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Control of Black Spot Disease by Ultraviolet-B Irradiation in Rose (*Rosa* × *hybrida*) Production

Ayumu Kono[1], Ayumu Kawabata[1], Akira Yamazaki[1], Yuma Ohkubo[1], Adriano Sofo[3] and Munetaka Hosokawa[1,2]*

[1]*Faculty of Agriculture, Kindai University, Nara 631-8505, Japan*
[2]*Agricultural Technology and Innovation Research Institute, Kindai University, Nara 631-8505, Japan*
[3]*Department of European and Mediterranean Cultures, Architecture, Environment, Cultural Heritage (DiCEM), University of Basilicata, Matera 75100, Italy*

We investigated the effect of ultraviolet-B (UV-B) irradiation on the development of black spot disease caused by *Diplocarpon rosae* Wolf., which is a major problematic disease in rose (*Rosa* × *hybrida*) production. The growth of *D. rosae* colonies was suppressed on potato dextrose agar (PDA) medium under UV-B irradiation (peak wavelength: 310 nm; full width at half maximum: 30 nm) at an intensity of 15 μW·cm^{-2} with 1 h daily treatment. In addition, black spot conidia were inoculated to the rose 'Danjiri Bayashi' leaves and the effective growth suppression of black spot symptoms was observed on the leaves under UV-B irradiation. Next, various rose cultivars were planted in two greenhouses: one for supplemental UV-B irradiation treatment and one as a control without the treatment. In the UV-B irradiation greenhouse, the roses were irradiated at an intensity of 3–5 μW·cm^{-2} every day from 23:00–23:30 and 0:00–0:30 (total: 1 h). No chemical pesticides other than a starch agent for aphid control were used throughout the experiment. With the exception of the data for 'Papa Meilland' in 2019, UV-B irradiation significantly reduced the number of leaves infected with black spot disease. In September 2019, the non-UV-B irradiated 'Danjiri Bayashi' and 'Papa Meilland' had severe black spot symptoms on over 20 leaves. The number of plants with black spot symptoms increased in July 2020 compared to 2019. On the other hand, in UV-B irradiated plants, fewer black spot symptoms were observed than in non-UV-B irradiated plants. Although some visible damage was observed in the UV-B irradiated plants, the chlorophyll and carotenoid contents in the leaves decreased, indicating that UV-B irradiation had a certain negative effect on the photosynthetic apparatus. Over a five-month period, the cumulative number of flowers in the UV-B irradiation greenhouse did not decrease, and actually increased, depending on the cultivar, compared to the control treatments. Our results suggest that supplemental UV-B irradiation is effective at suppressing black spot disease in roses and can contribute to the production of pesticide-free edible rose production.

Key Words: black spot, integrated pest management, powdery mildew, rose.

Introduction

Rose (*Rosa* × *hybrida*) is one of the most popular ornamental crops in the world and is used not only as cut flowers, but also in aroma oils, cosmetics, processed foods, flavor extracts, and medicinal remedies (Cutler, 2003). Roses are also planted in parks, rose gardens, and other places where citizens can relax. The edible flowers market is expanding with a compound annual growth rate (CAGR) of 5.2% and will reach a value of USD 512 million by 2030 (https://www.marketresearchfuture.com/reports/edible-flowers-market-6634). For edible flowers and roses planted in parks that are related to human health, it is desirable to use pesticide-free cultivation methods as far as possible. As is well known, because numerous pests and diseases occur in

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* Corresponding author (E-mail: mune@nara.kindai.ac.jp).

rose cultivation, rose production relies on various chemical pesticides. Black spot disease is one of the most serious diseases in roses and is caused by the filamentous fungus *Diplocarpon rosae* (*Marssonina rosae* anamorph) (Nauta and Spooner, 2000). Black spot disease on rose leaves weakens plants through yellowing in young leaves and continuous defoliation (Black et al., 1994).

Integrated pest management (IPM), proposed by Stern et al. (1959) and promoted worldwide, is a combination of techniques that reduces the need for the application of chemical pesticides and makes cultivation safe for producers, consumers, and the environment. With the production of pest-resistant plant varieties and the development of pest management techniques such as pheromone agents and optical application technology, agriculture that does not burden either people or the environment is approaching realization. A lot of research is still required to realize effective IPM in warm and humid areas with abundant biotas, such as Japan.

The effects of ultraviolet (UV) on microorganisms studied in the laboratory and individual leaf levels have been reported (Hockberger, 2002). UV can be divided into UV-A (315–400 nm), UV-B (280–315 nm), and UV-C (200–280 nm), depending on the wavelength. As the short-wavelength UV is absorbed by the ozone layer, ground-level solar UV comprises UV-A and a small amount of UV-B. UV at shorter wavelengths has a stronger negative effect on living organisms (Slieman and Nicholson, 2000). UV-B irradiation could suppress powdery mildew caused by *Podosphaera pannosa* in roses (Kanto et al., 2009, 2011) and strawberries (*Fragaria* × *ananassa*) (Matsuura et al., 2012), and white rust in chrysanthemum (Kooriyama et al., 2014). Other than fungi, Tanaka et al. (2016) found that they could use UV-B irradiation to control spider mites in strawberries. Control of spider mites by UV irradiation is a direct biological effect of UV-B irradiation (Ohtsuka and Osakabe, 2009; Sakai and Osakabe, 2010). Probit analysis showed that spider mite mortality has been reported to increase linearly with increasing cumulative UV-B irradiation (Murata and Osakabe, 2013). Murata and Osakabe (2014, 2017a, b) and Yoshioka et al. (2018) found an effective time of day to irradiate UV-B to avoid any photoreactivation that could allow spider mites to recover from UV-B damage. In this way, UV-B irradiation is a technique that is attracting attention in IPM because it has the potential to suppress multiple parasites at the same time.

If it is possible to eradicate black spot disease, it will be possible to control the main fungal diseases in rose production, and the amount of pesticide application could be greatly reduced. Therefore, we evaluated the effect of UV-B on black spot disease inoculation in laboratory tests and investigated the effect of UV-B on the occurrence of black spot disease over two years in a greenhouse simulating edible rose production.

Materials and Methods

Direct effect of UV-B on D. rosae colony growth

A leaf with black spot symptoms that occurred in a greenhouse at the Faculty of Agriculture, Kindai University was collected and briefly sterilized with a sodium hypochlorite solution (0.5% active chlorine), and the black spots on the leaves were pierced with an injection needle. Then, the tip of the needle was applied to a potato dextrose agar (PDA) medium (Nissui, Tokyo, Japan), and the PDA medium was cultured at 25°C. DNA was extracted from a developed colony and amplified by PCR using primers (Diplocarpon_rosae-F: CGCCTTGTCTCAAACACCCTCT, Diplocarpon_rosae-R: TCCACTGGCGATGACTCTTGTC) designed according to sequence information (MVNX01000453.1) of *D. rosae* (DortE4 strain). The amplified product was sequenced and the sequence of 589 bp completely coincided with the target sequence of MVNX01000453.1 was confirmed. Therefore, this colony was attributed to *D. rosae* and used for the experiment. These fungi were subcultured to fresh PDA media by syringe needle every three weeks and kept at 25°C. Petri dishes in which the colony size grew to 2–3 mm in diameter were irradiated with UV-B every day for three weeks. As a control, Petri dishes containing similarly grown colonies were covered with a UV cut film (Toshimasen; Achilles, Tokyo, Japan) and placed under a UV-B irradiation lamp (YGRKX21799; Panasonic Co., Osaka, Japan) at 15 $\mu W \cdot cm^{-2}$. UV-B irradiation was conducted at 23:00–23:30 and 0:00–0:30. The peak wavelength of the UV-B lamp was 310 nm, and the full width at half maximum (FWHM) was 30 nm. UV-B intensity was measured using a UV-B meter (HD2302.0; Delta OHM, Padova, Italy) connected to a UV-B probe (LP471UVB; Delta OHM). Daylength of 12 h (8:00–20:00) was provided by fluorescent lamps (FHF32EX-N-H; Panasonic Co.), illuminating at three wavelengths with peaks around 437, 545, and 610 nm and at an intensity of 190 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The room temperature was set at 25°C. After three weeks, the colony size was measured and the growth ratio calculated by dividing it by the colony size at the start of the culture.

Investigation of black spot disease occurrence on inoculated leaves

Conidia collected with distilled water from the culture dishes after three weeks of culture were used in the experiment. The conidia were collected by centrifugation at 5000 rpm for 10 min, sedimented conidia were resuspended in distilled water and adjusted to 3–6 conidia·10 μL^{-1} using a hemocytometer. Fully developed leaves (each leaf composed of three leaflets) at the position of 3–5th from the youngest leaf of 'Danjiri Bayashi' grown under fluorescent light (FHF32EX-N-H; Panasonic Co.) were collected, washed briefly with

tap water, and put into plastic containers (28 × 19 × 14.5 cm) laid with a well-moistened paper towel. A drop of 10 µL of conidial suspension was put on each leaflet (3 drops were put on each leaf) via a small wound made by a needle. The containers were kept under a UV-B lamp (YGRKX21799; Panasonic Co.) at 15 $\mu W \cdot cm^{-2}$ intensity irradiated at 23:00–23:30 and 0:00–0:30. For the non-UV-B irradiation control, the containers were covered with an ultraviolet cut film and placed under a UV-B lamp.

Evaluation of rose pest development

The experiment was conducted in two adjacent greenhouses (6 × 21 m) at Kindai University (Nara, Japan). Plants were cultivated based on edible rose production. Only flowers were harvested, and the height of the plant was maintained below 1 m while cutting growing branches. Plants were maintained with 3–5 branches throughout the experiment. Fallen leaves due to black spot disease were left in the greenhouses. Forty-five benches (1055 × 992 × 750 mm) filled with 600 L of polyester medium (Neo Agriearth Co., Ltd., Nara, Japan) were placed in each greenhouse and four rose plants were planted on each bench in May 2019. As shown in Table S1, various cultivars in each greenhouse were grown. The greenhouses were covered with shading material (shading rate of 30%) in the summer (July–September), and the plants were dormant without leaves in the winter. In both greenhouses, to prevent excessive temperature rises, an insect repellent net (0.4 mm) was put on the side of each greenhouse, and fan ventilation was conducted. A liquid nutrient (HYPONeX Japan, Osaka, Japan) was diluted to 1/1850, and 2 L of the solution was applied to each plant once daily. In addition, 20 g of slow-release fertilizer (Ecolong 413 100-day type; JCAM Agri Co., Ltd., Tokyo, Japan) was applied to each plant once every 2.5 months. UV-B irradiated was applied in one greenhouse and not in the other. In the UV-B irradiation greenhouse, UV-B lamps (YGRKX21799; Panasonic Co.) were hung over the plants. The UV-B irradiation intensity was 3–5 $\mu W \cdot cm^{-2}$ just above the plants, and they were irradiated for 1 h each day, from 23:00–23:30 and 0:00–0:30. In both greenhouses, we sprayed a specified concentration of a starch agent (Nenchaku-Kun; Sumitomo Chemical, Tokyo, Japan) regularly to control aphids. The starch agent, diluted to 1/100 concentration, was sprayed over entire plants once every two to three weeks. Spraying was done throughout the year except early August to mid-September when aphids were not present due to high temperatures. No chemical pesticides other than starch were used throughout the experiments. Data were collected on September 20, 2019, and July 15, 2020, targeting all rose cultivars. The number of leaves with symptoms of black spot disease was counted for each plant. For the χ-square test, plants were divided into two groups; the number of plants with 0–9 leaves infected and the number with ≥ 10 leaves infected. In addition, to investigate the occurrence of powdery mildew under the UV-B irradiation conditions, a similar survey was also conducted for powdery mildew in October 2020.

