Introduction

Avocado (*Persea americana*) is a sub-tropical fruit tree, native to Central and South America (Paull and Duarte, 2011); Mexico produces the largest quantities of avocado fruit worldwide (FAO, 2014). The fruit has a high oil content, with mono-unsaturated oleic acid as the major fatty acid (Paull and Duarte, 2011), and its palatability increases as the oil content increases. Avocado production has been increasing globally (FAO, 2014), and the amount of fruit imported to Japan, mainly from Mexico, has also been on the increase; in 2016, more than 70,000 t of avocados were imported, at a value of 200 billion yen (Ministry of Finance, Japan, 2017).

The major avocado variety imported to Japan from Mexico is ‘Hass’. Fruit imported to Japan is generally harvested at immature stage and ripened after harvest, due to the lengthy shipping period. Immature fruit can be softened by after-ripening but the flesh becomes ‘rubbery’ rather than buttery, in texture (Paull and Duarte, 2011). As the oil content increase as the fruit mature (Barmore, 1976); markets prefer such fruit (Whiley, 1984). If avocado can be harvested in Japan, the fruit can fully mature on the tree, and the palatability of domestically-produced fruit will be superior to that of imports.

Avocado can be divided into three horticultural races: West Indian, Guatemalan, and Mexican. The Mexican avocado race has the greatest tolerance for low temperatures; it can survive temperatures as low as −4 to −5 °C (Paull and Duarte, 2011). The Guatemalan race is less tolerant of low temperature than the Mexican race (McKellar *et al*., 1992), and the West Indian race is the most sensitive to low temperature. Therefore, in southwestern Japan, avocado cultivation might be possible at open field. ‘Bacon’, a Mexican × Guatemalan hybrid variety, is a promising candidate for cultivation in Japan, because the tree and fruit can survive temperatures as low as −4.4 °C and −3.8 °C, respectively, without damage (Bender, 2013).

Avocado cultivation has begun in southwestern Japan, though in a limited area. However, young trees have been reported to die in winter due to low temperatures, despite temperatures remaining above the published critical temperatures for avocado survival. Therefore, simple and inexpensive cultivation methods that will allow these trees to survive the winter season are now in demand. As young trees are generally less tolerant to low temperatures than mature ones, the cultivation methods for young trees are particularly needed. In general, low temperature damage becomes worse as light intensity increase (Sonoike, 1998), and empirically, low temperature damage of the sun leaf is known to be more severe than that of the shade leaf. Therefore, the hypothesis that under shading condition low temperature damage might lessen in avocado, was verified.

Materials and Methods

Twelve three-year-old grafted ‘Bacon’ avocado trees were used for the experiment. In November 2013, ‘Zutano’ seeds were sown for rootstock and in November 2014, ‘Beacon’ was grafted to the seedling at a nursery in Kagoshima prefecture, Japan. Both varieties are Mexican × Guatemalan hybrids, and have a relatively strong tolerance against cold stress (Knight, 1974). In November 2015, the grafted plants were transplanted to 5 L pots filled with typical upland field soil whose color is black in Miyazaki prefecture. The average height of the trees was 29.5 cm. Before the experiment, the plants were cultivated in a plastic greenhouse in which minimum temperature was kept at 7 °C. The plants were irrigated...
properly. No fertilizer was applied from November 2015 until the end of the experimental period.

The experiment was conducted from January 20 to March 16, 2016 in an experimental field in the University of Miyazaki (131.4°E, 31.8°N). Three different sunlight treatments, control, moderate shading, and heavy shading were applied. All the plants were moved into an arch-shaped steel pipe frame structure that was set up at open field. The height of the structure was 2 m with a width and length of 1.8 m. The plants were arranged in 3 rows; each containing 4 plants. The plants at south row were for control, at middle row were for moderate shading, and at north row were for heavy shading treatment. For the moderate shading treatment, the steel pipe frame structure was entirely covered with silver shade-cloth (Klark Corp., Aichi, Japan), while for the heavy shading treatment, the structure was entirely covered by the same cloth twice. In addition, the structure in all the 3 treatments was covered by thin nonwoven fabric to minimize environmental factors except for light intensity. Light intensities of moderate and heavy shading was 49% and 22% of control, respectively. Each treatment had four replicates.

