Does the thickness of net tissues affect the water-proofing ability of musk melon (*Cucumis melo* L.) fruit?

T. Nishizawa[1], T. Puthmee[2], R. Kawamata[1], T. Aikawa[3] and Y. Motomura[1]

[1]Faculty of Agriculture, Yamagata University, Tsuruoka 997-8555, Japan; [2]Rajamangala University of Technology Tawan-ok, Faculty of Agriculture and Natural Resources, Chonburi 20110, Thailand; [3]Horticultural Research Centre, Niigata Agricultural Research Institute, Niigata 957-0111, Japan.

Abstract

Water loss from the fissures of netted melon fruit decreases rapidly during fruit development, even if the fissures have not yet been plugged by lignified and suberized periderm tissues, suggesting that the water-proofing ability of net tissues in netted melon fruit may be independent of the thickness of net tissues. In this paper, therefore, the net tissues of F_1 hybrids of 'Earl's Favourite' musk melon (*Cucumis melo* L. var. *reticulatus*) fruits were rasped as far as the bottom of unrepaired cracks or removed until the horizontal level of the fruit skin, and changes in the transpiration rate from the net and cuticle areas were measured independently to clarify the role of suberized periderm tissues as a barrier to moisture loss. Rasping treatment did not affect the transpiration rate from the net area, while the rate markedly increased as a result of the net-removing treatment. The transpiration rate from the cuticle area remained constant throughout the period of transpiration measurement, irrespective of the treatment. These results indicate that moisture loss from the skin of musk melon fruit is effectively blocked if the suberized waxy periderm tissues have developed until just below the unrepaired cracks. In addition, microscopic analysis revealed that deformed cell walls in the hypodermal tissues were mainly distributed in the area surrounding the wounded nets. These results suggest that the moisture in hypodermal tissues is mainly lost from unrepaired net tissues, resulting in the surface depression of fruit rind, which is often observed in netted melon cultivars with very weak net development.

Keywords: lignin, melon, net, suberin, transpiration, water-proofing ability

INTRODUCTION

When plant tissues are wounded, phellogen (cork cambium), a kind of secondary meristem, is rapidly activated and covers the wound with lignified periderm tissues (Schreiber et al., 2005). In addition, epidermal layers of the periderm tissues (phellem tissues) are deposited in a waxy suberin matrix, and act as a barrier against moisture loss from the fruit surface (Ahsan et al., 2010). In netted melon fruits, naturally occurring fissures at the stage of early fruit development are healed by lignified and suberized periderm tissues called "nets" (Keren-Keiserman et al., 2004), resulting in a lower transpiration rate at fruit harvest (Puthmee et al., 2013). The degree of net development in netted melon fruits differs largely among cultivars. Among them, 'Earl's Favourite' and its hybrids (musk melon) are known to produce raised nets at commercial harvest (Ling et al., 1995).

In our previous study (Puthmee et al., 2013), we measured the transpiration rate from fissures and cuticles independently using three netted melon cultivars with different forms of net development, and revealed that the transpiration rate from the fissures reached a peak value during the early development of the fissures, while it decreased rapidly thereafter prior to complete development of the nets, irrespective of the cultivar. In addition, the transpiration rate from the nets at the fruit-maturing stage was similar among cultivars, regardless of the different thicknesses of suberized cell-wall layers. These results suggest that the water-proofing ability of the net tissues in netted melon fruit may not necessarily be correlated with netting characteristics such as the embossment and width of net elements.

Rather, the water-proofing ability of the net tissues may be as effective as that of cutinized cell walls, even if the net tissues are not raised above the fruit surface. In fact, the fissures of netted melon fruit were not completely covered by periderm tissues, and small cracks were still apparent along the nets even at the fruit-ripening stage (Lester, 1988; Puthmee et al., 2013), suggesting moisture loss through these unrepaired cracks.

Therefore, in this study, net tissues of musk melon fruit were rasped as far as the bottom of unrepaired cracks or removed until the horizontal level of the fruit skin, and changes in the transpiration rate were then measured. The results were compared with those of untreated fruit in order to clarify the role of suberized periderm tissues as a barrier against moisture loss.