Investigation of total antioxidant activity and electrolyte leakage of rose organs

To assess the effect of UV-B on damage or without apparent damage, an electrolyte leakage assay was carried out following Sukumaran and Weiser (1972), with some modifications. This value is greater if the organ is damaged by UV-B. For the experiment, five plants were randomly chosen for each treatment. A flower and leaf of the same age, exposition, and height from the ground were sampled from each plant. Four leaf discs were collected from each leaf (excluding the central rib) using a cork borer (0.2 cm^2) on paper towels. Immediately after cutting out the leaf disks, leaf disks from single plants were floated on 3 mL of ultrapure water in a test tube with the adaxial surface facing downward. Tubes with the lids on were left to sit for 30 min at 20°C. The water in the tube was replaced with 3 mL of fresh ultrapure water. The tubes were incubated at 20°C for 24 h in the dark without shaking. The conductivity of the solution was measured in each tube using an electrolytic conductivity meter (LAQUATWINEC33B; Horiba, Kyoto, Japan). Then, the tubes were capped to minimize evaporation, frozen at −80°C, and thawed at 20°C. After this, the conductivity of the solution was measured again. The percentage of electrolyte leakage as the ratio of the conductivity before freezing to that after freezing was calculated. The conductivity after freezing was assumed to represent complete (100%) electrolyte leakage.

Antioxidant analysis was carried out using a ferric reducing antioxidant power (FRAP) assay (Benzie and Strain, 1996), with some modifications. Leaf discs were collected in the same manner as described for the electrolyte leakage assay experiment. The discs were placed in 4 mL of extracting solution consisting of 98:2, methanol:37% HCl (37%) (v/v), following Sofo et al. (2012). The solution was let sit for 1 h at 20°C in the dark, before shaking at 100 rpm. FRAP reaction mix was prepared following Sofo et al. (2012). For all the leaf and petal samples, positive controls, and standards, the absorbance at 594 nm was measured after 60 min of incubation at 37°C. The results were expressed on a fresh weight (mg) basis.

Chlorophyll and carotenoid contents

Chlorophyll (Chl) and carotenoid (Car) contents (represented as [Chl] and [Car], respectively) of rose leaves were evaluated following the method of Lichtenthaler (1987), with slight modification. Leaf discs were collected in the same manner as described for the electrolyte leakage assay experiment. The disc area and

total weight were recorded. We dipped leaf discs in 5 mL of dimethylformamide and incubated them at 20°C for 24 h in the dark without shaking.

Chl a/b was calculated using the following equations:

$$[\text{Chl a}] = (12.00 \times \text{ABS}_{664} - 3.11 \times \text{ABS}_{647})$$
$$[\text{Chl b}] = (20.78 \times \text{ABS}_{647} - 4.88 \times \text{ABS}_{664})$$
$$[\text{Chl a + b}] = (17.67 \times \text{ABS}_{647} + 7.12 \times \text{ABS}_{664})$$

Total carotenoids (Car) were calculated using the following equation:

$$[\text{Car}] = (1000 \times \text{ABS}_{470} - 1.82 \times [\text{Chl a}] - 85.02 \times [\text{Chl b}])/198$$

The levels of both [Chl] and [Car] were expressed as μg on a fresh weight (mg) basis.

Cumulative numbers of flowers harvested

'Danjiri Bayashi' (non-UV-B irradiation: n = 30, UV-B irradiation: n = 42), 'Nighttime' (non-UV-B irradiation: n = 53, UV-B irradiation: n = 8), and 'Crimson Glory' (non-UV-B irradiation: n = 61, UV-B irradiation: n = 10) were used for data collection. From April 14, 2020, to September 21, 2020 (temperature data is shown in Fig. S1), flowers from the stalks were harvested approximately once weekly, and the number of harvested flowers was counted. After flower harvest, branches were left uncut back, and after lateral shoots developed, branch length was adjusted accordingly. The number of harvested flowers was counted per plant on each harvest day and divided by the number of plants of each cultivar. The time-course cumulative number and mean number of flowers per plant were compared between treatments.

Statistical analysis

For all statistical analyses between the two treatments (non-UV-B irradiation *vs* UV-B irradiation), Welch's *t*-test, Student's *t*-test, and the χ-square test were performed using R v.4.1.0. (R Core Team, 2021).

Results

A direct effect of UV-B on colony growth of D. rosae

The colony size (diameter) before treatment was 4.2 ± 0.9 mm (mean ± standard deviation). The sizes of *D. rosae* colonies cultured on PDA medium under temporal UV-B irradiation were smaller than those in the non-UV-B irradiation after three weeks of culture (Fig. 1A). In the leaf infection test, symptomatic lesions were observed in 30 out of 35 and 35 out of 36 inoculated portions in non-UV-B irradiation and UV-B irradiation leaves, respectively. Three weeks after inoculation, the lesion sizes (diameter) on the UV-irradiation leaves were smaller than those of the non-UV-B irradiation leaves (Fig. 1B).

Effect of UV-B irradiation on black spot disease

In 2019 and 2020, we observed black spot symptoms in the non-UV-B irradiation greenhouse. The heat map Figures for 2019 and 2020 (Fig. 2A, B) showing the severity of black spot disease in each plant revealed a clear difference between the two greenhouses. In addition, severely infected plants were found irrespective of their location in the greenhouses (Fig. 2A, B). In 2020, the incidence of black spots in both greenhouses was higher than that in 2019 (Fig. 2A–F). UV-B irradiation treatment significantly suppressed the development of rose black spot disease in both cultivars, 'Danjiri Bayashi' and 'Papa Meilland' (Fig. 2C–F). Significant differences were detected in all comparisons except 'Papa Meilland' in 2019 (Fig. 2E, F).

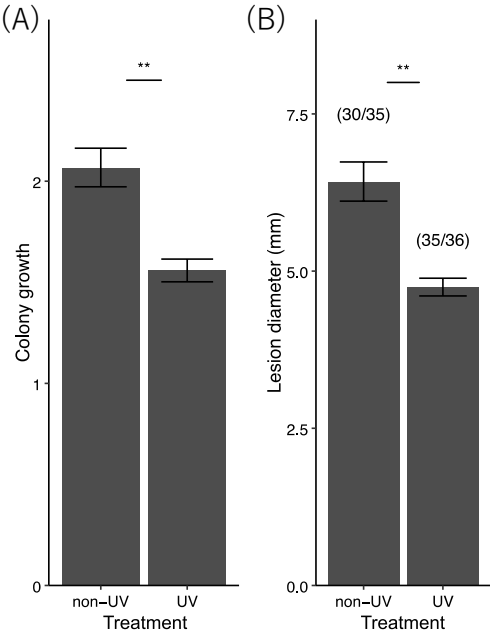

Fig. 1. Effect of UV-B irradiation on black spot colonies and symptoms. (A) Differences in colony size on PDA medium between non-UV-B irradiation and UV-B irradiation. Data are expressed as means ± standard deviation of eight colonies. Data are represented as the colony size ratio; colony diameter at three weeks after treatments/colony diameter at the start of treatment. (B) Differences in the diameter of lesions on leaves with non-UV-B irradiation and UV-B irradiation after inoculation of leaves with the *D. rosae* conidia. As shown in parentheses in the Figure, 30 of 35 and 35 of 36 inoculated portions had disease spots on the non-UV-B irradiation and UV-B irradiation leaves, respectively. Data are expressed as means ± standard deviation. In both experiments, leaves were collected three weeks after the start of UV-B irradiation treatment. ** means a significant difference at $P = 0.01$ in Welch's *t*-test.

Effect of UV-B irradiation on powdery mildew

We evaluated the severity of powdery mildew on October 2020 at which time the powdery mildew occurred severely in the UV-B irradiation greenhouse. The heat map Figures (Fig. S2) show that the outbreak of powdery mildew was suppressed completely in all cultivars in the UV-B irradiation greenhouse. Powdery mildew could be suppressed irrespective of the cultivar

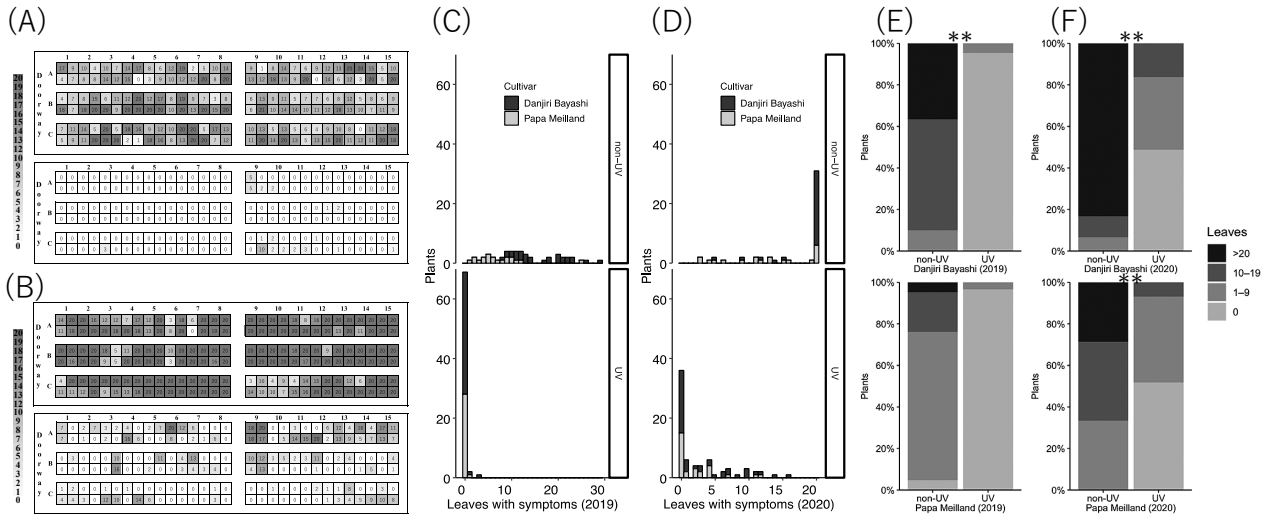

Fig. 2. Occurrence of black spot symptoms in non-UV-B irradiation and UV-B irradiation greenhouses. (A, B) Heat map indicating the distribution of rose plants at each disease level in each greenhouse. In both figures (A) and (B), the upper heatmap shows the non-UV-B irradiation greenhouse and the lower heatmap shows UV-B irradiation greenhouse. (A) Data for September 2019. (B) Data for July 2020. The biplanes were counted collectively as one leaf. Cultivation benches (1–15) planted with four plants were arranged in three rows of A–C (45 benches in total were arranged). Plants are numbered clockwise order from the upper left as 1, 2, 3, and 4. For example, plants planted on growth bench A-1 are A-1-1, A-1-2, A-1-3, and A-1-4 from the upper left. The number in the Figure with each color intensity is the number of leaves with black spot symptoms. Each cultivar's name is shown in Table S1. (C, D) Histograms of the number of leaves with black spot symptoms of 'Danjiri Bayashi' (non-UV-B irradiation: n = 30, UV-B irradiation: n = 43) and 'Papa Meilland' (non-UV-B irradiation: n = 20, UV-B irradiation: n = 29) in (C) 2019 and (D) 2020. (E, F) Occurrence of infected leaves in both 'Danjiri Bayashi' and 'Papa Meilland' treatments in 2019 (E) and 2020 (F). The upper panel is 'Danjiri Bayashi' and the lower is 'Papa Meilland'. For statistical analysis, plants were divided into two groups—the number of plants with 0–9 leaves infected and the number with ≥ 10 leaves infected—and the χ-square test was performed. The symbol indicates statistical difference (**: $P < 0.01$).

(Fig. S2). Severely infected plants were found irrespective of their location in the greenhouse (Fig. S2).

Organ damage due to UV-B irradiation

Branches growing close to the UV-B lamp rarely exhibited damage, such as leaf curling, but the UV-B irradiation did not induce any other outstanding damage as reported by Kobayashi et al. (2013). In both cultivars, 'Danjiri Bayashi' and 'Nighttime', no significant differences were detected in electrolyte leakage from leaves between the samples from the non-UV-B and the UV-B irradiation greenhouses (Fig. 3A). On the other hand, in 'Crimson Glory', there was a significant increase in electrolyte leakage from leaves with UV-B irradiation ($P < 0.05$) (Fig. 3A). A leaf and petal FRAP assay revealed no difference in the leaves between the non-UV-B and UV-B irradiation greenhouses in the three cultivars (Fig. 3B, C).

