CSPD development (Exp. 1)

The CSPD incidence in ‘Kurenainoyume’ was very severe and reached a 30.4% rate of total harvested fruit (Table 1). Although the Ca, B, and Ca + B treatments were applied 5 times during the fruit growing period from May 29 to July 13, none of the treatments reduced the CSPD to within the range of 35–40% (Table 1). The disorder index indicating the CSPD severity in each fruit was not reduced or prevented by any of the treatments either. The soluble solid content, titratable acidity, and flesh firmness showed similar values to those of the control for all treatments, while the fruit weight was enhanced and reduced, respectively, by Ca and B treatments (Table 1).

Since the exogenous application of Ca and B did not improve CSPD, we analyzed their contents to confirm the uptake of these elements into the flesh, skin, and leaves (Table 2) of the plants. The Ca content did not increase in any of the tissue samples from the treated fruit compared with the control samples, albeit some fluctuations were recorded (Table 2). Moreover, while the leaf Ca content was increased following Ca and Ca + B treatments, the B content of the B and Ca + B treatment groups was enhanced only in the skin, while none of the other treatments increased the B content in any of the tissues, which showed the same levels of B as those of the control (Table 2). These results suggest that the apple tissues might not take up the exogenously applied Ca and B and, therefore, we could not rule out the involvement of their deficiency in the development of CSPD.

Comparison of Ca contents in fruit flesh, skin, and leaves between Fujisaki and Kazuno (Exp. 2)

There was a significant difference in the CSPD incidence rate between fruit from the Fujisaki (30.4%) and Kazuno (0%, Table 3) orchards. Nevertheless, no differ-

Table 1. Effect of calcium (Ca) and boron (B) treatment on cork spot-like physiological disorder (CSPD) incidence (or development) and fruit quality.

| Treatment | Disordered fruit (%) | Disorder index  | Soluble solid (Brix °) | Flesh firmness (N) | Titratable acidity (%) | Fresh weight (g) |
|-----------|----------------------|-----------------|------------------------|--------------------|------------------------|-----------------|
| Control   | 30.7                 | 1.5             | 10.7 a                 | 70.6 a             | 0.88 a                 | 258.8 b         |
| Ca spray  | 40.0                 | 1.2             | 11.2 a                 | 66.5 a             | 0.94 a                 | 276.1 a         |
| B spray   | 38.9                 | 1.5             | 10.8 a                 | 67.7 a             | 0.92 a                 | 243.8 c         |
| Ca + B spray | 34.7               | 1.4             | 11.2 a                 | 72.0 a             | 0.91 a                 | 271.0 ab        |
| Chi-squared test  | NS                   | NS              | —                      | —                  | —                      | —               |

a: did not appear; 1: 1–2 spots; 2: 3–5 spots; and 3: more than 6 spots.

NS indicates non-significant differences, Chi-square test, $P < 0.05$ (n = 75).

Different letters in the same column indicate significant differences using Tukey-Kramer’s HSD tests at the 5% level (n = 75).
Diordre (%) Days after full bloom

Effect of pre-harvest fruit bagging treatment on CSPD development (Exp. 3)

Exogenous Ca and B treatments did not reduce the CSPD incidence. Therefore, we investigated the effect of pre-harvest fruit bagging on CSPD incidence. The non-bagged control fruit gradually developed CSPD from 100 to 154 DAFB (harvest time) and the rate finally reached 90%. In contrast, pre-harvest fruit bagging treatment with the 2-layer bag completely suppressed CSPD development (Fig. 3A). Furthermore, the disorder indexes of the bagged and control fruit were 0 and 2.8 in 2011 (Table 4). The fresh weight, flesh firmness, and titratable acidity values did not differ among the treatments, but the pre-harvest bagged fruit showed a slightly lower soluble solid content than the control fruit in 2011 (Table 3). These results suggest a relationship exists between light/temperature conditions and CSPD development.