On January 20, 28, February 3, and 9, the maximum quantum efficiency of photosystem II (Fv/Fm) was measured using a portable chlorophyll fluorometer (OS-30P; Opti-science, Inc., NH, USA). Fv/Fm can be used for the indicator of cold damage, because Fv/Fm decreases when complex such as photosystem II is damaged by stress such as cold. Before measurement, the leaves were subjected to a 30-min dark treatment, implemented by attaching plastic clips (Opti-science Inc.). Four fully mature sun leaves per plant were used for these measurements, all of which were conducted between 13:00 and 15:00. After the measurement on February 19, most of leaves, used for the measurement, fell off in the control and moderate shading treatment, thus the measurement was finished on that date. On March 16, dead stem length and number of fallen leaves were measured. Visually discolored black stem was judged to be dead stem. The air temperature of each treatment was measured using a thermometer with data logger (TR51i; T & D Corp., Nagano, Japan).

The data were analyzed by analysis of variance, and the statistical differences among treatments were subjected to further analysis using Tukey’s test. The significance level was $p < 0.05$.

**Results and Discussion**

The lowest minimum temperatures recorded during the experiment were ~5.2°C in the control treatment and ~4.5°C in both shading treatments, measured on January 25. On all other days, the minimum temperature did not drop below ~4°C (Fig. 1, 2). ‘Bacon’ trees have been reported to survive at ~4.4°C (Bender, 2013); thus, only this temperature might be critical for the trees. Radiative cooling may have been mild in the shading treatments due to the silver shade-cloth; the minimum temperature in the control treatment was 0.7°C lower than that in the two shading treatments. Moreover, due to the difference of the radiative cooling intensity, it was possible that the difference of leaf temperature between control and shading treatments was greater than 0.7°C. Hoarfrost on the leaves was not observed in any treatment during the experiment.

Leaves in the control treatment was severely damaged by the low temperature on January 25 (Fig. 3). On that day, immature leaves withered completely, many
small necrotic spots over the entire surface of almost all mature leaves were observed, the vein color turned brown and the stem became black. In the moderate shading treatment, the immature leaves of one of the four trees remained undamaged, and continued to mature afterwards. The immature leaves of the other three trees withered completely on that day. Small necrotic spots were observed over the entire surface of the mature sun leaves, although these spots in the moderate shading were fewer than that in the control treatment. Lower leaves that were shaded by the upper ones did not have necrotic spots. Leaves that had necrotic spots on that day fell off within 1 month. In the heavy shading treatment, immature leaves of two of the four trees remained undamaged and other immature leaves of two trees withered completely on that day. Necrotic spots were few, and two undamaged plants at immature leaves did not have necrotic spots.

The Fv/Fm values were the highest in the heavy shading treatment and the lowest in the control treatment during the experiment (Fig. 4); thus the damage of photosystem II might be the lightest in the heavy shading treatment and the heaviest in the control treatment. The Fv/Fm values of undamaged leaves have been reported to range from 0.8 to 0.83, regardless of plant species (Bjorkman and Demmig, 1987). In this study, on January 20, the Fv/Fm value of all treatments were almost identical at about 0.75, suggesting the trees suffered slight damage, which was not critical for
survival, from the pre-experimental condition and then decreased on January 28. The Fv/Fm values did not recover during the experimental period; thus, the damage of photosystem II may have been irreversible. The length of the dead stem and the number of fallen leaves were the lowest in the heavy shading treatment and the highest in the control treatment (Table 1). In the control treatment, almost all the leaves fell and in the moderate shading treatment, sun leaves, located at top of the stem, fell. The damage caused by the low temperature was the greatest in the control treatment and the lightest in the heavy shading treatment. Generally, photoinhibition increases as the extent of the utilization of absorbed light energy decrease at low temperature (Sonoike, 1998). Similarly, photoinhibition might increase as light intensity increased in this study.

In this study, low-temperature damage lessened under the shading treatment. The damage in the moderate shading treatment was more severe than that in the heavy shading, although the minimum temperature of the two shading treatments was the same. In the moderate shading treatment, the low-temperature damage on the sun leaves was more severe than that on the shade leaves, though the reasons were thought to be not only due to the difference of light intensity but also the difference of radiative cooling intensity; it was possible that the temperature of the sun leaves was lower than that of the shade leaves. Anyway, the shading treatment, a simple and inexpensive technique, effectively protected young avocado trees from low-temperature damage. On the other hand, we could not definitively conclude that the differences in symptoms between the control and two shading treatments were caused solely by the differences in light intensity, because the minimum temperature of the control treatment was 0.7 °C lower than that of the two shading treatments.

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References