Early Development of Powdery Mildew on Cucumber Leaves Acclimatized to Illumination with Different Red-to-far-red Ratios

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Abstract. The development of powdery mildew fungus (*Podosphaera xanthii*) is suppressed on cucumber (*Cucumis sativus* L.) seedlings acclimatized to higher red-to-far-red ratio (R:FR) than natural R:FR (≈ 1.2), but its early development and any limiting factors are still unclear. The present study evaluated conidial germination, initial invasion, and subsequent development of *P. xanthii* on cucumber seedlings raised under light-emitting diode (LED) lights with R:FRs of 1.2, 5.0, or 10. There were no differences in conidial germination or initial invasion between the treatments, so there was no effect of acclimatization to R:FR on either. But, the development of hyphae, hyphal cells, and haustoria after inoculation were suppressed on seedlings acclimatized to higher R:FR. Because differences occurred only after the initial invasion, nonstructural properties of the host leaves may have affected conidial development. Higher R:FR also suppressed conidial development under natural light filtered through a photo-selective film, which absorbs near-infrared (NIR)-light. However, this effect was reduced when the plants were moved to natural R:FR after inoculation, possibly because of reacclimatization of the seedlings.

Host-plant resistance to foliar diseases can be increased by artificial lights with particular wavelengths (Chen et al., 2015; Schuerger and Brown, 1997; Wang et al., 2010). The authors reported that the development of visible colonies of powdery mildew fungus (*P. xanthii*) was suppressed on cucumber (*C. sativus*) seedlings acclimatized to higher R:FR (>7.0) than that of natural light (R:FR ≈ 1.2) (Shibuya et al., 2011). Structural properties of the cucumber leaves caused by acclimatization to higher R:FR, such as thickened epidermis and increased leaf mass per area (LMA), may have inhibited the invasion of *P. xanthii* into epidermal cells. However, the early development of *P. xanthii*, which is important for estimating the factors that

limit it, on leaves acclimatized to higher R:FR is still unclear.

The early developmental stage of a *P. xanthii* colony can be divided into three processes: conidial germination, initial invasion, and subsequent development. The factors that limit each process differ: conidial germination is controlled by the microclimatic conditions of the host-leaf surface (Aust and Hoyningen-Huene, 1986); initial invasion by the structural properties of the host leaf, which resist penetration by the pathogen (Aust and Hoyningen-Huene, 1986); and subsequent development by the nonstructural properties [such as levels of salicylic acid (SA) and gene expression related to defense against pathogen attack] of the host leaf, which resist successive penetration and nutrient absorption by the pathogen (Aust and Hoyningen-Huene, 1986; Bélanger et al., 2002; Pérez-García et al., 2009). The present study evaluated the early development of *P. xanthii* on cucumber seedlings acclimatized to different R:FRs provided by LEDs, and estimated the relationships between colony development and leaf properties. In addition, the present study also investigated the practical application of higher R:FR in a greenhouse in which the R:FR was increased by an NIR-absorbing photo-selective film (NIR-absorbing film) which has been used to control the growth and development of horticultural crops (Rajapakse and Shahak,

2007; Rajapakse et al., 1999; Runkle and Heins, 2002).

Materials and Methods

Expt. 1: Evaluation of early conidial development of *P. xanthii* on cucumber seedlings grown under LEDs with different R:FRs, and evaluation of leaf properties

Preparation of plant materials. Cucumber ('Hokushin') seeds were sown individually in vermiculite in plastic cell trays (300 mm length $\times$ 240 mm width $\times$ 50 mm depth) partitioned into 80 square cells and germinated in growth chambers (LPH-220SP; Nippon Medical and Chemical Instruments Co., Ltd., Osaka, Japan) with illumination of R:FR = 1.2, 5.0, or 10 provided by LED lamps (ISL-305X302-RFGB; CCS Inc., Kyoto, Japan). The R:FR was determined as the ratio of photon flux density between 600 and 700 nm (R) and 700 and 800 nm (FR). The R:FR = 1.2, 5.0, or 10 are similar to those of natural light, natural light transmitted through NIR-absorbing film (which is used in the present study), and light from typical commercial fluorescent lamps, respectively. The spectrum of each treatment is shown in Fig. 1. The growth chambers were maintained at an air temperature of 28 °C, a relative humidity (RH) of 50%, and a photosynthetic photon flux density (PPFD) of 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ under a light:dark cycle of 16:8 h. To obtain seedlings of about the same developmental stage and leaf area ($\approx 17\text{ cm}^2$ per plant) for the inoculation tests, the seeds for the R:FR 1.2 treatment were sown 1 d after those for the R:FR 5.0 and 10 treatments. The cell trays were placed in a nutrient solution (A-type recipe of OAT Solution; OAT Agrio Co. Ltd., Tokyo, Japan) 5 to 10 mm deep.