MATERIALS AND METHODS

Plant materials

In August and October 2012, fruits of F_1 cultivars of 'Earl's Favourite' musk melon (*Cucumis melo* L. var. *reticulatus*), which are characterized by high net embossment, were obtained at the commercial harvesting stage from local distributors and used for the experiments. The mean mass of an individual fruit was 1.8-2.0 and 1.5-1.8 kg for experiments I and II, respectively (data not shown). The cut surface of the peduncle was shielded by a silicone caulk (Dow Corning 3140 RTV, MI, USA) to prevent moisture loss, and all the fruits were dipped in 10 L of 200 μL L^{-1} sodium hypochlorite solution for 30 min at ambient temperature in order to avoid disease-causing fruit decay during the experiment (Puthmee et al., 2013).

Experiment I. Effect of net rasping on the transpiration rate from net and cuticle areas

1. Determination of transpiration rate.

Three circles of 6-cm diameter (28 cm^2) were drawn on the fruit surface. Nets in the first circle were then rasped using a fine sandpaper (Scotch-Brite™ Ultra Fine Hand Pad, 3M, Tokyo, Japan) until the bottom of unrepaired cracks was reached (net-rasping). Nets in the second circle were shielded using the same silicone caulk as described above (net-shielding). Nets in the third circle were left untreated for measurement of the combined transpiration rate from the net and cuticle areas.

Three circles each were then covered by polyethylene terephthalate (PET) caps (28 and 90 cm^3 in opening space and volume, respectively), and the caps were affixed to the fruit surface using the same silicone caulk. The transpiration rate from the covered area of each circle was measured daily at 10°C and 63% RH for 7 days using a method described previously (Puthmee et al., 2013).

After the transpiration measurement, the caps were removed, the nets were stained by Schiff's reagent for 20 min, photos were taken under a stereomicroscope (SZ-ET, Olympus, Tokyo, Japan) and the net area of the rind was calculated using image-analysing software (Motic Image Plus 2.1S, Shimadzu, Kyoto, Japan) in order to calculate the transpiration rate per unit area.

The transpiration rate from the second circle was regarded as the transpiration from the cuticle area, and the difference in transpiration rates between the second and third circles was regarded as the transpiration rate from the net area. The difference in transpiration rates between the first and second circles was regarded as the transpiration rate from the rasped net area.

2. Determination of histological characteristics.

After measurement of transpiration rates, approximately 5-mm cubic samples were taken from the rasped and non-rasped net areas, and half of the cubic samples were sliced into 15-μm-thick sections to observe the autofluorescence of suberized cell walls using a confocal laser scanning microscope (CLSM) (LSM 700, Carl Zeiss Japan, Tokyo, Japan), as reported previously (Gerchikov et al., 2008; Puthmee et al., 2013). The remaining cubic

samples were used to observe the net structures of surfaces and cross-sections under a scanning electron microscope (SEM) (TM-3000, Hitachi High-Tech, Tokyo, Japan) (Puthmee et al., 2013).

Experiment II. Effect of net removal on the transpiration rate from net and cuticle areas

1. Determination of transpiration rate.

Three circles of 28 cm^2 each were drawn on the fruit surface, as described for experiment I, and nets in the first circle were peeled away using a surgical blade until reaching the horizontal level of the cuticle layer. Nets in the second and third circles were shielded with silicone caulk and not shielded, respectively, as described for experiment I.

Each circle was covered with a PET cap (6 cm in diameter), and the transpiration rate from the covered area was measured independently daily at 10°C and 63% RH for 6 days. Transpiration rates from the cuticle, net, and removed-net areas were calculated according to the method described in experiment I.

2. Determination of histological characteristics.

After measurement of the transpiration rates, approximately 5-mm cubic samples were taken from removed and non-removed net areas, and the net structures of surfaces and cross-sections were observed by SEM and CLSM, as described for experiment I.

Statistical analysis

Results for transpiration rates in experiments I and II were subjected to analysis of variance, and the means among treatments were compared with Tukey's test at $P<0.05$ using SAS for Windows version 6.12 (SAS system, Cary, NC, USA). All mean values and standard errors in the figures represent five replicates of each treatment.

RESULTS

Experiment I

1. Transpiration rate from the cuticle and net areas.

Net rasping did not affect the transpiration rate from the cuticle (Figure 1A). In addition, the transpiration rate was mostly stable (approximately 0.8 mg cm^{-2} h^{-1}) during the measurement period.