UV-B irradiation reduced the chlorophyll a and b contents in the leaves of 'Danjiri Bayashi', 'Nighttime', and 'Crimson Glory' (Fig. 3D). This decrease was particularly significant in 'Danjiri Bayashi' ($P < 0.05$). UV-B irradiation also reduced the carotenoid content in all three cultivars and was statistically significant ($P < 0.05$) in 'Danjiri Bayashi' (Fig. 3E). Visible damage caused by UV-B irradiation was not observed in flowers, and no visible difference in flower size was observed. From April 14th to September 21, 2020, there was a slight reduction in the cumulative number of flowers of 'Danjiri Bayashi' per plant under UV-B irradiation (Fig. 4B, E). The cumulative flower numbers were almost the same between the non-UV-B and UV-B irradiation treatments for 'Nighttime' (Fig. 4C, F), whereas that of 'Crimson Glory' increased with UV-B irradiation compared to non-UV-B irradiation (Fig. 4A, D).

Discussion

Suppression of black spot disease by UV-B irradiation

UV-B irradiation has been reported to be highly effective against pathogens such as powdery mildew (Kanto et al., 2009, 2011; Kobayashi et al., 2013; Suthaparan et al., 2012; Willocquet et al., 1996) and spider mites (Murata and Osakabe, 2013; Ohtsuka and Osakabe, 2009; Sakai and Osakabe, 2010; Sakai et al., 2012). We have discovered that UV-B irradiation is also highly effective at suppressing black spot disease in roses. Black spot disease is the most serious problem not only in roses (Black et al., 1994; Dobbs, 1984), but also in apples (MacHardy, 1996), pears (Bauske and Buchholz, 1967), and other fruit crops, which are different genus from *Diplocarpon*.

Although the growth of *D. rosae* colonies on the PDA medium was affected by UV-B irradiation (Fig. 1A), UV-B irradiation only slowed the growth of colony size and did not stop colony growth (Fig. 1A).

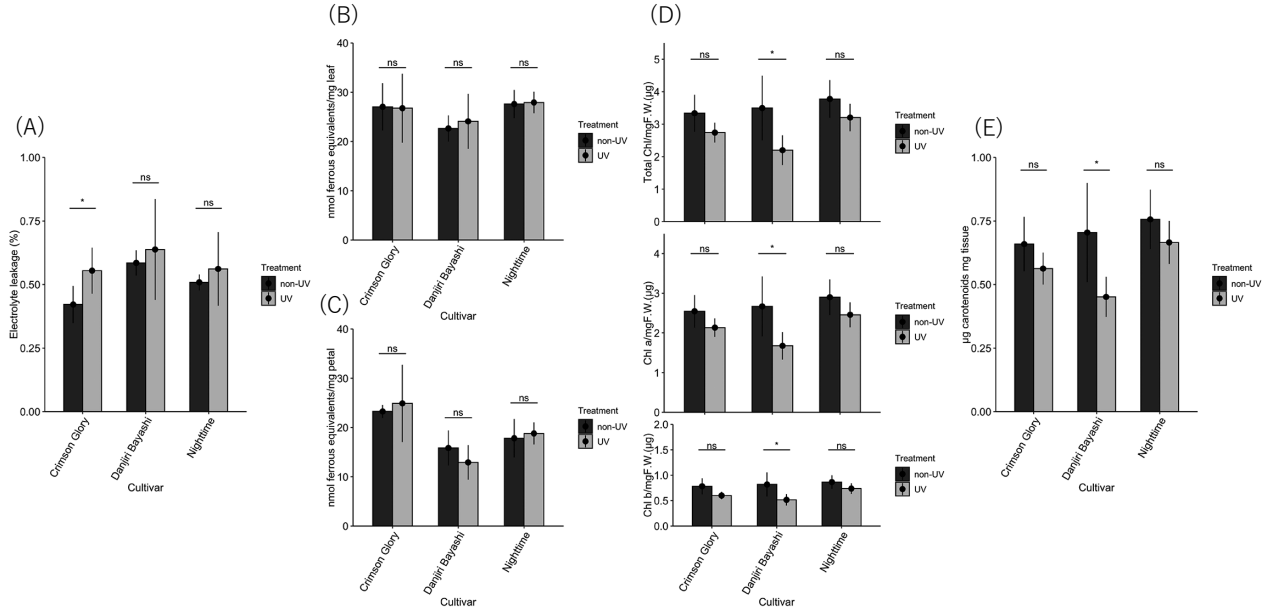

Fig. 3. Differences in physiological traits and plant damage between the non-UV-B irradiation and UV-B irradiation greenhouses. (A) Ion leakage, (B, C) FRAP assay (B: leaves, C: petals). (D) Chlorophyll contents (upper: chlorophyll a; middle: chlorophyll b; lower: chlorophyll a + chlorophyll b. (E) Carotenoid content in leaves of rose plants grown in the non-UV-B irradiation greenhouse or in UV-B irradiation greenhouse. For each cultivar, five plants from the non-UV-B irradiation and UV-B irradiation greenhouses were used. Bars represent standard deviations, while * represents significant differences according to Welch's *t*-test ($P < 0.05$). n.s. means no significant difference.

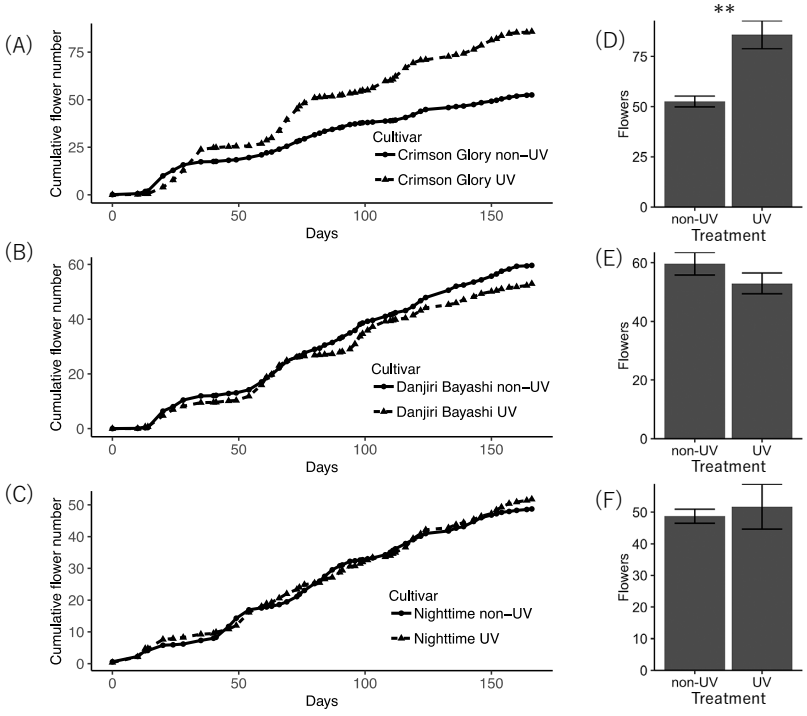

Fig. 4. A cumulative number of flowers per plant of each cultivar in the non-UV-B irradiation greenhouse and UV-B irradiation greenhouses. (A) 'Crimson Glory' (non-UV-B irradiation: n = 61; UV-B irradiation: n = 10). (B) 'Danjiri Bayashi' (non-UV-B irradiation: n = 30; UV-B irradiation: n = 42). (C) 'Nighttime' (non-UV-B irradiation: n = 53; UV-B irradiation: n = 8). Data were collected from April 14, 2020, to September 21, 2020 (157 days). (D–F) Yields of three cultivars. Bars represent standard deviations, while ** represents significant differences according to the Student's *t*-test ($P < 0.01$). (D) 'Crimson Glory' (E) 'Danjiri Bayashi' (F) 'Nighttime'.

Kobayashi et al. (2013) used a UV-B intensity of 6.5–14.0 $\mu W \cdot cm^{-2}$ and reported slight damage on some rose branches. In addition, Suthaparan et al. (2012) also irradiated roses with UV-B for 1 or 2 h with 10–20 $\mu W \cdot cm^{-2}$ to suppress powdery mildew, but damage such as smaller leaves and dwarf plants were observed.

In our colony growing experiment, the UV-B irradiation intensity was set at 15 μW·cm^{-2} with a duration time of 1 h because we considered a higher intensity and duration than these conditions could not realistically be used for rose production. We speculate that UV-B irradiation does not kill the fungus, but has an effect on the growth of *D. rosae*. Similar results were obtained when leaves were inoculated with the conidia, with the effect of UV-B appearing as a difference in the size of the lesions, although the UV-B irradiation did not eliminate the occurrence of the lesions (Fig. 1B).

On the other hand, clearer differences were observed in the field experiments between the non-UV-B irradiation and UV-B irradiation greenhouses (Fig. 2A–F). The irradiation intensity was 5 μW·cm^{-2}, which was lower than in the *in vitro* infection experiment, 15 μW·cm^{-2}. UV-B irradiation had a positive effect on suppressing black spots. There are two possible reasons for the dramatic effect of the greenhouse experiment. One is the difference in infection methods. In the *in vitro* inoculation experiment, the leaves were wounded and the conidia were directly inoculated inside the leaves. This is because, from preliminary experiments, no lesions were observed when conidia were placed on either the adaxial or abaxial side the leaf. On the other hand, under the usual cultivation conditions in the greenhouses, a slight wound occurs in the leaves before infection, and conidia carried by the wind adhere to the leaves and germinate under suitable conditions. From germination to the formation of conidia can be divided into two phases (Gachomo et al., 2006); the early phase and the late phase. In the early phase, conidia germinate and develop appressoria, subcuticular vesicles, subcuticular hyphae, and haustoria. It seems that UV-B irradiation is effective by directly affecting the conidia in the early phase. The other possibility is resistance induced in plants by long-term UV-B irradiation. UV-B irradiation has been shown to induce expression of the phenylpropanoid biosynthetic enzyme phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS) genes (Vogt, 2010), and phenylpropanoid metabolites may contribute to the suppression of powdery mildew (Kanto et al., 2011, Ota et al., 2021). Even in our experiments, it is highly possible that UV-B irradiation was involved in metabolism related to infection resistance, including the phenylpropanoid system. As a preliminary test, we inoculated black spot conidia to the leaves collected from the UV-B irradiation greenhouse and confirmed that infection of *D. rosae* was possible. Thus, it is considered that the resistance induced in the plant body alone cannot explain the dramatic effect in the field experiment. In addition to these possibilities, the possibility of a physical barrier due to the synthesis of cuticles by UV-B should be considered.

Suppression of powdery mildew by UV-B irradiation

Powdery mildew, which is one of the most economically serious plant diseases, appears as white powder on the leaves and stems of roses. In our experiment, we observed powdery mildew outbreaks in some plants in the non-UV-B irradiation greenhouse in 2020 (Fig. S2). On the other hand, we observed almost no powdery mildew symptoms in the UV-B irradiation plants. This positive effect of UV-B is consistent with that of Suthaparan et al. (2012) and Kobayashi et al. (2013). In addition, although it was thought that insufficient intensity of UV-B could reach the inside of the plant canopy due to shadows of the leaves and branches, powdery mildew could be completely suppressed in all plants. Compared with black spot disease, powdery mildew was easier to suppress under UV irradiation conditions. Of course, it is necessary to consider the characteristics of the pathogen itself, but as pointed out by Kobayashi et al. (2013), induced resistance by UV-B irradiation may be one of the causes of complete suppression.