Inoculation with *P. xanthii* and subsequent growth conditions. Inoculations with *P. xanthii* were conducted by dropping or spraying inoculum of the pathogen on leaf surface. Inoculum of *P. xanthii* was prepared by gently transferring the conidia on cucumber leaves to distilled water using a brush, and was inoculated within 1 h. The conidia of *P. xanthii* had been maintained on cucumber leaves, which were placed in a growth chamber (LPH-220SP) at an air temperature of 28 °C, an RH of 50%, and a PPFD of 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ provided by fluorescent lamps (FL20SEX-N-HG; NEC Lighting Ltd., Tokyo, Japan; R:FR = 10) under a light:dark cycle of 16:8 h. The inoculum density was calculated as the mean number of conidia per 10 μL under an optical microscope at $\times 50$ magnification in five samples. The density for the evaluation of early conidial development was 1.40×10^6 conidia/mL.

For the evaluation of conidial germination, after cotyledons had expanded, they were cut into pieces 20 mm², and two 10- μL droplets of inoculum were dropped onto the adaxial surface by a micropipette (Nichipet EX; Nichiryo, Co. Ltd., Saitama, Japan). The leaf pieces were then placed in polystyrene cups (94-mm inner diameter, 57-mm depth)

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Fig. 1. Light spectra of light-emitting diodes (LEDs), natural light, and light under the near-infrared-light-absorbing film. Photon fluxes per unit wavelength are expressed relative to the maximum.

containing water-soaked cotton to restrict water loss. The cups were then placed in the same growth chamber (LPH-220SP) at 28 °C, 99% RH (in the cups), and a PPFD of 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ provided by fluorescent lamps (FL20SEX-N-HG) under a light:dark cycle of 16:8 h, for 24 to 72 h.

For the evaluation of conidial infection, hyphal development, haustorial formation, and visible colony development, seedlings were individually transplanted into plastic pots (60-mm diameter, 55-mm height) and were spray-inoculated onto the adaxial surface. They were then placed in the same growth chamber (LPH-220SP) at 28 °C, 50% RH, and a PPFD of 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ provided by fluorescent lamps (FL20SEX-N-HG) under a light:dark cycle of 16:8 h, for 24, 48, 72 h, or 7 d.

Evaluation of conidial germination. Conidial germination was evaluated on cucumber

cotyledons acclimatized to R:FRs of 1.2 or 10. Germination at 0 h was evaluated by examining the conidia in the inoculum immediately before inoculation under a digital microscope (VHX-1000; Keyence Corp., Osaka, Japan) with reflected light. The adaxial cotyledon surfaces of three seedlings in each treatment group were observed under a scanning electron microscope (SU-1510; Hitachi High-Technologies Corp., Tokyo, Japan) 24 and 48 h after inoculation, according to Itagaki et al. (2014). About 700 to 900 conidia in each treatment group were observed at each time. Conidia with germ tubes were considered as germinated, and the percentage of conidial germination was calculated.

Evaluation of conidial infection, hyphal growth, and formation of secondary haustoria. Conidial infection, hyphal development, and haustorial formation were evaluated on cotyledons acclimatized to R:FRs of 1.2 or 10. The

cotyledons of two seedlings in each treatment were detached and fixed (including degreening) in formalin:acetic acid:alcohol = 1:1:1 v/v/v solution for 2 weeks, 24, 48, and 72 h after inoculation. After the cotyledons were degreened, they were gently washed in water and stained with lactophenol blue solution (Merck Millipore Co., Darmstadt, Germany). The formation of conidial germ tubes, hyphal growth, and haustoria were observed under an optical microscope (BX-50; Olympus Corp., Tokyo, Japan). Thirty conidia on a cotyledon were examined in each of four trials.