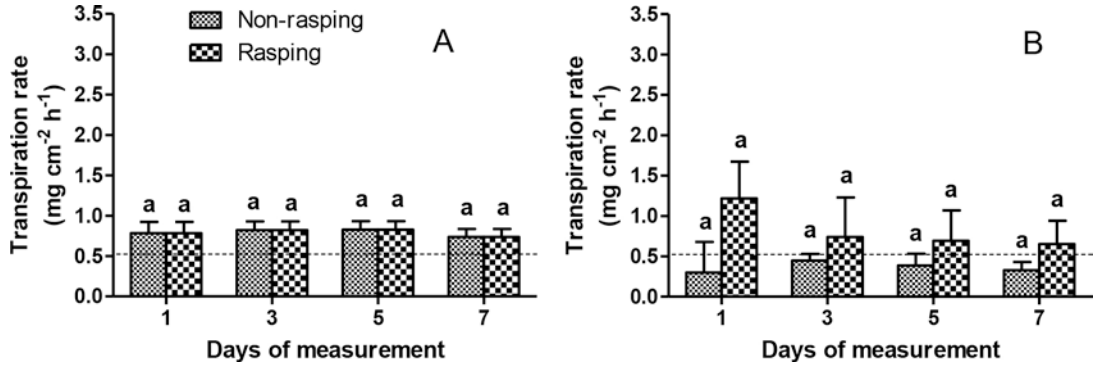

Figure 1. Effect of net rasping on the transpiration rate from the cuticle (A) and net (B) areas of musk melon fruit (experiment I). Transpiration rates were measured at 10°C for 7 days. Different letters within columns indicate a significant difference at $P \leq 0.05$ by Tukey's test. Bars indicate mean±SD (n=5).

The transpiration rate from the non-rasped nets (<0.5 mg cm^{-2} h^{-1}) was mostly lower

than that from the cuticle (Figure 1B). Although the transpiration rate from the rasped nets (0.7-1.2 mg cm^{-2} h^{-1}) tended to be a little higher than that from the non-rasped nets, there was no significant difference between the rasped and non-rasped nets during the measurement period.

2. Histological comparison between non-rasped and rasped net areas.

Although SEM images of the surface of the non-rasped net area showed that it was covered by a waxy matrix, some unrepaired cracks still remained along the nets (Figure 2A, C). When the net surface was rasped until the bottom of the unrepaired cracks, some of the outermost phellem cell-wall layers were missing, but the waxy matrix remained on the net surface (Figure 2B, D).