Positive and negative effects of UV-B on plant physiology

In Tanaka et al. (2016), the UV-B irradiation intensity was 3.1 μW·cm^{-2} for strawberries, whereas in Kobayashi et al. (2013) it was 6.5–14.0 μW·cm^{-2} roses. Suthaparan et al. (2012) reported that UV-B irradiation of either 10–20 μW·cm^{-2} for 1–2 h effectively suppressed powdery mildew in roses, with some injuries. According to Kobayashi et al. (2013), 6.5–14.0 μW·cm^{-2} daytime irradiation for 4 or 6 h induced injuries in young leaves, whereas the same intensity for 2 h at night did not induce injuries. The irradiation intensity we applied in this study was lower than or equivalent to these reports, and our irradiation time was 1 h. There are reports that UV-B treatment increases (Smith et al., 2000; Suthaparan et al., 2012) and decreases (Balakrishnan et al., 2005; Zhao et al., 2003) chlorophyll content in leaves. In order to evaluate the appropriateness of UV-B intensity and duration time, it is necessary to collect data on physiological indicators, as well as visually observable disorders, of roses. The amount of electrolyte leakage indicated that there was little significant damage (Fig. 3A). In addition, the FRAP assay did not detect any leaf or petal damage (Fig. 3B, C). UV-B irradiation reduced the amount of chlorophyll and carotenoid in the leaves of 'Danjiri Bayashi', 'Nighttime', and 'Crimson Glory', and a marked decrease was observed in 'Danjiri Bayashi' (Fig. 3D, E). From these results, we suspected that UV-B irradiation damaged the photosynthetic apparatus to a certain extent, even under our irradiation conditions. Suthaparan et al. (2012) reported that UV-B increased chlorophyll content in leaves, which differs from our results. This may be because of the obvious damage to their plants, such as smaller leaves, and due to the difference in analytical methods. In a yield survey using the number of flowers as an indicator of UV-B damage to the plants, no decrease in yield due to UV-B irradia-

tion was observed in any of the cultivars (Fig. 4A–F). The number of harvested flowers increased in 'Crimson Glory' (Fig. 4A, D). Several studies have reported that UV irradiation increases yield (Darras et al., 2012; Xu et al., 2017), although the mechanisms are unknown.

UV-B lamps for IPM

This study revealed that black spot disease, which causes serious damage to roses, can be suppressed by UV-B irradiation, in addition to powdery mildew, as previously reported by Suthaparan et al. (2012) and Kobayashi et al. (2013). It is important to note that the low irradiation intensity and low energy dose caused almost no damage to the roses and prevented infection without reducing the yield. Spider mite control is also important for rose cultivation. Tanaka et al. (2016) controlled spider mites on strawberries with a UV-B irradiation intensity of 3.1 $\mu W \cdot cm^{-2}$. The irradiation intensity was the same as the intensity in this study. Since the control effect of UV-B irradiation has a direct effect on spider mites, it is possible to expect such effects from the UV-B irradiation method that we used in this study.

This time, we conducted an experiment simulating the production of edible roses in a greenhouse where various cultivars were planted. Multiple fungi, not only powdery mildew but also black spot, could be controlled by UV-B irradiation. This provides basic knowledge for safe rose production, especially edible roses, with a reduced amount of pesticides. In addition, the knowledge regarding UV-B irradiation is expected to be applied in parks and gardens where spraying pesticides is undesirable.

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Control of Black Spot Disease by Ultraviolet-B Irradiation in Rose (*Rosa* × *hybrida*) Production

Ayumu Kono[1], Ayumu Kawabata[1], Akira Yamazaki[1], Yuma Ohkubo[1], Adriano Sofo[3] and Munetaka Hosokawa[1,2]*

[1]*Faculty of Agriculture, Kindai University, Nara 631-8505, Japan*
[2]*Agricultural Technology and Innovation Research Institute, Kindai University, Nara 631-8505, Japan*
[3]*Department of European and Mediterranean Cultures, Architecture, Environment, Cultural Heritage (DiCEM), University of Basilicata, Matera 75100, Italy*

We investigated the effect of ultraviolet-B (UV-B) irradiation on the development of black spot disease caused by *Diplocarpon rosae* Wolf., which is a major problematic disease in rose (*Rosa* × *hybrida*) production. The growth of *D. rosae* colonies was suppressed on potato dextrose agar (PDA) medium under UV-B irradiation (peak wavelength: 310 nm; full width at half maximum: 30 nm) at an intensity of 15 μW·cm^{-2} with 1 h daily treatment. In addition, black spot conidia were inoculated to the rose 'Danjiri Bayashi' leaves and the effective growth suppression of black spot symptoms was observed on the leaves under UV-B irradiation. Next, various rose cultivars were planted in two greenhouses: one for supplemental UV-B irradiation treatment and one as a control without the treatment. In the UV-B irradiation greenhouse, the roses were irradiated at an intensity of 3–5 μW·cm^{-2} every day from 23:00–23:30 and 0:00–0:30 (total: 1 h). No chemical pesticides other than a starch agent for aphid control were used throughout the experiment. With the exception of the data for 'Papa Meilland' in 2019, UV-B irradiation significantly reduced the number of leaves infected with black spot disease. In September 2019, the non-UV-B irradiated 'Danjiri Bayashi' and 'Papa Meilland' had severe black spot symptoms on over 20 leaves. The number of plants with black spot symptoms increased in July 2020 compared to 2019. On the other hand, in UV-B irradiated plants, fewer black spot symptoms were observed than in non-UV-B irradiated plants. Although some visible damage was observed in the UV-B irradiated plants, the chlorophyll and carotenoid contents in the leaves decreased, indicating that UV-B irradiation had a certain negative effect on the photosynthetic apparatus. Over a five-month period, the cumulative number of flowers in the UV-B irradiation greenhouse did not decrease, and actually increased, depending on the cultivar, compared to the control treatments. Our results suggest that supplemental UV-B irradiation is effective at suppressing black spot disease in roses and can contribute to the production of pesticide-free edible rose production.

Key Words: black spot, integrated pest management, powdery mildew, rose.

Introduction

Rose (*Rosa* × *hybrida*) is one of the most popular ornamental crops in the world and is used not only as cut flowers, but also in aroma oils, cosmetics, processed foods, flavor extracts, and medicinal remedies (Cutler, 2003). Roses are also planted in parks, rose gardens, and other places where citizens can relax. The edible flowers market is expanding with a compound annual growth rate (CAGR) of 5.2% and will reach a value of USD 512 million by 2030 (https://www.marketresearchfuture.com/reports/edible-flowers-market-6634). For edible flowers and roses planted in parks that are related to human health, it is desirable to use pesticide-free cultivation methods as far as possible. As is well known, because numerous pests and diseases occur in

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* Corresponding author (E-mail: mune@nara.kindai.ac.jp).

rose cultivation, rose production relies on various chemical pesticides. Black spot disease is one of the most serious diseases in roses and is caused by the filamentous fungus *Diplocarpon rosae* (*Marssonina rosae* anamorph) (Nauta and Spooner, 2000). Black spot disease on rose leaves weakens plants through yellowing in young leaves and continuous defoliation (Black et al., 1994).

Integrated pest management (IPM), proposed by Stern et al. (1959) and promoted worldwide, is a combination of techniques that reduces the need for the application of chemical pesticides and makes cultivation safe for producers, consumers, and the environment. With the production of pest-resistant plant varieties and the development of pest management techniques such as pheromone agents and optical application technology, agriculture that does not burden either people or the environment is approaching realization. A lot of research is still required to realize effective IPM in warm and humid areas with abundant biotas, such as Japan.

The effects of ultraviolet (UV) on microorganisms studied in the laboratory and individual leaf levels have been reported (Hockberger, 2002). UV can be divided into UV-A (315–400 nm), UV-B (280–315 nm), and UV-C (200–280 nm), depending on the wavelength. As the short-wavelength UV is absorbed by the ozone layer, ground-level solar UV comprises UV-A and a small amount of UV-B. UV at shorter wavelengths has a stronger negative effect on living organisms (Slieman and Nicholson, 2000). UV-B irradiation could suppress powdery mildew caused by *Podosphaera pannosa* in roses (Kanto et al., 2009, 2011) and strawberries (*Fragaria* × *ananassa*) (Matsuura et al., 2012), and white rust in chrysanthemum (Kooriyama et al., 2014). Other than fungi, Tanaka et al. (2016) found that they could use UV-B irradiation to control spider mites in strawberries. Control of spider mites by UV irradiation is a direct biological effect of UV-B irradiation (Ohtsuka and Osakabe, 2009; Sakai and Osakabe, 2010). Probit analysis showed that spider mite mortality has been reported to increase linearly with increasing cumulative UV-B irradiation (Murata and Osakabe, 2013). Murata and Osakabe (2014, 2017a, b) and Yoshioka et al. (2018) found an effective time of day to irradiate UV-B to avoid any photoreactivation that could allow spider mites to recover from UV-B damage. In this way, UV-B irradiation is a technique that is attracting attention in IPM because it has the potential to suppress multiple parasites at the same time.

If it is possible to eradicate black spot disease, it will be possible to control the main fungal diseases in rose production, and the amount of pesticide application could be greatly reduced. Therefore, we evaluated the effect of UV-B on black spot disease inoculation in laboratory tests and investigated the effect of UV-B on the occurrence of black spot disease over two years in a greenhouse simulating edible rose production.

Materials and Methods

Direct effect of UV-B on D. rosae colony growth

A leaf with black spot symptoms that occurred in a greenhouse at the Faculty of Agriculture, Kindai University was collected and briefly sterilized with a sodium hypochlorite solution (0.5% active chlorine), and the black spots on the leaves were pierced with an injection needle. Then, the tip of the needle was applied to a potato dextrose agar (PDA) medium (Nissui, Tokyo, Japan), and the PDA medium was cultured at 25°C. DNA was extracted from a developed colony and amplified by PCR using primers (Diplocarpon_rosae-F: CGCCTTGTCTCAAACACCCTCT, Diplocarpon_rosae-R: TCCACTGGCGATGACTCTTGTC) designed according to sequence information (MVNX01000453.1) of *D. rosae* (DortE4 strain). The amplified product was sequenced and the sequence of 589 bp completely coincided with the target sequence of MVNX01000453.1 was confirmed. Therefore, this colony was attributed to *D. rosae* and used for the experiment. These fungi were subcultured to fresh PDA media by syringe needle every three weeks and kept at 25°C. Petri dishes in which the colony size grew to 2–3 mm in diameter were irradiated with UV-B every day for three weeks. As a control, Petri dishes containing similarly grown colonies were covered with a UV cut film (Toshimasen; Achilles, Tokyo, Japan) and placed under a UV-B irradiation lamp (YGRKX21799; Panasonic Co., Osaka, Japan) at 15 $\mu W \cdot cm^{-2}$. UV-B irradiation was conducted at 23:00–23:30 and 0:00–0:30. The peak wavelength of the UV-B lamp was 310 nm, and the full width at half maximum (FWHM) was 30 nm. UV-B intensity was measured using a UV-B meter (HD2302.0; Delta OHM, Padova, Italy) connected to a UV-B probe (LP471UVB; Delta OHM). Daylength of 12 h (8:00–20:00) was provided by fluorescent lamps (FHF32EX-N-H; Panasonic Co.), illuminating at three wavelengths with peaks around 437, 545, and 610 nm and at an intensity of 190 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The room temperature was set at 25°C. After three weeks, the colony size was measured and the growth ratio calculated by dividing it by the colony size at the start of the culture.

Investigation of black spot disease occurrence on inoculated leaves

Conidia collected with distilled water from the culture dishes after three weeks of culture were used in the experiment. The conidia were collected by centrifugation at 5000 rpm for 10 min, sedimented conidia were resuspended in distilled water and adjusted to 3–6 conidia·10 μL^{-1} using a hemocytometer. Fully developed leaves (each leaf composed of three leaflets) at the position of 3–5th from the youngest leaf of 'Danjiri Bayashi' grown under fluorescent light (FHF32EX-N-H; Panasonic Co.) were collected, washed briefly with

tap water, and put into plastic containers (28 × 19 × 14.5 cm) laid with a well-moistened paper towel. A drop of 10 µL of conidial suspension was put on each leaflet (3 drops were put on each leaf) via a small wound made by a needle. The containers were kept under a UV-B lamp (YGRKX21799; Panasonic Co.) at 15 µW·cm^{-2} intensity irradiated at 23:00–23:30 and 0:00–0:30. For the non-UV-B irradiation control, the containers were covered with an ultraviolet cut film and placed under a UV-B lamp.