Conidial infection was evaluated 24 h after inoculation (Fig. 2A and B). After conidial germination (Fig. 2A), a primary haustorium forms in the host epidermal cell under the germ tube and draws nutrients from the cell. When the conidium was successful in invasion and formation of the primary haustorium, primary hyphae arose from the germ tube or from another side of the conidium (Fig. 2B), and secondary haustoria were formed. Next, secondary hyphae arose from the primary hyphae (Fig. 2C) and then tertiary hyphae branched from the secondary hyphae. On the basis of this infection process, conidium with more than one primary hypha was considered evidence of successful infection (Kuzuya et al., 2006; Suthaparan et al., 2014). The percentage of conidial infection is calculated as $\sum (\text{conidia with a germ tube and primary hyphae}) \times 100 / [\sum (\text{conidia with germ tube only}) + \sum (\text{conidia with germ tube and primary hyphae})]$.

The total number of secondary haustoria per conidium and the location of the haustoria were observed 24 and 48 h after inoculation. Hyphal growth was evaluated as the total number of primary hyphae, secondary hyphae that branch from the primary hyphae, tertiary hyphae that branch from the secondary hyphae, 24–72 h after inoculation, and hyphal cells per conidium 24 and 48 h after inoculation (Fig. 2C and D). The hyphal cells were discriminated by the septa formed in the hyphae (Fig. 2D). The percentage of secondary haustoria which formed in hyphal cells next to conidia was calculated.

Evaluation of visible colony development 7 d after inoculation. The number of visible colonies on 10 seedlings in each treatment group (R:FRs of 1.2, 5.0, and 10) was evaluated 7 d after inoculation, and the colony density was calculated from that number and the leaf area. This experiment was repeated three times in the same conditions. The inoculum densities were 0.96×10^6 conidia/mL in trial 1, 0.92×10^6 conidia/mL in trial 2, and 1.40×10^6 conidia/mL in trial 3.

Evaluation of haustorial formation 7 d after inoculation. For the evaluation of the late developmental stage of *P. xanthii*, haustorial formation 7 d after inoculation was observed according to Itagaki et al. (2014). Cross-sections (120- μm thick) of leaf pieces (5 mm²) with a representative colony were prepared from five seedlings in each treatment group on a plant microtome (MTH-1; Nippon Medical and Chemical Instruments).

Fig. 2. Development of *Podosphaera xanthii* conidia on cucumber seedlings acclimatized to red-to-far-red ratio of 1.2. (A) Conidium with a germ tube. (B) Conidium with germ tube and primary hypha 12 h after inoculation. (C) Conidium with primary and secondary hyphae 48 h after inoculation. (D) Hyphal cell 48 h after inoculation. The images of (C) and (D) are taken from the same conidium at different magnification and depth of focus. Haustoria were observed at different focus positions but are not clearly shown in these figures.

After staining with lactophenol blue, about 200 adaxial epidermal cells in each cross-section (≈ 1000 cells in each treatment group) were examined under a digital microscope (VHX-1000) with transmitted light to estimate the percentage of cells with haustoria.

Evaluation of leaf properties acclimatized to different R:FRs. The relative chlorophyll (Chl) content, dry weight, and leaf area of 10 seedlings from each R:FR treatment were measured. Relative Chl content was evaluated with a Chl meter (SPAD-502; Konica Minolta Inc., Tokyo, Japan). Leaf area was measured on an image scanner using the image analysis software LIA for win32 (K. Yamamoto, Nagoya University, Nagoya, Japan). The LMA was calculated in three inoculation tests, which evaluated colony development 7 d after inoculation. The carbon (C) and nitrogen (N) contents in trial 1 of LMA measurement were determined

with a CN Elemental Analyzer (2400 Series II; PerkinElmer Inc., Waltham, MA), and the C:N ratio was calculated. SA contents were determined for cotyledons, which were obtained from the seedlings acclimatized to R:FR = 1.2 or 10, with the high-performance liquid chromatography/mass spectrometry system according to the method described by Segarra et al. (2006). Three samples of cotyledons [5.0 to 6.5 g fresh weight (FW) in each sample] in each treatment were prepared for the SA determination.