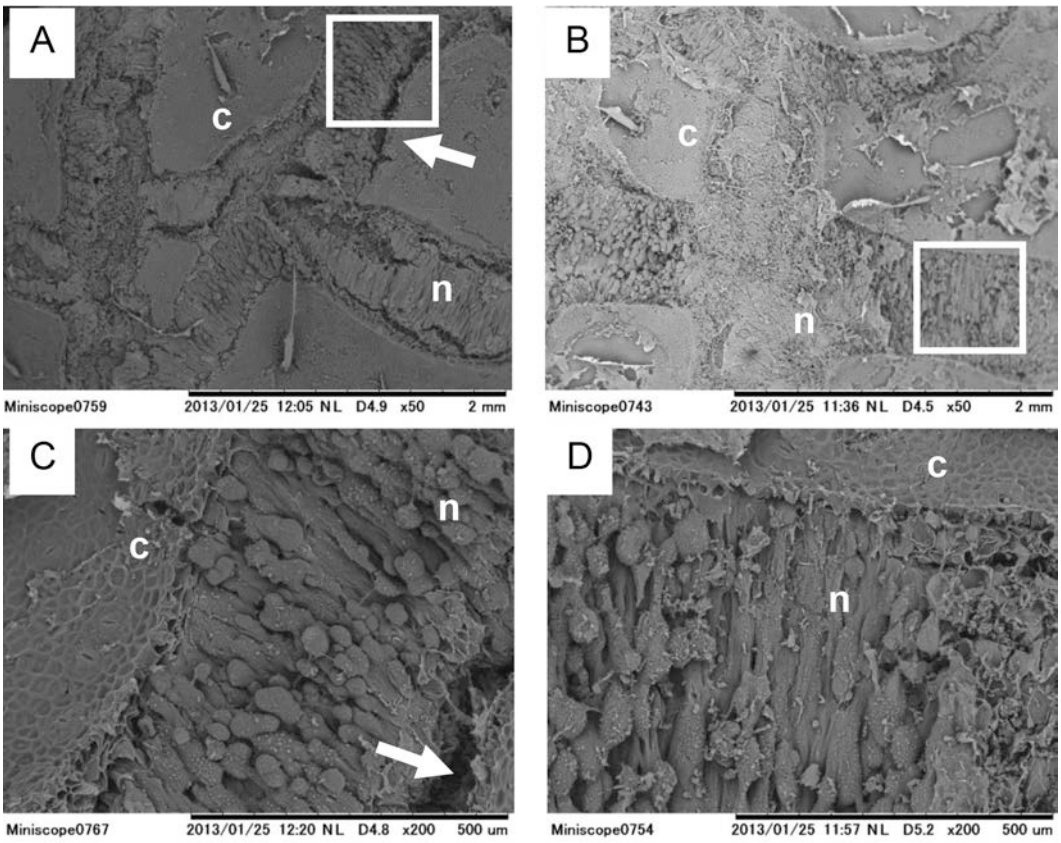

Figure 2. Effect of net rasping on the surface structure of musk melon fruit (experiment I). (A, C) Non-rasped nets; (B, D) rasped nets. Arrows show unrepaired cracks, and n and c show the net and cuticle, respectively. (C) and (D) are enlargements of each framework. Bars, 2 mm (A, B) and 500 μm (C, D).

SEM images of the cross-sections showed that the shape of periderm cell walls was similar between non-rasped and rasped nets, but several layers of the hypodermal cell walls, which were located just below the periderm tissues, showed mild shrinkage due to net-rasping treatment (Figure 3).

The images of CLSM showed that a few suberized phellem cell-wall layers remained in the uppermost net tissues even if the net surface was rasped until the bottom of the unrepaired cracks (Figure 4).

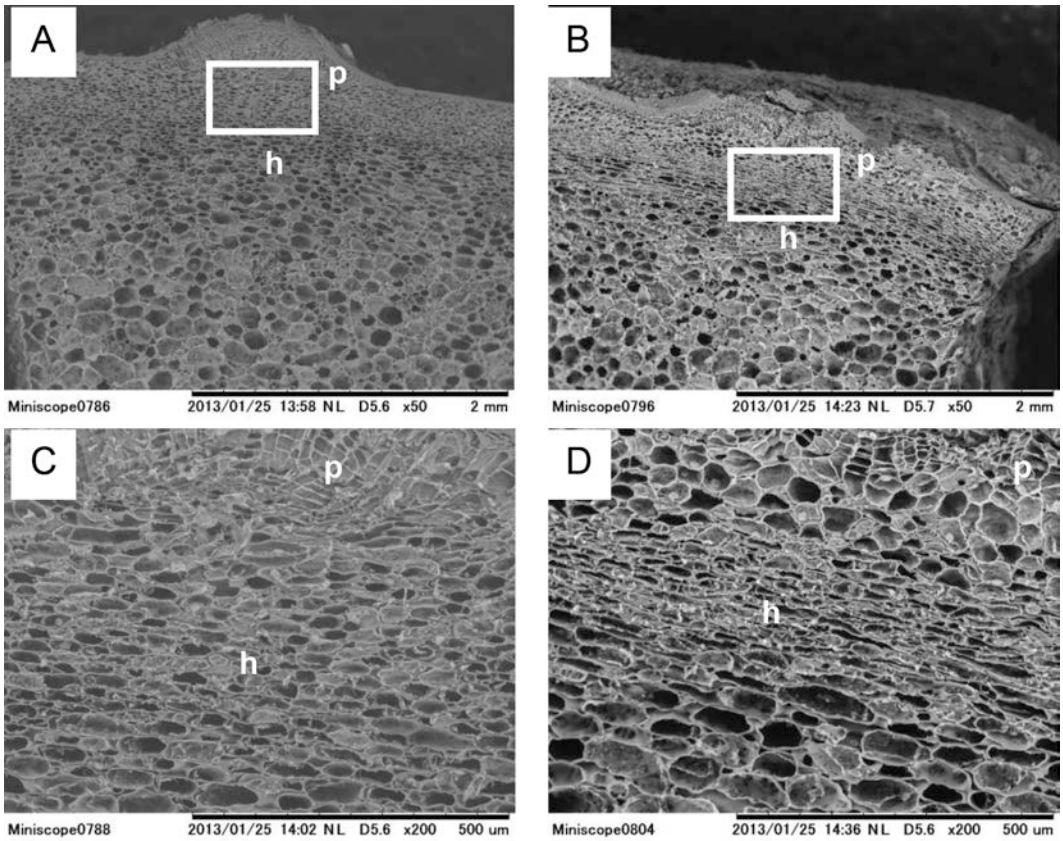

Figure 3. Effect of net rasping on the cross-section of musk melon fruit (experiment I). (A, C) Non-rasped nets; (B, D) rasped nets. p and h are periderm and hypodermal tissues, respectively. (C) and (D) are enlargements of each framework. Bars, 2 mm (A, B) and 500 μm (C, D).