Next, we investigated the effects of light/temperature using 3 types of paper with different light permeability levels in 2012 (Fig. 2). A similar trend was observed in CSPD development in 2012; the non-bagged control fruit developed severe CSPD, while fruit from the 2-layer bag treatment group did not exhibit any CSPD symptoms (Fig. 3B). Considering paper types, the rate of CSPD incidence in the fruit increased in those covered with bags that were permeable to short wavelength light (Figs. 2 and 3B). Treatment with the 1-layer red

Table 3. Comparison of calcium (Ca) content in fruit flesh, skin, and leaves, sunshine duration, maximum temperature, number of days over 30°C, and average temperature between cork spot-like physiological disorder (CSPD) occurring often (Fujisaki) and rarely (Kazuno) in orchards.

| Orchard | Disordered fruit (%)<sup>a</sup> | Ca (mmol·kg<sup>-1</sup> DW) | Sunshine duration (h) | Maximum temperature (°C) | Number of days over 30°C (days) | Average temperature (°C) |
|---------|-----------------------------|-----------------------------|------------------------|--------------------------|-------------------------------|--------------------------|
|         | Flesh | Skin | Leaf | Flesh | Skin | Leaf |                           |                           |                             |
| Fujisaki | 30.4 | 5.93 | 15.7 | 390.5 | 8.86 | a  | 326.2 | 34.7 | 31 | 25.0 |
| Kazuno  | 0.0  | 5.41 | 15.4 | 321.9 | 8.05 | a  | 282.7 | 35.6 | 20 | 24.1 |
| t-test<sup>b</sup> | NS | NS | NS | * | NS | NS | --- | --- | --- | --- |

<sup>a</sup> Cork spot-like physiological disorder (CSPD) incidence (development) in Fujisaki and Kazuno were calculated using 75 (n = 75) and 10 (n = 10) fruit, respectively.

<sup>b</sup> NS and * indicate non-significant and significant differences between CSPD occurring often and rarely in orchards, P < 0.01, t-test (n = 10).

Meteorological data for Fujisaki (quoted Fig. 4).

Table 2. Effect of calcium (Ca) and boron (B) treatment on their content in fruit flesh, skin, and leaves.

| Treatment     | Ca (mmol·kg<sup>-1</sup> DW) | B (mmol·kg<sup>-1</sup> DW) |
|---------------|-------------------------------|-----------------------------|
|               | Flesh | Skin | Leaf | Flesh | Skin | Leaf | Flesh | Skin | Leaf |
| Control       | 5.93 ab<sup>c</sup> | 15.7 a | 390.5 b | 8.86 a | 7.39 c | 8.18 a |
| Ca spray      | 6.66 a  | 15.7 a | 529.7 a | 8.70 a  | 7.53 c | 8.53 a |
| B spray       | 5.01 b  | 13.1 a | 447.4 ab | 9.87 a  | 9.04 b | 8.18 a |
| Ca + B spray  | 5.48 a  | 15.6 a | 507.9 a  | 9.54 a  | 11.17 a | 8.67 a |

<sup>c</sup> Different letters in the same column indicate significant differences using Tukey-Kramer’s HSD tests at the 5% level (n = 10).
and white bags resulted in 20% and 45% CSPD development, respectively, in the fruit at harvest (Fig. 3B). None of the examined fruit quality parameters (fresh weight, flesh firmness, titratable acidity, and soluble solid content) differed among the treatments, except for the disorder index in 2012 (Table 4). The measured temperature in the treatment bag did not differ much among the treatments (Table 5). Furthermore, when we considered the meteorological differences between Fujisaki and Kazuno again, especially the sunshine duration from July to August, it was apparent that Kazuno had a lower sunshine duration than Fujisaki (Table 3). Thus, the light conditions rather than temperature might be closely related to CSPD development.

**Effect of meteorological factors on CSPD development**

We hypothesized that the main environmental factor affecting the development of CSPD incidence might be the light conditions. However, since there were no differences in the temperature conditions of the treatments using the 3 kinds of paper bags (Table 5), we could not evaluate the effect of temperature on CSPD incidence. Therefore, to confirm our hypothesis, we analyzed the correlation between the incidence of disordered fruit at harvest from 2010 to 2015 and the sunshine duration, as well as the maximum and average temperatures or the number of days that temperatures were over 30°C in July and August, or a combination of these factors. A correlation was observed between the CSPD incidence at harvest and the sunshine duration at $P < 0.05$ (Fig. 4A). However, other temperature-related factors did not show any correlation with CSPD (Fig. 4B–D). When the investigation duration was changed, the temperature-related factors did not show any correlations with CSPD; however, the sunshine duration in July showed a higher correlation with CSPD than that in other combinations of duration from July to September (Figs. S1–S4).