Expt. 2: Inoculation of cucumber seedlings grown under modified natural light

Cucumber seeds were sown in vermiculite in plastic pots and germinated in the growth chamber (LPH-220SP) at 28 °C and 80% RH. The seedlings were then placed under an NIR-absorbing film (prototype, NIRF-L-PP-10; YANMAR Co., Ltd., Osaka,

Japan, and PANAC ADVANCE Co., Ltd., Tokyo, Japan) under natural light in a greenhouse. The film transmits $\approx 40\%$ of PPFD. A polyvinyl-chloride (PVC) film (Nobiace Mirai; Mitsubishi Plastics Agri Dream Co., Ltd., Tokyo, Japan) was used as the control. In the control treatment, a shielding net (4S-SU45; Sekisui Nano Coat Technology Co., Ltd., Aichi, Japan) that does not change the R:FR was used to reduce the PPFD by the same amount. The R:FR under the PVC film was 1.2, and that under the NIR-absorbing film was 4.7. The spectrum of each film is shown in Fig. 1. The average total PPFD in the greenhouse was $18.0 \text{ mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$. The average day/night air temperature and RH were $\approx 30/23$ °C and 70%/90%, respectively, in both treatments. To obtain seedlings of about the same developmental stage and leaf area ($\approx 22 \text{ cm}^2$ per plant at the cotyledon stage; ≈ 24 and 40 cm^2 per plant in cotyledons and first true leaf, respectively, at the first-true-leaf stage) in both treatments, the seedlings for the control treatment were sown 1 or 2 d after the seedlings for the cotyledon or first-true-leaf stage, respectively, in the NIR-absorbing film treatment.

Plants were spray-inoculated with 1.21×10^6 conidia/mL at the cotyledon stage or the first-true-leaf stage. Fifteen seedlings in each treatment group were then grown as before. In addition, eight control seedlings and eight NIR-absorbing-film seedlings were switched. The number of visible colonies was evaluated 7 d after inoculation, and the colony density was calculated. The experiment was conducted from 24 Aug. to 14 Sept. 2015 at Osaka Prefecture University ($34^\circ 32' 34.8'' \text{N}$, $135^\circ 30' 20.8'' \text{E}$).

Statistical analysis

For Expt. 1, differences in the rates of conidial germination and conidial infection between treatments (R:FRs of 1.2 and 10) were analyzed by chi-squared test of independence in 2×2 contingency tables. Differences in the total numbers of hyphal cells, primary to tertiary hyphae, and the rate of secondary haustorial formation in hyphal cells next to conidia between treatments (R:FRs of 1.2 and 10) were analyzed by Student's *t* test. Differences in haustorial formation 7 d after inoculation between treatments (R:FRs of 1.2 and 10) were analyzed by chi-squared test of independence in 2×2 contingency tables. The effects of acclimatization to R:FR on colony density, relative Chl content, LMA, and C:N ratio under LEDs (R:FR of 1.2, 5.0, or 10) were determined by one-way analysis of variance (ANOVA) for each trial ($n = 10$). SA content between treatments (R:FRs of 1.2 and 10) were analyzed by Student's *t* test.

For Expt. 2, differences in colony density 7 d after inoculation were analyzed by Tukey–Kramer test for each growing stage and leaf position ($n = 8\text{--}15$). The effect of the interaction of acclimatization to R:FR between before and after inoculation on colony density was determined by two-way ANOVA.

Results

Expt. 1: Evaluation of early conidial development of *P. xanthii* on cucumber seedlings grown under LEDs with different R:FRs, and evaluation of leaf properties. The conidial germination did not differ between the treatments either 24 or 48 h after inoculation: The rate of conidial germination on cucumber seedlings acclimatized to R:FR = 1.2 and 10 was 38.8% and 36.8% at 24 h after inoculation, and 55.4% and 53.0% at 48 h after inoculation, respectively. The conidial infection also did not differ between the treatments: The rate of conidial infection on cucumber seedlings acclimatized to R:FR = 1.2 and 10 was 88.3% and 79.2% at 24 h after inoculation, respectively. These results indicate that acclimatization R:FRs of 1.2 to 10 did not significantly affect the germination or initial invasion by *P. xanthii* conidia.