Evaluation of rose pest development

The experiment was conducted in two adjacent greenhouses (6 × 21 m) at Kindai University (Nara, Japan). Plants were cultivated based on edible rose production. Only flowers were harvested, and the height of the plant was maintained below 1 m while cutting growing branches. Plants were maintained with 3–5 branches throughout the experiment. Fallen leaves due to black spot disease were left in the greenhouses. Forty-five benches (1055 × 992 × 750 mm) filled with 600 L of polyester medium (Neo Agriearth Co., Ltd., Nara, Japan) were placed in each greenhouse and four rose plants were planted on each bench in May 2019. As shown in Table S1, various cultivars in each greenhouse were grown. The greenhouses were covered with shading material (shading rate of 30%) in the summer (July–September), and the plants were dormant without leaves in the winter. In both greenhouses, to prevent excessive temperature rises, an insect repellent net (0.4 mm) was put on the side of each greenhouse, and fan ventilation was conducted. A liquid nutrient (HYPONeX Japan, Osaka, Japan) was diluted to 1/1850, and 2 L of the solution was applied to each plant once daily. In addition, 20 g of slow-release fertilizer (Ecolong 413 100-day type; JCAM Agri Co., Ltd., Tokyo, Japan) was applied to each plant once every 2.5 months. UV-B irradiated was applied in one greenhouse and not in the other. In the UV-B irradiation greenhouse, UV-B lamps (YGRKX21799; Panasonic Co.) were hung over the plants. The UV-B irradiation intensity was 3–5 µW·cm^{-2} just above the plants, and they were irradiated for 1 h each day, from 23:00–23:30 and 0:00–0:30. In both greenhouses, we sprayed a specified concentration of a starch agent (Nenchaku-Kun; Sumitomo Chemical, Tokyo, Japan) regularly to control aphids. The starch agent, diluted to 1/100 concentration, was sprayed over entire plants once every two to three weeks. Spraying was done throughout the year except early August to mid-September when aphids were not present due to high temperatures. No chemical pesticides other than starch were used throughout the experiments. Data were collected on September 20, 2019, and July 15, 2020, targeting all rose cultivars. The number of leaves with symptoms of black spot disease was counted for each plant. For the χ-square test, plants were divided into two groups; the number of plants with 0–9 leaves infected and the number with ≥ 10 leaves infected. In addition, to investigate the occurrence of powdery mildew under the UV-B irradiation conditions, a similar survey was also conducted for powdery mildew in October 2020.

Investigation of total antioxidant activity and electrolyte leakage of rose organs

To assess the effect of UV-B on damage or without apparent damage, an electrolyte leakage assay was carried out following Sukumaran and Weiser (1972), with some modifications. This value is greater if the organ is damaged by UV-B. For the experiment, five plants were randomly chosen for each treatment. A flower and leaf of the same age, exposition, and height from the ground were sampled from each plant. Four leaf discs were collected from each leaf (excluding the central rib) using a cork borer (0.2 cm^2) on paper towels. Immediately after cutting out the leaf disks, leaf disks from single plants were floated on 3 mL of ultrapure water in a test tube with the adaxial surface facing downward. Tubes with the lids on were left to sit for 30 min at 20°C. The water in the tube was replaced with 3 mL of fresh ultrapure water. The tubes were incubated at 20°C for 24 h in the dark without shaking. The conductivity of the solution was measured in each tube using an electrolytic conductivity meter (LAQUATWINEC33B; Horiba, Kyoto, Japan). Then, the tubes were capped to minimize evaporation, frozen at −80°C, and thawed at 20°C. After this, the conductivity of the solution was measured again. The percentage of electrolyte leakage as the ratio of the conductivity before freezing to that after freezing was calculated. The conductivity after freezing was assumed to represent complete (100%) electrolyte leakage.

Antioxidant analysis was carried out using a ferric reducing antioxidant power (FRAP) assay (Benzie and Strain, 1996), with some modifications. Leaf discs were collected in the same manner as described for the electrolyte leakage assay experiment. The discs were placed in 4 mL of extracting solution consisting of 98:2, methanol:37% HCl (37%) (v/v), following Sofo et al. (2012). The solution was let sit for 1 h at 20°C in the dark, before shaking at 100 rpm. FRAP reaction mix was prepared following Sofo et al. (2012). For all the leaf and petal samples, positive controls, and standards, the absorbance at 594 nm was measured after 60 min of incubation at 37°C. The results were expressed on a fresh weight (mg) basis.

Chlorophyll and carotenoid contents

Chlorophyll (Chl) and carotenoid (Car) contents (represented as [Chl] and [Car], respectively) of rose leaves were evaluated following the method of Lichtenthaler (1987), with slight modification. Leaf discs were collected in the same manner as described for the electrolyte leakage assay experiment. The disc area and

total weight were recorded. We dipped leaf discs in 5 mL of dimethylformamide and incubated them at 20°C for 24 h in the dark without shaking.

Chl a/b was calculated using the following equations:

$$[\text{Chl a}] = (12.00 \times \text{ABS}_{664} - 3.11 \times \text{ABS}_{647})$$
$$[\text{Chl b}] = (20.78 \times \text{ABS}_{647} - 4.88 \times \text{ABS}_{664})$$
$$[\text{Chl a + b}] = (17.67 \times \text{ABS}_{647} + 7.12 \times \text{ABS}_{664})$$

Total carotenoids (Car) were calculated using the following equation:

$$[\text{Car}] = (1000 \times \text{ABS}_{470} - 1.82 \times [\text{Chl a}] - 85.02 \times [\text{Chl b}])/198$$

The levels of both [Chl] and [Car] were expressed as μg on a fresh weight (mg) basis.

Cumulative numbers of flowers harvested

'Danjiri Bayashi' (non-UV-B irradiation: n = 30, UV-B irradiation: n = 42), 'Nighttime' (non-UV-B irradiation: n = 53, UV-B irradiation: n = 8), and 'Crimson Glory' (non-UV-B irradiation: n = 61, UV-B irradiation: n = 10) were used for data collection. From April 14, 2020, to September 21, 2020 (temperature data is shown in Fig. S1), flowers from the stalks were harvested approximately once weekly, and the number of harvested flowers was counted. After flower harvest, branches were left uncut back, and after lateral shoots developed, branch length was adjusted accordingly. The number of harvested flowers was counted per plant on each harvest day and divided by the number of plants of each cultivar. The time-course cumulative number and mean number of flowers per plant were compared between treatments.

Statistical analysis

For all statistical analyses between the two treatments (non-UV-B irradiation *vs* UV-B irradiation), Welch's *t*-test, Student's *t*-test, and the χ-square test were performed using R v.4.1.0. (R Core Team, 2021).

Results

A direct effect of UV-B on colony growth of D. rosae

The colony size (diameter) before treatment was 4.2 ± 0.9 mm (mean ± standard deviation). The sizes of *D. rosae* colonies cultured on PDA medium under temporal UV-B irradiation were smaller than those in the non-UV-B irradiation after three weeks of culture (Fig. 1A). In the leaf infection test, symptomatic lesions were observed in 30 out of 35 and 35 out of 36 inoculated portions in non-UV-B irradiation and UV-B irradiation leaves, respectively. Three weeks after inoculation, the lesion sizes (diameter) on the UV-irradiation leaves were smaller than those of the non-UV-B irradiation leaves (Fig. 1B).

Effect of UV-B irradiation on black spot disease

In 2019 and 2020, we observed black spot symptoms in the non-UV-B irradiation greenhouse. The heat map Figures for 2019 and 2020 (Fig. 2A, B) showing the severity of black spot disease in each plant revealed a clear difference between the two greenhouses. In addition, severely infected plants were found irrespective of their location in the greenhouses (Fig. 2A, B). In 2020, the incidence of black spots in both greenhouses was higher than that in 2019 (Fig. 2A–F). UV-B irradiation treatment significantly suppressed the development of rose black spot disease in both cultivars, 'Danjiri Bayashi' and 'Papa Meilland' (Fig. 2C–F). Significant differences were detected in all comparisons except 'Papa Meilland' in 2019 (Fig. 2E, F).

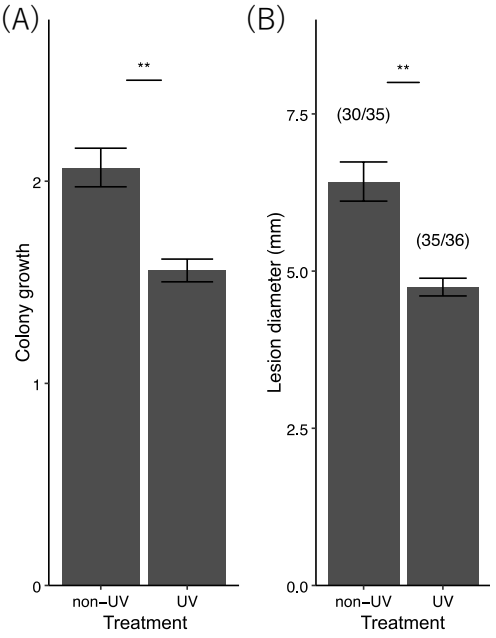

Fig. 1. Effect of UV-B irradiation on black spot colonies and symptoms. (A) Differences in colony size on PDA medium between non-UV-B irradiation and UV-B irradiation. Data are expressed as means ± standard deviation of eight colonies. Data are represented as the colony size ratio; colony diameter at three weeks after treatments/colony diameter at the start of treatment. (B) Differences in the diameter of lesions on leaves with non-UV-B irradiation and UV-B irradiation after inoculation of leaves with the *D. rosae* conidia. As shown in parentheses in the Figure, 30 of 35 and 35 of 36 inoculated portions had disease spots on the non-UV-B irradiation and UV-B irradiation leaves, respectively. Data are expressed as means ± standard deviation. In both experiments, leaves were collected three weeks after the start of UV-B irradiation treatment. ** means a significant difference at $P = 0.01$ in Welch's *t*-test.

Effect of UV-B irradiation on powdery mildew

We evaluated the severity of powdery mildew on October 2020 at which time the powdery mildew occurred severely in the UV-B irradiation greenhouse. The heat map Figures (Fig. S2) show that the outbreak of powdery mildew was suppressed completely in all cultivars in the UV-B irradiation greenhouse. Powdery mildew could be suppressed irrespective of the cultivar

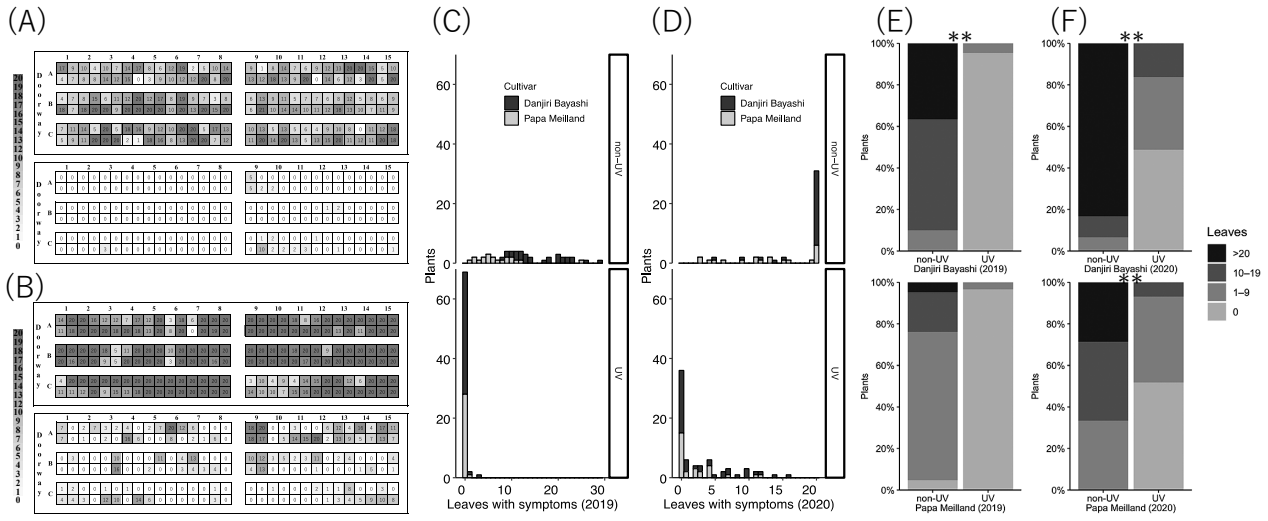

Fig. 2. Occurrence of black spot symptoms in non-UV-B irradiation and UV-B irradiation greenhouses. (A, B) Heat map indicating the distribution of rose plants at each disease level in each greenhouse. In both figures (A) and (B), the upper heatmap shows the non-UV-B irradiation greenhouse and the lower heatmap shows UV-B irradiation greenhouse. (A) Data for September 2019. (B) Data for July 2020. The biplanes were counted collectively as one leaf. Cultivation benches (1–15) planted with four plants were arranged in three rows of A–C (45 benches in total were arranged). Plants are numbered clockwise order from the upper left as 1, 2, 3, and 4. For example, plants planted on growth bench A-1 are A-1-1, A-1-2, A-1-3, and A-1-4 from the upper left. The number in the Figure with each color intensity is the number of leaves with black spot symptoms. Each cultivar's name is shown in Table S1. (C, D) Histograms of the number of leaves with black spot symptoms of 'Danjiri Bayashi' (non-UV-B irradiation: n = 30, UV-B irradiation: n = 43) and 'Papa Meilland' (non-UV-B irradiation: n = 20, UV-B irradiation: n = 29) in (C) 2019 and (D) 2020. (E, F) Occurrence of infected leaves in both 'Danjiri Bayashi' and 'Papa Meilland' treatments in 2019 (E) and 2020 (F). The upper panel is 'Danjiri Bayashi' and the lower is 'Papa Meilland'. For statistical analysis, plants were divided into two groups—the number of plants with 0–9 leaves infected and the number with ≥ 10 leaves infected—and the χ-square test was performed. The symbol indicates statistical difference (**: $P < 0.01$).