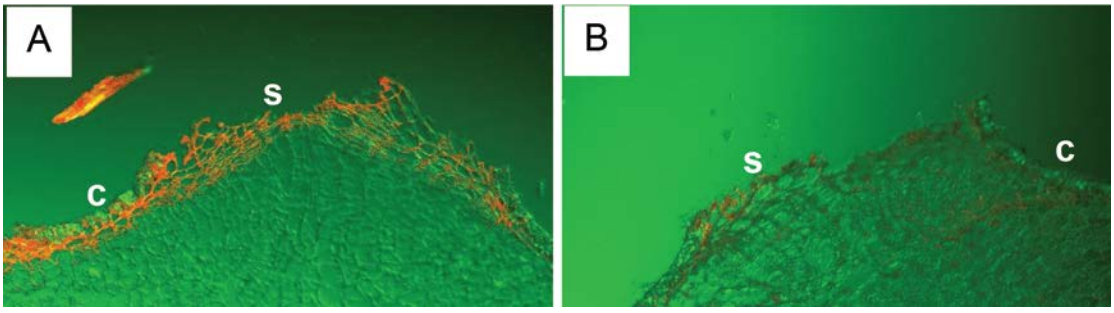

Figure 4. Effect of net rasping on the suberized phellem cell wall layers of musk melon fruit (experiment I). (A) Non-rasped nets; (B) rasped nets. S and c indicate suberized phellem cell walls and cuticle, respectively.

Experiment II

1. Transpiration rate from the cuticle and net areas.

The transpiration rate from the cuticle area was maintained at approximately 1 mg cm^{-2} h^{-1} during the measurement period (Figure 5A). In addition, the net-removing treatment did not affect the transpiration rate from the cuticle area.

The transpiration rate from the non-removed net area was approximately 0.5 mg cm^{-2} h^{-1}, while the rate from the removed net area was more than 8-fold higher (approximately

4-7 mg cm^{-2} h^{-1}) compared with that from the non-removed net area during the measurement period (Figure 5B).

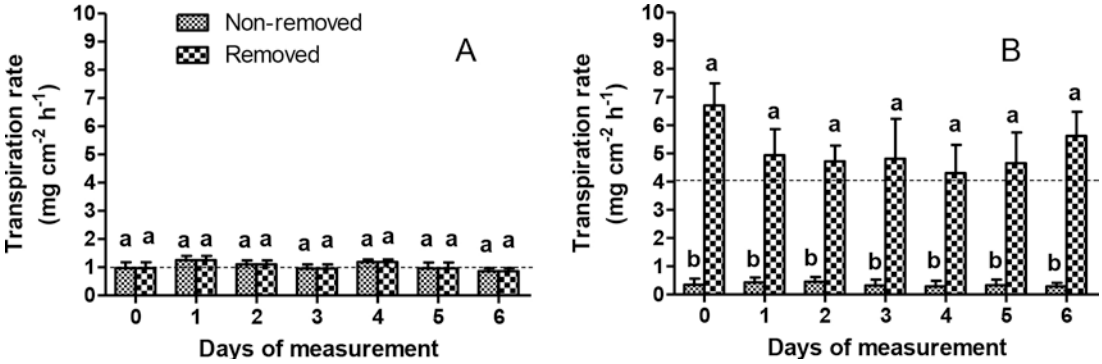

Figure 5. Effect of net removal on the transpiration rate from the cuticle (A) and net (B) areas of musk melon fruit (experiment II). Transpiration rates were measured at 10°C for 6 days. Different letters within columns indicate a significant difference at $P \leq 0.05$ by Tukey's test. Bars indicate mean±SD (n=5).

2. Histological comparison between non-removed and removed net areas.

Surface SEM images showed that the surface of the non-removed area was covered by a waxy matrix, and the shape of each cell was indistinct (Figure 6A). On the other hand, the round to oval shape of each cell was clearly recognized in the removed surface (Figure 6B).