Although there was no significant difference in the number of primary hyphae at 24 h after inoculation, seedlings acclimatized to R:FR = 10 had significantly fewer primary hyphae than those acclimatized to R:FR = 1.2 at 48 and 72 h after inoculation (Fig. 3A): The number of primary hyphae on seedlings acclimatized to R:FR = 10 was $0.90\times$ ($P = 0.011$) and $0.88\times$ ($P = 0.046$) at 48 and 72 h after inoculation, respectively, than at R:FR = 1.2. Although there was no significant difference in number of secondary and tertiary hyphae at 24 and 48 h after inoculation, seedlings acclimatized to R:FR = 10 had significantly fewer secondary and tertiary hyphae than those acclimatized to R:FR = 1.2 at 72 h after inoculation (Fig. 3B and C): The number of secondary and tertiary hyphae on seedlings acclimatized to R:FR = 10 was $0.79\times$ ($P = 0.029$) and $0.59\times$ ($P = 0.013$), respectively, than those acclimatized to R:FR = 1.2 at 72 h after inoculation. Although there was no significant difference in hyphal cell number at 24 h after inoculation, seedlings acclimatized to R:FR = 10 had significantly fewer hyphal cells (at $0.88\times$, $P = 0.021$) than those acclimatized to R:FR = 1.2 at 48 h after inoculation (Fig. 3D). Seedlings acclimatized to R:FR = 10 had significantly fewer secondary haustoria (at $0.72\times$, $P = 0.049$) than those acclimatized to R:FR = 1.2 at 48 h (secondary haustoria were not developed at 24 h) (Fig. 3E). Seedlings acclimatized to R:FR = 10 had a significantly lower rate of secondary haustoria formed in hyphal cells next to conidia (at $0.69\times$, $P = 0.031$) than those acclimatized to R:FR = 1.2 (Fig. 3F). Thus, the hyphal and haustorial development of *P. xanthii* colonies differed between treatments only after the initial invasion.

Seedlings acclimatized to R:FR = 10 had a significantly lower rate of epidermal cells containing haustoria (at $0.52\times$) 7 d after inoculation than those acclimatized to R:FR = 1.2 (Table 1). Visible colony development was suppressed on seedlings acclimatized to higher R:FRs at 7 d after inoculation (Table 2): colony densities at R:FR = 5.0 and 10 were $0.80\times$ and $0.73\times$ than at R:FR = 1.2 (Fig. 4).

Fig. 3. Numbers of (A) primary hyphae, (B) secondary hyphae, (C) tertiary hyphae, (D) hyphal cells, (E) secondary haustoria, and (F) rate of secondary haustorial formation in hyphal cells next to conidia on cucumber seedlings acclimatized to red-to-far-red ratio (R:FR) = 1.2 or 10, at 24, 48, and 72 h after inoculation. Mean $\pm$ SE ($n = 4$); NS = not significant at $P > 0.05$; *significant difference between treatments at $P = 0.05$ by Student's t test.

Relative Chl content increased as acclimatization to R:FR increased: The relative Chl content of seedlings acclimatized to R:FR = 1.2, 5.0, and 10 were 48.1 ± 0.6 , 54.7 ± 0.4 , and 60.4 ± 0.6 (Chl meter value, SPAD-502; Means $\pm$ SE; $P < 0.001$ by Tukey–Kramer test). LMA increased as acclimatization to R:FR increased (Table 2). The LMA of seedlings acclimatized to R:FR = 5.0 and 10 were $1.13\times$ and $1.18\times$ than those acclimatized to R:FR = 1.2 (Fig. 4). The C:N ratio decreased as acclimatization to R:FR increased (Table 2): The C:N ratio of seedlings acclimatized to R:FR = 5.0 and

10 were $0.91\times$ and $0.92\times$ than those acclimatized to R:FR = 1.2, and there was no significant difference between acclimatization to R:FR = 5.0 and 10 (Fig. 4). SA content did not differ between seedlings acclimatized to R:FR = 1.2 and 10: The SA content was 111.4 ± 20.5 ng·g⁻¹ FW (Means $\pm$ SE) for seedlings acclimatized to R:FR = 1.2, and was 139.0 ± 21.4 ng·g⁻¹ FW for seedlings acclimatized to R:FR = 10.