**Effect of shading treatment on CSPD development (Exp. 4)**

Since we identified the importance of light conditions in CSPD incidence, we investigated the effect of shading on development as a practical treatment strategy with high efficiency. The shading treatment, which was conducted in the summer time, effectively reduced CSPD development (Table 6). The disorder index was also improved by the shading treatment. However, the fruit quality parameters were significantly lower in the

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**Table 4.** Effect of pre-harvest fruit bagging treatment on fruit quality of ‘Kurenainoyume’ apples at harvest.

| Year   | Treatment$^a$ | Disorder index$^b$ | Soluble solid (Brix$^b$) | Flesh firmness (N) | Titratable acidity (%) | Fresh weight (g) |
|--------|---------------|--------------------|--------------------------|-------------------|------------------------|-----------------|
| 2011   | Non-bagged control | 2.8               | 12.3                     | 72.9              | 1.09                   | 263.3           |
|        | 2-layer bag    | 0.0               | 11.3                     | 74.9              | 1.00                   | 249.4           |
| Test   | ****          |                   | NS$^v$                   | NS$^v$            | NS$^v$                 |                 |
| 2012   | Non-bagged control | 2.9               | 12.0 a$^w$               | 69.9 a            | 0.95 a                 | 315.9 a         |
|        | 2-layer bag    | 0.0               | 11.5 a                   | 75.4 a            | 0.86 a                 | 279.2 a         |
|        | 1-layer red bag | 0.2               | 11.4 a                   | 77.5 a            | 0.99 a                 | 257.1 a         |
|        | 1-layer white bag | 0.9               | 11.7 a                   | 73.2 a            | 0.86 a                 | 294.9 a         |
| Test   | ****          |                   | NS$^v$                   | NS$^v$            | NS$^v$                 |                 |

$^a$ Fruit were harvested 175 and 170 days after full bloom in 2011 and 2012, respectively.

$^b$ 2-layer bag: light impermeable double-layered paper bags, 1-layer red bag: light permeable single-layered red waxed paper bags, 1-layer white bag: light permeable single-layered white waxed paper bags.

$^c$ Different letters within the same column indicate significant differences using Tukey-Kramer’s HSD tests at the 5% level ($n = 15$).

$^d$ NS indicates no significant differences between non-bagged control and 2-layer bagged fruit at $P < 0.05$, respectively, $t$-test ($n = 15$).

**Table 5.** Average and maximum temperatures outside and inside different kinds of paper bags during pre-harvest fruit bagging treatment.

| Year   | Treatment$^a$ | Average | Maximum |
|--------|---------------|---------|---------|
| 2011   | outside bag   | 23.6    | 34.4    |
|        | 2-layer bag   | 23.7    | 35.4    |
|        | $t$-test$^*$  | NS      | NS      |
| 2012   | outside bag   | 23.1 a$^w$ | 36.2 a |
|        | 2-layer bag   | 23.3 a  | 36.6 a  |
|        | 1-layer red bag | 23.7 a  | 38.4 a  |
|        | 1-layer white bag | 23.6 a  | 38.6 a  |

$^a$ 2-layer bag: light impermeable double-layered paper bags, 1-layer red bag: light permeable single-layered red waxed paper bags, 1-layer white bag: light permeable single-layered white waxed paper bags.

$^w$ Means of 3 independent thermometer readings from 8:00 to 17:00 from July 1 to August 31.

$^v$ NS indicates no significant differences between non-bagged control and 2-layer bagged fruit at $P < 0.05$, $t$-test ($n = 3$).

$^v$ Different letters in the same column indicate significant differences using Tukey-Kramer’s HSD tests at the 5% level ($n = 3$).
treatment groups than they were in the control group, which produced smaller fruit with a lower soluble solid content (Table 6). The flesh firmness was increased by the treatment, but the titratable acidity was unchanged. Considering the low soluble solid content, which is one of the most important parameters for consumers, we concluded that shading treatment was not a practical method.