(Fig. S2). Severely infected plants were found irrespective of their location in the greenhouse (Fig. S2).

Organ damage due to UV-B irradiation

Branches growing close to the UV-B lamp rarely exhibited damage, such as leaf curling, but the UV-B irradiation did not induce any other outstanding damage as reported by Kobayashi et al. (2013). In both cultivars, 'Danjiri Bayashi' and 'Nighttime', no significant differences were detected in electrolyte leakage from leaves between the samples from the non-UV-B and the UV-B irradiation greenhouses (Fig. 3A). On the other hand, in 'Crimson Glory', there was a significant increase in electrolyte leakage from leaves with UV-B irradiation ($P < 0.05$) (Fig. 3A). A leaf and petal FRAP assay revealed no difference in the leaves between the non-UV-B and UV-B irradiation greenhouses in the three cultivars (Fig. 3B, C).

UV-B irradiation reduced the chlorophyll a and b contents in the leaves of 'Danjiri Bayashi', 'Nighttime', and 'Crimson Glory' (Fig. 3D). This decrease was particularly significant in 'Danjiri Bayashi' ($P < 0.05$). UV-B irradiation also reduced the carotenoid content in all three cultivars and was statistically significant ($P < 0.05$) in 'Danjiri Bayashi' (Fig. 3E). Visible damage caused by UV-B irradiation was not observed in flowers, and no visible difference in flower size was observed. From April 14th to September 21, 2020, there was a slight reduction in the cumulative number of flowers of 'Danjiri Bayashi' per plant under UV-B irradiation (Fig. 4B, E). The cumulative flower numbers were almost the same between the non-UV-B and UV-B irradiation treatments for 'Nighttime' (Fig. 4C, F), whereas that of 'Crimson Glory' increased with UV-B irradiation compared to non-UV-B irradiation (Fig. 4A, D).

Discussion

Suppression of black spot disease by UV-B irradiation

UV-B irradiation has been reported to be highly effective against pathogens such as powdery mildew (Kanto et al., 2009, 2011; Kobayashi et al., 2013; Suthaparan et al., 2012; Willocquet et al., 1996) and spider mites (Murata and Osakabe, 2013; Ohtsuka and Osakabe, 2009; Sakai and Osakabe, 2010; Sakai et al., 2012). We have discovered that UV-B irradiation is also highly effective at suppressing black spot disease in roses. Black spot disease is the most serious problem not only in roses (Black et al., 1994; Dobbs, 1984), but also in apples (MacHardy, 1996), pears (Bauske and Buchholz, 1967), and other fruit crops, which are different genus from *Diplocarpon*.

Although the growth of *D. rosae* colonies on the PDA medium was affected by UV-B irradiation (Fig. 1A), UV-B irradiation only slowed the growth of colony size and did not stop colony growth (Fig. 1A).

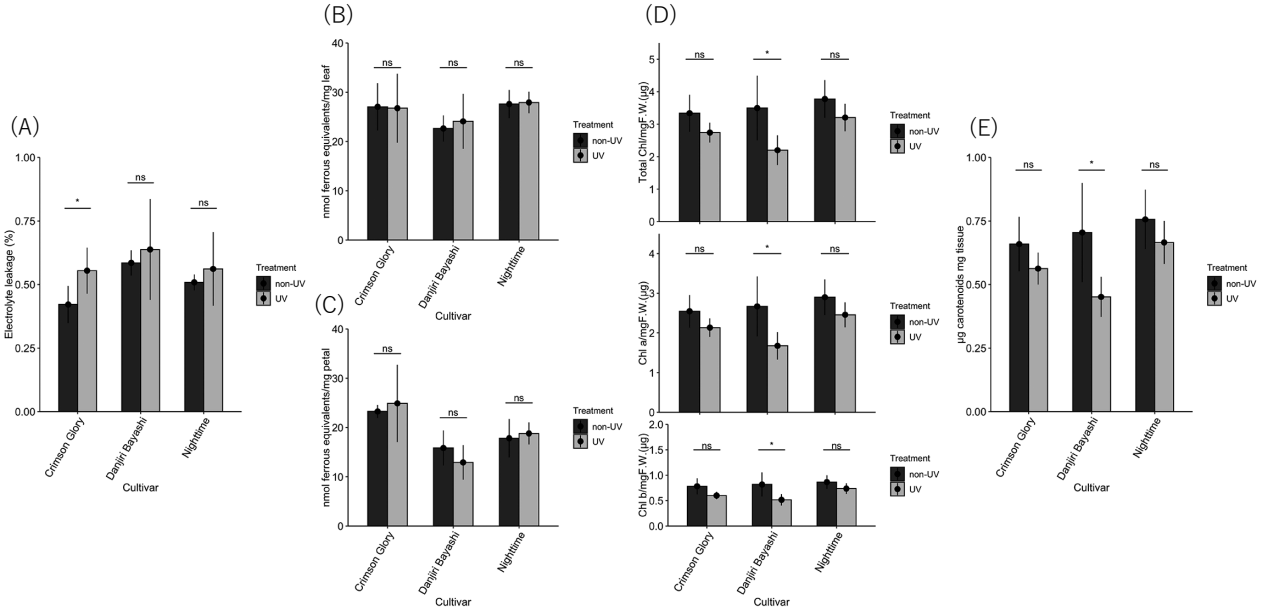

Fig. 3. Differences in physiological traits and plant damage between the non-UV-B irradiation and UV-B irradiation greenhouses. (A) Ion leakage, (B, C) FRAP assay (B: leaves, C: petals). (D) Chlorophyll contents (upper: chlorophyll a; middle: chlorophyll b; lower: chlorophyll a + chlorophyll b. (E) Carotenoid content in leaves of rose plants grown in the non-UV-B irradiation greenhouse or in UV-B irradiation greenhouse. For each cultivar, five plants from the non-UV-B irradiation and UV-B irradiation greenhouses were used. Bars represent standard deviations, while * represents significant differences according to Welch's *t*-test ($P < 0.05$). n.s. means no significant difference.

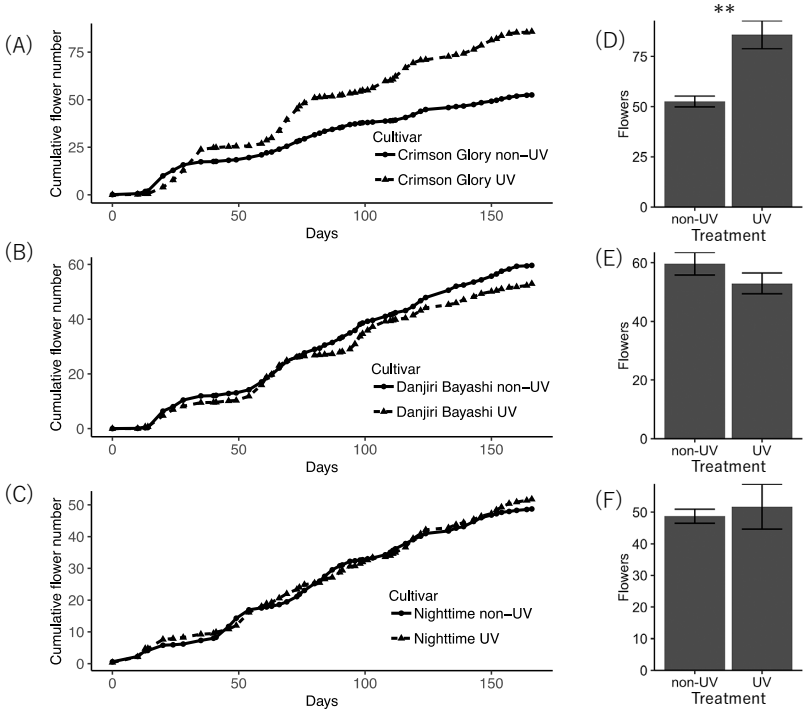

Fig. 4. A cumulative number of flowers per plant of each cultivar in the non-UV-B irradiation greenhouse and UV-B irradiation greenhouses. (A) 'Crimson Glory' (non-UV-B irradiation: n = 61; UV-B irradiation: n = 10). (B) 'Danjiri Bayashi' (non-UV-B irradiation: n = 30; UV-B irradiation: n = 42). (C) 'Nighttime' (non-UV-B irradiation: n = 53; UV-B irradiation: n = 8). Data were collected from April 14, 2020, to September 21, 2020 (157 days). (D–F) Yields of three cultivars. Bars represent standard deviations, while ** represents significant differences according to the Student's *t*-test ($P < 0.01$). (D) 'Crimson Glory' (E) 'Danjiri Bayashi' (F) 'Nighttime'.

Kobayashi et al. (2013) used a UV-B intensity of 6.5–14.0 $\mu W \cdot cm^{-2}$ and reported slight damage on some rose branches. In addition, Suthaparan et al. (2012) also irradiated roses with UV-B for 1 or 2 h with 10–20 $\mu W \cdot cm^{-2}$ to suppress powdery mildew, but damage such as smaller leaves and dwarf plants were observed.

In our colony growing experiment, the UV-B irradiation intensity was set at 15 μW·cm^{-2} with a duration time of 1 h because we considered a higher intensity and duration than these conditions could not realistically be used for rose production. We speculate that UV-B irradiation does not kill the fungus, but has an effect on the growth of *D. rosae*. Similar results were obtained when leaves were inoculated with the conidia, with the effect of UV-B appearing as a difference in the size of the lesions, although the UV-B irradiation did not eliminate the occurrence of the lesions (Fig. 1B).

On the other hand, clearer differences were observed in the field experiments between the non-UV-B irradiation and UV-B irradiation greenhouses (Fig. 2A–F). The irradiation intensity was 5 μW·cm^{-2}, which was lower than in the *in vitro* infection experiment, 15 μW·cm^{-2}. UV-B irradiation had a positive effect on suppressing black spots. There are two possible reasons for the dramatic effect of the greenhouse experiment. One is the difference in infection methods. In the *in vitro* inoculation experiment, the leaves were wounded and the conidia were directly inoculated inside the leaves. This is because, from preliminary experiments, no lesions were observed when conidia were placed on either the adaxial or abaxial side the leaf. On the other hand, under the usual cultivation conditions in the greenhouses, a slight wound occurs in the leaves before infection, and conidia carried by the wind adhere to the leaves and germinate under suitable conditions. From germination to the formation of conidia can be divided into two phases (Gachomo et al., 2006); the early phase and the late phase. In the early phase, conidia germinate and develop appressoria, subcuticular vesicles, subcuticular hyphae, and haustoria. It seems that UV-B irradiation is effective by directly affecting the conidia in the early phase. The other possibility is resistance induced in plants by long-term UV-B irradiation. UV-B irradiation has been shown to induce expression of the phenylpropanoid biosynthetic enzyme phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS) genes (Vogt, 2010), and phenylpropanoid metabolites may contribute to the suppression of powdery mildew (Kanto et al., 2011, Ota et al., 2021). Even in our experiments, it is highly possible that UV-B irradiation was involved in metabolism related to infection resistance, including the phenylpropanoid system. As a preliminary test, we inoculated black spot conidia to the leaves collected from the UV-B irradiation greenhouse and confirmed that infection of *D. rosae* was possible. Thus, it is considered that the resistance induced in the plant body alone cannot explain the dramatic effect in the field experiment. In addition to these possibilities, the possibility of a physical barrier due to the synthesis of cuticles by UV-B should be considered.