Expt. 2: Inoculation of cucumber seedlings grown under modified natural light. Visible colony development was significantly less when seedlings were grown under the higher

Table 1. Haustorial formation in epidermal cells of cucumber seedlings acclimatized to red-to-far-red ratio (R:FR), 7 d after inoculation with *Podosphaera xanthii* (n = 5), in leaf cross-sections (200 cells/seedling).

Treatment	Total no. epidermal cells	No. epidermal cells containing haustoria	Percentage of epidermal cells containing haustoria
R:FR 1.2	1000	463	46.3
R:FR 10	1015	246	24.2
Significance ^z	—	—	***

^z***Significant difference between percentages in each treatment group at $P = 0.001$ by chi-squared test of independence in 2×2 contingency tables.

Table 2. Results of one-way analysis of variance to test the effects of acclimatization red-to-far-red ratio on colony density of *Podosphaera xanthii* 7 d after inoculation, leaf mass per area (LMA), and carbon-to-nitrogen ratio (C:N ratio).

		Trial 1	Trial 2	Trial 3
Colony density	df	2	2	2
	F	11.2	14.4	3.8
	P	<0.001	<0.001	0.035
LMA	df	2	2	2
	F	10.5	33.3	12.5
	P	<0.001	<0.001	<0.001
C:N ratio	df	2	—	—
	F	16.3	—	—
	P	<0.001	—	—

Fig. 4. Colony density of *Podosphaera xanthii* 7 d after inoculation (means of three trials), leaf mass per area (LMA) (means of three trials), and carbon-to-nitrogen ratio (C:N ratio) (trial 1, n = 10) of cucumber seedlings acclimatized to red-to-far-red ratio (R:FR) = 1.2, 5.0, or 10. The results of analysis of variance are shown in Table 2.

R:FR throughout the experiment (Fig. 5; “4.7/4.7”) than in the control treatment (“1.2/1.2”): the colony density was 0.83× that of the control at the cotyledon stage, 0.81× on the cotyledons at the first-true-leaf stage, and 0.58× on the first true leaves.

R:FR before inoculation had significant effects on colony development, and also after inoculation except on the cotyledons at the first-true-leaf stage (Table 3). The suppressive effect of acclimatization to R:FR was reduced when seedlings were moved from higher R:FR to natural R:FR after inoculation (Fig. 5; “4.7/1.2”). The suppressive effect of acclimatization to R:FR were observed when seedlings were moved from natural R:FR to higher R:FR after inoculation (“1.2/4.7”); at the cotyledon stage and on the first true leaves, the colony density was reduced to the same level as that of seedlings grown under higher R:FR throughout the experiment (“4.7/4.7”), although there was no significant difference from the control treatment at the cotyledon stage. There were no interaction effects of R:FR before and after inoculation (Table 3).

Discussion

Germination of *P. xanthii* conidia and initial invasion were not suppressed, but subsequent invasion was suppressed on cucumber seedlings acclimatized to higher R:FR. Colony development and haustorial formation 7 d after inoculation were also suppressed, possibly as a result of the earlier suppression of hyphal and haustorial formation (Morishita et al., 2003; Pérez-García et al., 2009). Acclimatization decreased the formation of secondary haustoria, which formed in hyphal cells next to conidia, perhaps by delaying or suppressing their formation.

Leaf properties of seedlings acclimatized to higher R:FR (greater Chl content and greater LMA) were similar to those of sun leaves (Lichtenthaler et al., 1981). These properties probably resulted from the heightened acclimatization to light intensity induced by higher R:FR (Shibuya et al., 2010, 2012, 2015), which might have affected the pathogen development. The seedlings acclimatized to higher R:FR had an increase in LMA, which generally correlates with an increase in structural defenses against pathogen invasion (Feng et al., 2009; Toome et al., 2010; Wright and Cannon, 2001), as observed in the previous study (Shibuya et al., 2011). However, the initial invasion of