**Effect of pre-harvest fruit bagging treatment period on CSPD development (Exp. 5)**

In 2013, we planned to investigate the effect of the duration of the pre-harvest fruit bagging treatment on CSPD to optimize fruit bagging treatment, but there were few positive effects of pre-harvest fruit bagging on the rate of CSPD in that particular year; the rate of CSPD was only 11.8% even in the uncovered control. The climatic conditions, especially sunshine duration from July to August in 2013 were shorter than those of the other years according to the data from the Japan Meteorological Agency. Specifically, sunshine duration in 2013 was 285.9 h, while that of the other years (2010, 2011, 2012, 2014, and 2015), which showed high CSPD incidence, ranged from 349.7 to 440.3 h. Therefore, the shorter sunshine duration in 2013 than that usually observed in previous years could have led to this phenomenon.

![Fig. 4.](image)

**Fig. 4.** Effect of meteorological factors on cork spot-like physiological disorder (CSPD) incidence (or development) in ‘Kurenainoyume’ apples. Correlations were calculated using data from July to August 2010 to 2015. Correlation between incidence of disordered fruit at harvest and A: Sunshine duration, B: Maximum temperature, C: Number of days that temperature was over 30°C, and D: Average temperature.

![Fig. 5.](image)

**Fig. 5.** Effect of duration of pre-harvest fruit bagging treatment on CSPD incidence (or development). A: Rate of disordered fruit, B: Disorder indexes (0: no spot appear; 1: 1–2 spots; 2: 3–5 spots; and 3: more than 6 spots). Different letters in the same graph indicate significant differences, Chi-squared test, 5% level (n = 15). DAFB, days after full bloom.

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**Table 6.** Effect of shading treatment on fruit quality of ‘Kurenainoyume’ apples.

| Treatment | Disordered fruit (%) | Disorder index\(^z\) | Soluble solid (Brix\(^{\circ}\)) | Flesh firmness (N) | Titratable acidity (%) | Fresh weight (g) |
|-----------|----------------------|----------------------|-----------------|------------------|----------------------|-----------------|
| Control   | 80.0                 | 2.3                  | 12.8            | 64.8             | 1.17                 | 383.1           |
| Shading   | 13.3                 | 0.7                  | 11.9            | 68.5             | 1.27                 | 348.6           |
| Test      | **x**                | **y**                | ***x**          | ***x**           | NS\(^x\)            | **x**           |

\(^z\) 0: did not appear; 1: 1–2 spots; 2: 3–5 spots; and 3: more than 6 spots.

\(^y\) ** indicates significant differences, Chi-square test, P < 0.01 (n = 30).

\(^x\) NS and ** indicate non-significant and significant differences, P < 0.01, respectively, t-test (n = 30).
In 2014, we carried out a planned experiment that was not executed in 2013. The CSPD rate of the control reached 86.7%, and the disorder index was 2.5 (Fig. 5). All bagging treatments using 2-layer bags lowered the CSPD occurrence, and the reduction rate differed among the bagging durations (Fig. 5). The conventional bagging duration of 50–140 DAFB completely inhibited CSPD development. In addition, the 65–140 DAFB treatment also entirely prevented CSPD. When bagging started at 50 DAFB, the incidence of CSPD increased in an inverse manner depending on the bagging duration with the exception of the 50–110 DAFB treatment. Similarly, when bagging was terminated (removal of the bag) at 140 DAFB, CSPD decreased as the bagging duration was prolonged (Fig. 5). Thus, the longer bagging period showed a lower CSPD incidence than the shorter period, which was concomitant with the lower disorder index observed with longer bagging treatments (Fig. 5). 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### Discussion

Bitter pit, cork spot, and corky core are the major CSPDs affecting the apple fruit skin surface (Blanco et al., 2010; Faust and Shear, 1968). Generally, these CSPD symptoms are induced by Ca or B deficiency, and the exogenous application of supplemental sprays containing these mineral nutrients improves the symptoms (Faust and Shear, 1968; Vang-Petersen, 1980). It is worth noting that typical sunburn symptoms have seldom been reported in Aomori Prefecture to date, possibly due to the relatively lower temperatures during fruit development in Aomori compared with other apple-producing areas located in the southern regions. Moreover, as indicated below, the appearance of CSPD of ‘Kurenainoyume’ is quite different from that of typical sunburn symptoms. These points suggest that nobody paid attention to sunburn at that time. Therefore, considering these previous reports and conditions, we first tried to prevent the development of CSPD in ‘Kurenainoyume’ by exogenous treatments with Ca or B solutions, or both. Contrary to our expectations, the development of CSPD in ‘Kurenainoyume’ could not be controlled by the application of these solutions (Table 1).