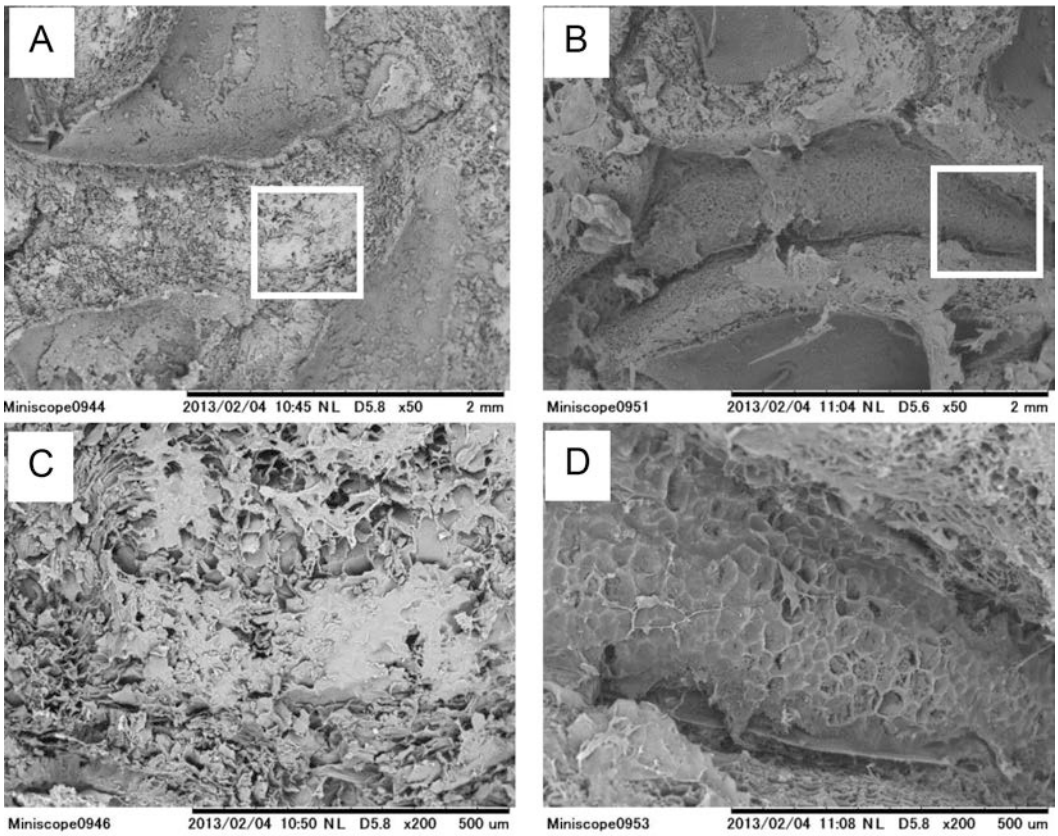

Figure 6. Effect of net removal on the surface structure of musk melon fruit (experiment II). (A, C) Non-removed nets; (B, D) removed nets. (C) and (D) are enlargements of each framework. Bars, 2 mm (A, B) and 500 μm (C, D).

Cross-section SEM images showed that the hypodermal cell walls below the periderm tissues collapsed as a result of net removal, while oval cell walls were recognized in the hypodermal cell walls in the absence of net removal (Figure 7).