Suppression of powdery mildew by UV-B irradiation

Powdery mildew, which is one of the most economically serious plant diseases, appears as white powder on the leaves and stems of roses. In our experiment, we observed powdery mildew outbreaks in some plants in the non-UV-B irradiation greenhouse in 2020 (Fig. S2). On the other hand, we observed almost no powdery mildew symptoms in the UV-B irradiation plants. This positive effect of UV-B is consistent with that of Suthaparan et al. (2012) and Kobayashi et al. (2013). In addition, although it was thought that insufficient intensity of UV-B could reach the inside of the plant canopy due to shadows of the leaves and branches, powdery mildew could be completely suppressed in all plants. Compared with black spot disease, powdery mildew was easier to suppress under UV irradiation conditions. Of course, it is necessary to consider the characteristics of the pathogen itself, but as pointed out by Kobayashi et al. (2013), induced resistance by UV-B irradiation may be one of the causes of complete suppression.

Positive and negative effects of UV-B on plant physiology

In Tanaka et al. (2016), the UV-B irradiation intensity was 3.1 μW·cm^{-2} for strawberries, whereas in Kobayashi et al. (2013) it was 6.5–14.0 μW·cm^{-2} roses. Suthaparan et al. (2012) reported that UV-B irradiation of either 10–20 μW·cm^{-2} for 1–2 h effectively suppressed powdery mildew in roses, with some injuries. According to Kobayashi et al. (2013), 6.5–14.0 μW·cm^{-2} daytime irradiation for 4 or 6 h induced injuries in young leaves, whereas the same intensity for 2 h at night did not induce injuries. The irradiation intensity we applied in this study was lower than or equivalent to these reports, and our irradiation time was 1 h. There are reports that UV-B treatment increases (Smith et al., 2000; Suthaparan et al., 2012) and decreases (Balakrishnan et al., 2005; Zhao et al., 2003) chlorophyll content in leaves. In order to evaluate the appropriateness of UV-B intensity and duration time, it is necessary to collect data on physiological indicators, as well as visually observable disorders, of roses. The amount of electrolyte leakage indicated that there was little significant damage (Fig. 3A). In addition, the FRAP assay did not detect any leaf or petal damage (Fig. 3B, C). UV-B irradiation reduced the amount of chlorophyll and carotenoid in the leaves of 'Danjiri Bayashi', 'Nighttime', and 'Crimson Glory', and a marked decrease was observed in 'Danjiri Bayashi' (Fig. 3D, E). From these results, we suspected that UV-B irradiation damaged the photosynthetic apparatus to a certain extent, even under our irradiation conditions. Suthaparan et al. (2012) reported that UV-B increased chlorophyll content in leaves, which differs from our results. This may be because of the obvious damage to their plants, such as smaller leaves, and due to the difference in analytical methods. In a yield survey using the number of flowers as an indicator of UV-B damage to the plants, no decrease in yield due to UV-B irradia-

tion was observed in any of the cultivars (Fig. 4A–F). The number of harvested flowers increased in 'Crimson Glory' (Fig. 4A, D). Several studies have reported that UV irradiation increases yield (Darras et al., 2012; Xu et al., 2017), although the mechanisms are unknown.

UV-B lamps for IPM

This study revealed that black spot disease, which causes serious damage to roses, can be suppressed by UV-B irradiation, in addition to powdery mildew, as previously reported by Suthaparan et al. (2012) and Kobayashi et al. (2013). It is important to note that the low irradiation intensity and low energy dose caused almost no damage to the roses and prevented infection without reducing the yield. Spider mite control is also important for rose cultivation. Tanaka et al. (2016) controlled spider mites on strawberries with a UV-B irradiation intensity of 3.1 $\mu W \cdot cm^{-2}$. The irradiation intensity was the same as the intensity in this study. Since the control effect of UV-B irradiation has a direct effect on spider mites, it is possible to expect such effects from the UV-B irradiation method that we used in this study.

This time, we conducted an experiment simulating the production of edible roses in a greenhouse where various cultivars were planted. Multiple fungi, not only powdery mildew but also black spot, could be controlled by UV-B irradiation. This provides basic knowledge for safe rose production, especially edible roses, with a reduced amount of pesticides. In addition, the knowledge regarding UV-B irradiation is expected to be applied in parks and gardens where spraying pesticides is undesirable.

Acknowledgements

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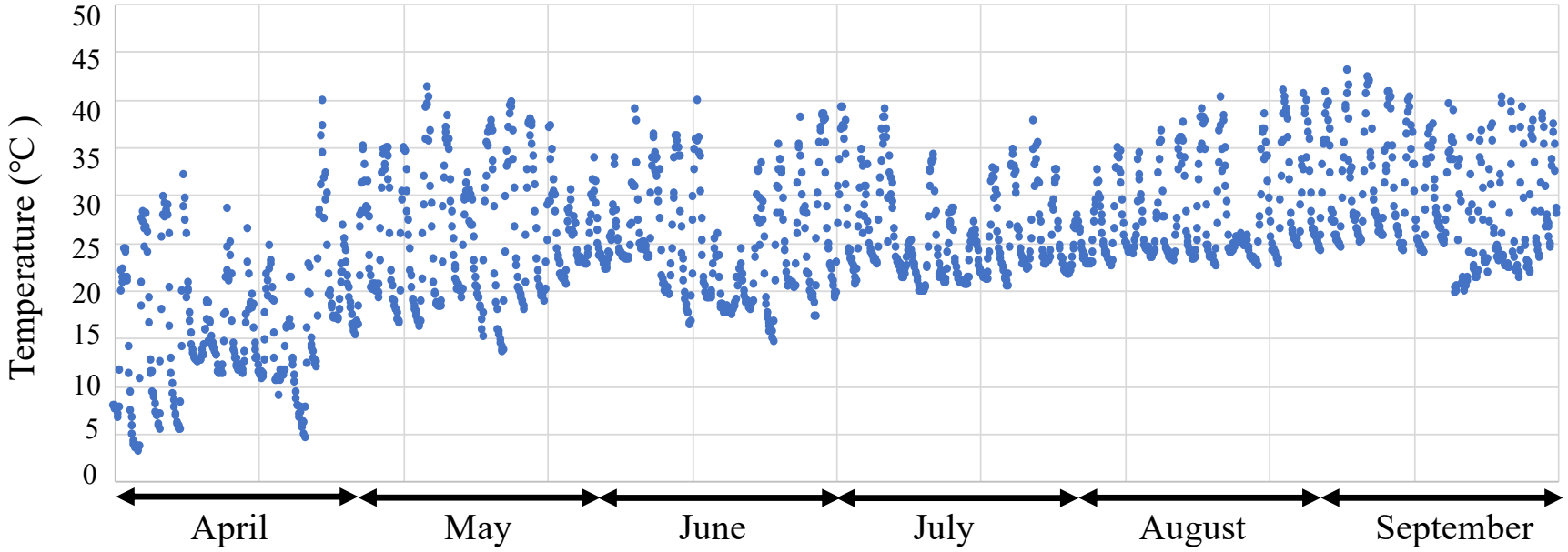

Fig. S1 Temperatures in the greenhouses (35° N, 136° E) in the period April–September 2020.

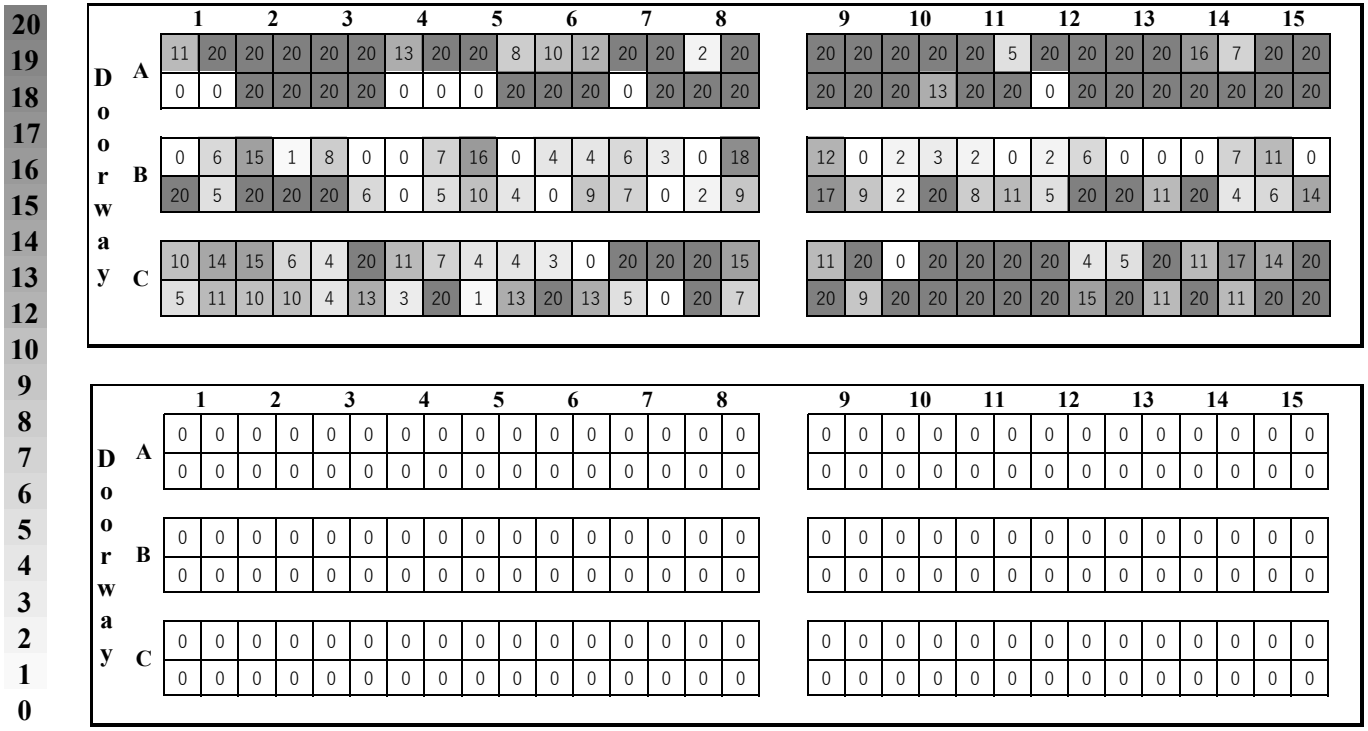

Fig. S2 Occurrence of powdery mildew symptoms in non-UV-B irradiation greenhouse and in UV-B irradiation greenhouse. Heat map showing the distribution of rose plants at each disease level in each greenhouse. The upper heatmap shows non-irradiation and the lower heatmap shows UV-B irradiation. Data for October 2020. The biplanes were counted collectively as one leaf. Cultivation benches (1–15) planted with four plants were arranged in three rows of A–C (45 benches in total were arranged at each greenhouse). Plants are numbered clockwise order from the upper left as 1, 2, 3, and 4. For example, plants planted on the growth bench A-1 are A-1-1, A-1-2, A-1-3, and A-1-4 from the upper left. The number in the figure with each color intensity is the number of leaves with powdery mildew symptoms. Each cultivar's name is shown in Table S1.