P. xanthii, which is generally affected by the structural properties of host leaves, did not differ between the treatments, and therefore the structural properties might not have limited the development of *P. xanthii* colony. Instead, the nonstructural properties such as the nutritive value of host cells or chemical defense levels may have limited the early development of *P. xanthii*. The C:N ratio, which generally correlates with nutritive value and chemical defense against leaf pathogens (Hermans et al., 2006; Martin et al., 2002; Mathur et al., 2013; McElrone et al., 2005), decreased with increasing acclimatization to R:FR, but did not explain the changes in colony development; a higher C:N ratio generally suppressed pathogen development, but colony development was enhanced in the present study. Therefore, other leaf chemical components may be involved. The authors had considered that SA-mediated disease resistance or gene expression related to defense against pathogen attack might have increased, as acclimatization to R:FR lower than that of natural light (<1.2) suppresses both responses (Ballaré et al., 2012; Cerrudo et al., 2012; Demotes-Mainard et al., 2015), and acclimatization to higher R:FR than natural caused the opposite responses in physiological properties of cucumber seedlings (Shibuya et al., 2010, 2012, 2015). However, SA content was not significantly affected by acclimatization to R:FRs of 1.2–10 in the present study. Wang et al. (2010) demonstrated that increase in SA content due to specific wavelengths of light occurs mainly after pathogen infection. Thus, physiological responses in plant metabolism including occurrence of SA-mediated resistance should be closely examined to elucidate the mechanism of improved resistance under higher R:FR.

Acclimatization to higher R:FR under the NIR-absorbing film also suppressed the development of *P. xanthii* colony. Thus, the NIR-absorbing film, which has been used for controlling the plant growth and development, would also be useful for reducing the severity of powdery mildew. The suppression effect of the colony development was reduced when seedlings were moved from higher R:FR to natural R:FR after inoculation. This result may due to reacclimatization of the plants to new light conditions (Anderson et al., 1995; Bailey et al., 2004; Niinemets et al., 2006), although direct effect of specific wavelengths of light on the pathogen (Suthaparan et al., 2010) could also be involved. Reacclimatization may affect subsequent development, because it takes several days for leaves to adapt to new light conditions (Frantz and Bugbee, 2005). For the same reason, suppression may not have been fully achieved when the seedlings were moved from natural R:FR to higher R:FR after inoculation. Cotyledons at the first-true-leaf stage did not show the effect of R:FR after inoculation, maybe because of differences in leaf maturity and reacclimatization capacity, since mature leaves are less physiologically plastic than immature leaves (Niinemets et al.,

Fig. 5. Colony density of *Podosphaera xanthii* on cucumber seedlings acclimatized to red-to-far-red ratio (R:FR) = 1.2 (natural light) or 4.7 (under near-infrared-light-absorbing film). Plants were inoculated at the cotyledon or first-true-leaf stage. Codes indicate R:FR before and after inoculation. Bars are means $\pm$ SE of 15 replicates for 1.2/1.2 and 4.7/4.7 treatment, and eight replicates for 1.2/4.7 and 4.7/1.2 treatment. Bars with the same letter are not significantly different at $P < 0.05$ by Tukey–Kramer test. The results of two-way ANOVA are shown in Table 3.

Table 3. Results of two-way analysis of variance to test the effects of acclimatization red-to-far-red ratio on colony density 7 d after inoculation with *Podosphaera xanthii* at cotyledon and first-true-leaf stages.

Treatment		Cotyledon stage		True-leaf stage	
		Cotyledons	Cotyledons	First true leaf	
Before inoculation	df	1	1	1	
	F	8.00	20.72	20.84	
	P	0.007	<0.001	<0.001	
7 d after inoculation	df	1	1	1	
	F	9.76	2.59	31.97	
	P	0.003	0.115	<0.001	
Before and after inoculation	df	1	1	1	
	F	0.290	0.004	0.029	
	P	0.593	0.947	0.866	

2006). This difference could not be explained by the direct light effect described above.

In conclusion, the present results reveal that acclimatization of cucumber seedlings to higher R:FR suppressed the subsequent invasion by *P. xanthii*, possibly owing to

changes in nonstructural properties of leaves. The experimental approach and main findings in the present study would be effective in the future studies to clarify the roles of specific light wavelengths in determining plant–pathogen interactions. Plants grown

under an NIR-absorbing film in a greenhouse showed the same effect, but the effect was negated when plants were moved back to natural R:FR, possibly because of reacclimatization. Therefore, when illumination with higher R:FR is used to control powdery mildew, both the developmental process of the pathogen’s colony and the light acclimatization process of the host plant should be considered.

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