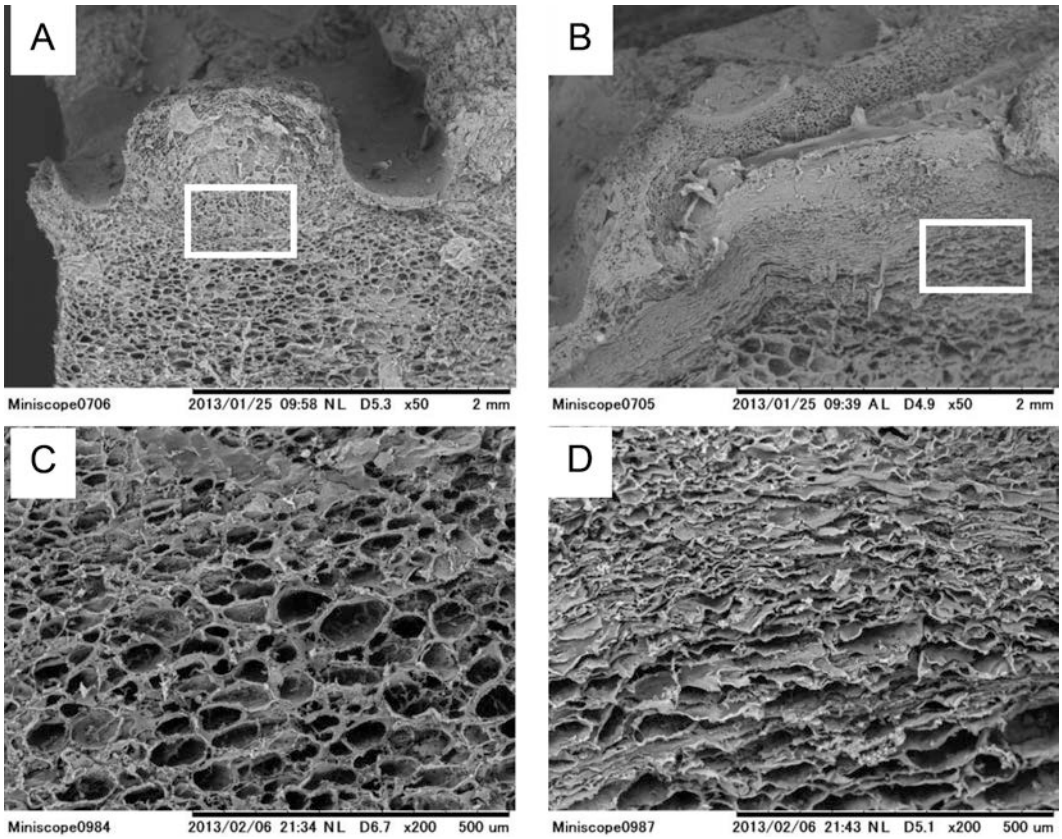

Figure 7. Effect of net removal on the cross-section of musk melon fruit (experiment II). (A, C) Non-removed nets; (B, D) removed nets. (C) and (D) are enlargements of each framework. Bars, 2 mm (A, B) and 500 μm (C, D).

Cross-section CLSM images showed that the periderm tissues of non-removed nets were well covered by suberized phellem cell wall layers, while those layers were partly missing on the removed nets (Figure 8).

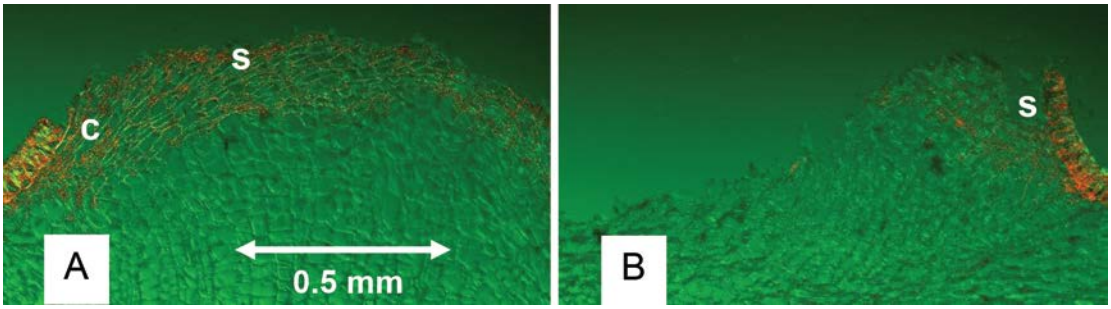

Figure 8. Effect of net removal on the suberized phellem cell-wall layers of musk melon fruit (experiment II). (A) Non-removed nets; (B) removed nets. S and c indicate suberized phellem cell walls and cuticle, respectively.

DISCUSSION

In netted melon fruit, fissures that naturally occur during fruit development are

covered by suberized phellem cell-wall layers, which are made by phellogens developing on both sides of the fissures (Gerchikov et al., 2008). However, small cracks are still apparent along the nets even at the fruit-ripening stage (Lester, 1988; Puthmee et al., 2013), indicating the incomplete repair of the fissures. Nevertheless, the transpiration rate from the fissures declines to the same level as in the cuticular membrane prior to complete development of the nets (Puthmee et al., 2013).

In our experiments, the net surface of commercially harvested musk melon fruit was also covered by a waxy matrix (Figure 2), and the net tissues were well protected against moisture loss even if the net surface was rasped until the bottom of the unrepaired cracks (Figure 1), because the rasped net surface was still covered by a few suberized phellem cell-wall layers (Figure 4). On the other hand, moisture loss remained high when the nets were peeled away from the fruit surface (Figure 5), and the fruit surface was not completely covered by suberized phellem cell-wall layers (Figure 8). These results indicate that the thickness of periderm tissues, which is associated with net embossment, is not correlated with the transpiration rate from net tissues. Rather, the development of only a few layers of suberized phellem cell walls under the unrepaired cracks is sufficient to prevent moisture loss. In netted melon fruit, the cutinized cell wall with wax deposition develops only one layer on the fruit surface (Figures 4 and 8), as reported by other researchers (Combrink et al., 2001; Webster and Craig, 1976), regardless of its high water-proofing ability, as well as suberized periderm tissues (Figures 1 and 5).