Table S1 The list of rose cultivars used for the experiments

UV-B non-irradiation greenhouse						UV-B irradiation greenhouse					
Position	Cultivar	Position	Cultivar	Position	Cultivar	Position	Cultivar	Position	Cultivar	Position	Cultivar
A-1-1	Crimson Glory	B-1-1	Ophelia	C-1-1	Samurai	A-1-1	Danjiri Bayashi	B-1-1	Goethe Rose	C-1-1	Papa Meilland
A-1-2	The Poet's Wife	B-1-2	Crimson Glory	C-1-2	Nighttime	A-1-2	Just Joey	B-1-2	Goethe Rose	C-1-2	Danjiri Bayashi
A-1-3	Yves Piaget	B-1-3	Crimson Glory	C-1-3	Nighttime	A-1-3	Danjiri Bayashi	B-1-3	Danjiri Bayashi	C-1-3	Papa Meilland
A-1-4	Nighttime	B-1-4	Nighttime	C-1-4	Papa Meilland	A-1-4	Just Joey	B-1-4	Goethe Rose	C-1-4	Papa Meilland
A-2-1	Nighttime	B-2-1	Crimson Glory	C-2-1	Nighttime	A-2-1	Just Joey	B-2-1	Goethe Rose	C-2-1	Papa Meilland
A-2-2	Papa Meilland	B-2-2	Crimson Glory	C-2-2	Crimson Glory	A-2-2	Papa Meilland	B-2-2	Crimson Glory	C-2-2	Papa Meilland
A-2-3	Crimson Glory	B-2-3	Nighttime	C-2-3	Papa Meilland	A-2-3	Danjiri Bayashi	B-2-3	Papa Meilland	C-2-3	Papa Meilland
A-2-4	Nighttime	B-2-4	Papa Meilland	C-2-4	Nighttime	A-2-4	Papa Meilland	B-2-4	Crimson Glory	C-2-4	Papa Meilland
A-3-1	Nighttime	B-3-1	Crimson Glory	C-3-1	Danjiri Bayashi	A-3-1	Papa Meilland	B-3-1	Papa Meilland	C-3-1	Danjiri Bayashi
A-3-2	Crimson Glory	B-3-2	Danjiri Bayashi	C-3-2	Crimson Glory	A-3-2	Papa Meilland	B-3-2	Danjiri Bayashi	C-3-2	Danjiri Bayashi
A-3-3	Charlotte Austin	B-3-3	Nighttime	C-3-3	Nighttime	A-3-3	Papa Meilland	B-3-3	Goethe Rose	C-3-3	Danjiri Bayashi
A-3-4	Crimson Glory	B-3-4	Papa Meilland	C-3-4	Nighttime	A-3-4	Papa Meilland	B-3-4	Crimson Glory	C-3-4	Danjiri Bayashi
A-4-1	Nighttime	B-4-1	Nighttime	C-4-1	Danjiri Bayashi	A-4-1	Danjiri Bayashi	B-4-1	Goethe Rose	C-4-1	Danjiri Bayashi
A-4-2	Crimson Glory	B-4-2	Nighttime	C-4-2	Crimson Glory	A-4-2	Papa Meilland	B-4-2	Papa Meilland	C-4-2	Danjiri Bayashi
A-4-3	Jubilee Celebration	B-4-3	Crimson Glory	C-4-3	Papa Meilland	A-4-3	Danjiri Bayashi	B-4-3	Goethe Rose	C-4-3	Papa Meilland
A-4-4	Samurai	B-4-4	Danjiri Bayashi	C-4-4	Nighttime	A-4-4	Crimson Glory	B-4-4	Goethe Rose	C-4-4	Danjiri Bayashi
A-5-1	Crimson Glory	B-5-1	Nighttime	C-5-1	Crimson Glory	A-5-1	Papa Meilland	B-5-1	Papa Meilland	C-5-1	Danjiri Bayashi
A-5-2	Crimson Glory	B-5-2	Nighttime	C-5-2	Danjiri Bayashi	A-5-2	Double Delight	B-5-2	Crimson Glory	C-5-2	Danjiri Bayashi
A-5-3	Just Joey	B-5-3	Crimson Glory	C-5-3	Nighttime	A-5-3	Goethe Rose	B-5-3	Crimson Glory	C-5-3	Rocse
A-5-4	Nighttime	B-5-4	Danjiri Bayashi	C-5-4	Nighttime	A-5-4	Just Joey	B-5-4	Crimson Glory	C-5-4	Danjiri Bayashi
A-6-1	Papa Meilland	B-6-1	Nighttime	C-6-1	Danjiri Bayashi	A-6-1	Double Delight	B-6-1	Double Delight	C-6-1	Danjiri Bayashi
A-6-2	Nighttime	B-6-2	Crimson Glory	C-6-2	Crimson Glory	A-6-2	Double Delight	B-6-2	Double Delight	C-6-2	Danjiri Bayashi
A-6-3	Marco Polo	B-6-3	Fragrant Apricot	C-6-3	Nighttime	A-6-3	Double Delight	B-6-3	Nighttime	C-6-3	Danjiri Bayashi
A-6-4	Crimson Glory	B-6-4	Danjiri Bayashi	C-6-4	Nighttime	A-6-4	Bathsheba	B-6-4	Marco Polo	C-6-4	Danjiri Bayashi
A-7-1	Papa Meilland	B-7-1	Crimson Glory	C-7-1	Nighttime	A-7-1	Fragrant Apricot	B-7-1	Fragrant Apricot	C-7-1	Danjiri Bayashi
A-7-2	Nighttime	B-7-2	Crimson Glory	C-7-2	Papa Meilland	A-7-2	Fragrant Apricot	B-7-2	Fragrant Apricot	C-7-2	Danjiri Bayashi
A-7-3	Goethe Rose	B-7-3	Nighttime	C-7-3	Danjiri Bayashi	A-7-3	Yves Piaget	B-7-3	Fragrant Apricot	C-7-3	Danjiri Bayashi
A-7-4	Crimson Glory	B-7-4	Danjiri Bayashi	C-7-4	Crimson Glory	A-7-4	Fragrant Apricot	B-7-4	Fragrant Apricot	C-7-4	Danjiri Bayashi
A-8-1	Princess Alexandra of Kent	B-8-1	The Poet's Wife	C-8-1	Danjiri Bayashi	A-8-1	Papa Meilland	B-8-1	Danjiri Bayashi	C-8-1	Danjiri Bayashi
A-8-2	Nighttime	B-8-2	Crimson Glory	C-8-2	Crimson Glory	A-8-2	Papa Meilland	B-8-2	Danjiri Bayashi	C-8-2	Danjiri Bayashi
A-8-3	Papa Meilland	B-8-3	Nighttime	C-8-3	Nighttime	A-8-3	Danjiri Bayashi	B-8-3	Danjiri Bayashi	C-8-3	Danjiri Bayashi
A-8-4	Crimson Glory	B-8-4	Danjiri Bayashi	C-8-4	Nighttime	A-8-4	Papa Meilland	B-8-4	Papa Meilland	C-8-4	Danjiri Bayashi
A-9-1	Nighttime	B-9-1	Crimson Glory	C-9-1	Papa Meilland	A-9-1	Haikara	B-9-1	Crimson Glory	C-9-1	Graefin Diana
A-9-2	Papa Meilland	B-9-2	Nighttime	C-9-2	Nighttime	A-9-2	Crimson Glory	B-9-2	Graefin Diana	C-9-2	Haikara
A-9-3	Crimson Glory	B-9-3	Crimson Glory	C-9-3	Danjiri Bayashi	A-9-3	Velvet Fragrance	B-9-3	Haikara	C-9-3	Graefin Diana
A-9-4	Nighttime	B-9-4	Danjiri Bayashi	C-9-4	Crimson Glory	A-9-4	Haikara	B-9-4	Velvet Fragrance	C-9-4	Velvet Fragrance
A-10-1	Nighttime	B-10-1	Crimson Glory	C-10-1	Anne-sophie PIC	A-10-1	Goethe Rose	B-10-1	Nighttime	C-10-1	Ophelia
A-10-2	Crimson Glory	B-10-2	Danjiri Bayashi	C-10-2	Danjiri Bayashi	A-10-2	Goethe Rose	B-10-2	Just Joey	C-10-2	Ophelia
A-10-3	Nighttime	B-10-3	Papa Meilland	C-10-3	Nighttime	A-10-3	Bathsheba	B-10-3	Ophelia	C-10-3	Just Joey
A-10-4	Danjiri Bayashi	B-10-4	Nighttime	C-10-4	Crimson Glory	A-10-4	Goethe Rose	B-10-4	Danjiri Bayashi	C-10-4	Ophelia
A-11-1	Crimson Glory	B-11-1	Crimson Glory	C-11-1	Papa Meilland	A-11-1	Goethe Rose	B-11-1	Just Joey	C-11-1	Danjiri Bayashi
A-11-2	Crimson Glory	B-11-2	Papa Meilland	C-11-2	Nighttime	A-11-2	Goethe Rose	B-11-2	Goethe Rose	C-11-2	Just Joey
A-11-3	Nighttime	B-11-3	Danjiri Bayashi	C-11-3	Danjiri Bayashi	A-11-3	Goethe Rose	B-11-3	Just Joey	C-11-3	Nighttime
A-11-4	Danjiri Bayashi	B-11-4	Nighttime	C-11-4	Crimson Glory	A-11-4	Goethe Rose	B-11-4	Goethe Rose	C-11-4	Just Joey
A-12-1	Crimson Glory	B-12-1	Crimson Glory	C-12-1	Papa Meilland	A-12-1	Papa Meilland	B-12-1	Danjiri Bayashi	C-12-1	Munstead Wood
A-12-2	Crimson Glory	B-12-2	Papa Meilland	C-12-2	Danjiri Bayashi	A-12-2	Bathsheba	B-12-2	Nighttime	C-12-2	Munstead Wood
A-12-3	Crimson Glory	B-12-3	Nighttime	C-12-3	Nighttime	A-12-3	The Poet's Wife	B-12-3	Just Joey	C-12-3	Munstead Wood
A-12-4	Danjiri Bayashi	B-12-4	Danjiri Bayashi	C-12-4	Crimson Glory	A-12-4	Boscobel	B-12-4	Princess Alexandra of Kent	C-12-4	Munstead Wood
A-13-1	Crimson Glory	B-13-1	Crimson Glory	C-13-1	Double Delight	A-13-1	Papa Meilland	B-13-1	Nighttime	C-13-1	Jubilee Celebration
A-13-2	Crimson Glory	B-13-2	Crimson Glory	C-13-2	Danjiri Bayashi	A-13-2	Papa Meilland	B-13-2	Scepter'd Isle	C-13-2	Samurai
A-13-3	Papa Meilland	B-13-3	Danjiri Bayashi	C-13-3	Nighttime	A-13-3	Rose Pompadour	B-13-3	Just Joey	C-13-3	Jubilee Celebration
A-13-4	Danjiri Bayashi	B-13-4	Nighttime	C-13-4	Crimson Glory	A-13-4	Scepter'd Isle	B-13-4	Abracadabura	C-13-4	Jubilee Celebration
A-14-1	Crimson Glory	B-14-1	Munstead Wood	C-14-1	Papa Meilland	A-14-1	Guy Savoy	B-14-1	The Poet's Wife	C-14-1	Danjiri Bayashi
A-14-2	Crimson Glory	B-14-2	Crimson Glory	C-14-2	Crimson Glory	A-14-2	Papa Meilland	B-14-2	Charlotte Austin	C-14-2	Goethe Rose
A-14-3	Papa Meilland	B-14-3	Danjiri Bayashi	C-14-3	Nighttime	A-14-3	Desdemona	B-14-3	Papa Meilland	C-14-3	Danjiri Bayashi
A-14-4	Danjiri Bayashi	B-14-4	Nighttime	C-14-4	Crimson Glory	A-14-4	Desdemona	B-14-4	Marie Henriette	C-14-4	Jude The Obscure
A-15-1	Crimson Glory	B-15-1	Crimson Glory	C-15-1	Nighttime	A-15-1	Crimson Glory	B-15-1	Rouge Pierre de Ronsard	C-15-1	Danjiri Bayashi
A-15-2	Crimson Glory	B-15-2	Crimson Glory	C-15-2	Crimson Glory	A-15-2	Charlotte Austin	B-15-2	Madame de Stael	C-15-2	The Poet's Wife
A-15-3	Papa Meilland	B-15-3	Nighttime	C-15-3	Nighttime	A-15-3	Charlotte Austin	B-15-3	Pierre de Ronsard	C-15-3	Danjiri Bayashi
A-15-4	Danjiri Bayashi	B-15-4	Danjiri Bayashi	C-15-4	Crimson Glory	A-15-4	Charlotte Austin	B-15-4	Paul Bocuse	C-15-4	Danjiri Bayashi

In each house, cultivation benches (1-15) planted with 4 plants were arranged in 3 rows of A–C (45 benches in total were arranged) as shown in Fig. 1 and Fig. S1. Plants are numbered clockwise order from the upper left as 1, 2, 3 and 4.