Drazeta et al. (2001) reported that the susceptibility of this cultivar to bitter pit was related to the dysfunction of the xylem inflow to the fruit because Ca only moves in the xylem (Marschner, 1983). It could be speculated that dysfunction of xylem in ‘Kurenainoyume’ could be related to the similar Ca content in the treatment and control regardless of the treatment with exogenous Ca at 5 times the normal levels (Table 2), which failed to reduce CSPD development (Table 1). However, this inference was not supported by the Ca content of the ‘Kurenainoyume’ in Fujisaki and Kazuno, where the CSPD rate was higher in Fujisaki than it was in Kazuno. Moreover, there were no significant differences in the Ca contents of the two locations, although leaf values were lower in Kazuno than they were in Fujisaki (Table 3). In addition, earlier application of Ca was shown to be effective in reducing and preventing bitter pit, but this treatment did not increase the fruit Ca content at harvest (Nielsen et al., 2005). Considering the current results, we inferred that at least Ca and B deficiencies were not the main reasons

### Table 7. Effect of duration of fruit bagging using double-layered light-impermeable fruit bags on fruit quality.

| Bagged duration (DAFB) | Soluble solid (Brix°) | Flesh firmness (N) | Titratable acidity (%) | Fresh weight (g) |
|-----------------------|-----------------------|--------------------|------------------------|------------------|
| No bagged control     | 12.5 a                | 75.0 a             | 1.13 a^z               | 377.7 a          |
| 50–80                 | 12.8 a                | 76.5 a             | 1.09 abc               | 381.8 a          |
| 50–95                 | 12.5 a                | 77.0 a             | 1.12 ab                | 381.1 a          |
| 50–110                | 12.7 a                | 78.2 a             | 1.14 a                 | 372.4 a          |
| 50–125                | 12.4 a                | 80.8 a             | 1.05 abc               | 343.2 a          |
| 50–140                | 12.5 a                | 79.6 a             | 1.10 abc               | 362.2 a          |
| 65–140                | 12.5 a                | 75.4 a             | 1.02 bc                | 347.0 a          |
| 80–140                | 12.3 a                | 79.1 a             | 0.99 c                 | 351.9 a          |
| 95–140                | 12.8 a                | 79.6 a             | 1.06 abc               | 361.1 a          |
| 110–140               | 12.3 a                | 79.3 a             | 1.02 bc                | 336.8 a          |
| 125–140               | 12.4 a                | 75.1 a             | 1.11 ab                | 337.8 a          |
| 65–110                | 12.6 a                | 80.6 a             | 1.09 abc               | 341.9 a          |
| 95–125                | 12.5 a                | 80.4 a             | 1.02 bc                | 369.1 a          |

^z Different letters in the same column indicate significant differences using Tukey-Kramer’s HSD tests at the 5% level (n = 15); DAFB, days after full bloom.
for CSPD development in the ‘Kurenainoyume’ apple. Therefore, CSPD observed in ‘Kurenainoyume’ cannot be improved by using the conventional methods used to control bitter pit.

Pre-harvest fruit bagging treatment is a common cultivation technique used in Japan to improve fruit quality, especially the outward appearance (Arakawa and Komori, 2006). In recent years, the usefulness of this technique has been recognized by other countries and, therefore, it has been used in commercial orchards in Australia, China, and India (Sharma et al., 2013, 2014). We also used this technique to prevent CSPD development in the ‘Kurenainoyume’ apple. Surprisingly, CSPD development in ‘Kurenainoyume’ was completely prevented by pre-harvest fruit bagging, especially the use of a 2-layer bag (Fig. 3). Although the use of fruit bagging to reduce or prevent sunburn has been reported (Schrader, 2011), to the best of our knowledge, the fruit bagging technique has not been used for fruit showing symptoms similar to those of bitter pit, cork spot, and corky core.