Although both lignification and suberization also often occur in naturally grown non-netted fruits, especially when the rind is wounded, the development of healing periderm tissues is usually far less marked than that of netted melon cultivars (Gerchikov et al., 2008). Nevertheless, there is no evidence to suggest that the transpiration rate from the healing periderm tissues in non-netted melon fruit is higher than that from the net tissues in netted melon fruit.

Hypodermal cell walls just below the periderm tissues of the rasped fruit shrunk a little compared with those of the non-rasped fruit (Figure 3). Although there was no significant difference in the transpiration rates from the nets between the rasped and non-rasped fruits, the transpiration rate of the former tended to be higher than that of the latter during the measurement period. This was more apparent when the nets were peeled away, and the hypodermal cell walls below the removed net tissues were collapsed (Figure 7). However, such deformed cell walls were distributed mainly in the area surrounding the wounded net tissues in both treatments (Figures 3 and 7). In quickly growing netted melon fruit such as ‘Life’, lignification and suberization in the periderm tissues are limited because of the shorter netting period (Puthmee et al., 2013), and surface depression often occurs along the nets after harvest (Nishizawa et al., 2009). These results suggest that moisture in the fruit rind is lost primarily from the hypodermal cells that are located in the area surrounding the periderm tissues, resulting in surface depression along the nets.

ACKNOWLEDGEMENTS

This study was supported by JSPS KAKENHI grant number 22580026.

Literature cited

Ahsan, N., Pervenn, S., and Akhand, A.A. (2010). Influence of temperature and growth regulators on wound periderm formation of potato tuber. J. Adv. Lab. Res. Biol. *1* (*2*), 115–119.

Combrink, N.J.J., Agenbag, G.A., Langenhoven, P., Jacobs, G., and Marais, E.M. (2001). Anatomical and compositional changes during fruit development of ‘Galia’ melons. S. Afr. J. Plant Soil *18* (*1*), 7–14 https://doi.org/10.1080/02571862.2001.10634393.

Gerchikov, N., Keren-Keiserman, A., Perl-Treves, R., and Ginzberg, I. (2008). Wounding of melon fruit as a model system to study rind netting. Sci. Hortic. (Amsterdam) *117* (*2*), 115–122 https://doi.org/10.1016/j.scienta.2008.03.015.

Keren-Keiserman, A., Tanami, Z., Shoseyov, O., and Ginzberg, I. (2004). Differing rind characteristics of developing fruits of smooth and netted melons (*Cucumis melo*). J. Hortic. Sci. Biotechnol. *79* (*1*), 107–113 https://doi.org/10.1080/14620316.2004.11511721.

Lester, G.E. (1988). Comparisons of 'Honey Dew' and netted muskmelon fruit tissues in relation to storage life. HortScience *23*, 180–182.

Ling, K., Kawamura, T., and Kobori, O. (1995). On the information engineering of analysis for the net in the musk melon. Jpn. J. Farm Work Res. *30* (*2*), 84–91 https://doi.org/10.4035/jsfwr.30.84.

Nishizawa, T., Okafuji, K., and Murayama, H. (2009). Storability and development of physiological disorder of netted melon 'Life' fruit as influenced by storage conditions. Acta Hortic. *837*, 147–154 https://doi.org/10.17660/ActaHortic.2009.837.17.

Puthmee, T., Takahashi, K., Sugawara, M., Kawamata, R., Motomura, Y., and Nishizawa, T. (2013). The role of net development as a barrier to moisture loss in netted melon fruit (*Cucumis melo* L.). HortScience *48*, 1463–1469.

Schreiber, L., Franke, R., and Hartmann, K. (2005). Wax and suberin development of native and wound periderm of potato (*Solanum tuberosum* L.) and its relation to peridermal transpiration. Planta *220* (*4*), 520–530 https://doi.org/10.1007/s00425-004-1364-9. PubMed

Webster, B.D., and Craig, M.E. (1976). Net morphogenesis and characteristics of the surface of muskmelon fruit. J. Am. Soc. Hortic. Sci. *101*, 412–441.