Sunburn has become a significant problem in apple cultivating regions because of global warming (Iamsub et al., 2009; Schrader et al., 2003; Wünsche et al., 2004). The appearance of CSPD in ‘Kurenainoyume’ is quite different from that of sunburn, but it is expected that similar factors might contribute to the development of these disorders. Schrader et al. (2001) reported that sunburn was induced by both high skin temperature and solar radiation. Intense sunlight induces thermal dissipation and antioxidation by upregulating the xanthophyll and ascorbate-glutathione cycles, respectively (Chen et al., 2008, 2009; Ma and Cheng, 2003, 2004). However, when this upregulation is inadequate, a physiological disorder is induced by photooxidation (Chen et al., 2008). In this study, the temperature inside the paper bag did not differ significantly among treatments (Table 5). In contrast, our results (Tables 3, 5, and 6; Figs. 2–5 and S1–S4) indicated that light permeability and sunshine duration, but not temperature, could be the primary factors for CSPD development in ‘Kurenainoyume’, which differed from the case for sunburn. Therefore, we propose that the reduction and prevention of excessive exposure to sunlight (= sunshine duration) might be a critical factor in reducing or preventing CSPD development in ‘Kurenainoyume’. It is noteworthy that we did not monitor solar radiation itself due to the unavailability of a public climate database and, thus, we could only determine the effects of sunshine duration.

Although the incidence of CSPD in apple orchards fluctuated among the investigated years, a higher correlation was apparent with the sunshine duration in July to August than there was with the other temperature-related factors (Fig. 4). Moreover, the shading treatment reduced the development of CSPD dramatically (Table 5). These findings also support our hypothesis that the sunshine duration critically affects the development of CSPD by ‘Kurenainoyume’. Chen et al. (2008) reported that apple skin sunburn was caused by damage to the PSII complexes induced by high temperatures coupled with strong light exposure. The pre-harvest fruit bagging using the 2-layer bag reduced the accumulation of chlorophyll on the fruit surface and the fruit exhibited an albino-like appearance. The lack of a photosynthetic apparatus might prevent photooxidation, at least by strong light exposure. Further research will be necessary to elucidate the mechanisms underlying the development of CSPD in ‘Kurenainoyume’.

The current urgent issue is the need to develop a practical technique to prevent CSPD development in ‘Kurenainoyume’. As mentioned before, shading treatment reduced CSPD development; however, it also reduced the fruit fresh weight and soluble solid content (Table 6). In contrast, the pre-harvest fruit bagging treatment using a 2-layer bag could be an effective, practical tool for preventing CSPD (Fig. 3) with very limited quality reduction (Table 4) although it is slightly labor intensive. The conventional practice of pre-harvest fruit bagging was conducted at 50 to 140 DAFB (early-July to late-September), when no CSPD had developed (Figs. 3 and 5). In the present experiment, we also showed that pre-harvest fruit bagging 65 to 140 DAFB (mid-July to late-September) completely prevented CSPD development (Fig. 3) while retaining the fruit quality (Table 7), and this period was just 15 days shorter than that used in conventional practice. However, this 15-day difference (delay in the bagging procedures) has a meaningful impact on growers. Specifically, the delay makes it possible for the growers to distribute the labor force for pre-harvest fruit bagging, which is required not only for ‘Kurenainoyume’, but also for most cultivars for good skin coloration and a long shelf life.

In conclusion, we demonstrated that CSPD development in ‘Kurenainoyume’ apples could not be prevented by Ca or B treatments or both during fruit development. However, the possible involvement of nutritional elements other than Ca and B, cannot be completely ruled out. Instead, we thought that light conditions could be a primary factor for CSPD development considering the correlation with the incidence of CSPD and sunshine duration. The tree shading treatment reduced CSPD development dramatically; however, the fruit quality indexes were reduced by this treatment. The pre-harvest fruit bagging treatment using a 2-layer bag effectively reduced CSPD development without notable inferiority of the fruit quality. Therefore, the pre-harvest fruit bagging treatment from mid-July to end-September is an effective strategy for preventing CSPD development in ‘Kurenainoyume’. The mechanisms underlying the light-induced CSPD development in ‘Kurenainoyume’ remain unclear. Elucidation of the effects of photooxidation and photo-
protection, along with measurement of solar radiation could provide a new strategy to resolve this question.

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