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Effects of supplementary UV-B radiation on development of damping-off in spinach caused by the soil-borne fungus *Fusarium oxysporum*

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The effects of UV-B radiation (290–320 nm) on development of damping-off of spinach (*Spinacia oleracea*) caused by the fungus *Fusarium oxysporum* were examined in a growth cabinet. The incidence of disease greatly increased when experimental plants were grown in visible radiation with supplementary UV-B radiation. This increase was suppressed by increasing the irradiation of visible radiation. *Fusarium oxysporum* was isolated from the roots of all damping-off plants and the roots of some unwilted plants, indicating that spinach infected with the pathogen did not necessarily suffer from damping-off in 15 d. Supplementary UV-B radiation suppressed the increase in growth components such as the number of leaves, the plant height and the fresh weight of aboveground plant parts, but did not affect the fresh weight of roots. The ratio of the number of plants infected with pathogen to the total number of plants was over 80% irrespective of light conditions. It was suggested that the defense response of spinach to this pathogen was greatly influenced by the physiological state of aboveground plant parts resulting from supplementary UV-B radiation.

Key Words——damping-off; *Fusarium oxysporum*; soil-borne fungus; spinach (*Spinacia oleracea*); UV-B radiation.

Recently, interest in various aspects of global environmental conditions has increased. One area which has received much attention is the effect on the life and ecology of plants of increased levels of solar ultraviolet-B (UV-B: 290–320 nm) radiation reaching the Earth's surface due to the depletion of stratospheric ozone. In response to this problem, numerous studies on the effects of UV-B radiation on the growth, development, pigment biosynthesis and other aspects of plants have been conducted (Bornman and Teramura, 1993). However, compared with biochemical and physiological studies in plants, there have been relatively few reports on the effects of supplementary UV-B radiation on the interaction between pathogens and host plants.

Diverse processes in fungi are greatly modulated by light as an environmental signal, as seen in the formation of reproductive structures, the growth of hyphae, phototropism, phototaxis, pigment biosynthesis, and so on. Generally, most of these photophysiological responses of fungi are controlled either by near-UV and blue radiation or by near-UV radiation alone (Carlile, 1970; Leach, 1971; Tan, 1978). The action spectra of photoresponses in the former case show peaks in the blue/UV-A region of the spectrum, while those in the latter exhibit peaks in the UV-B region between 280 and 320 nm (Kumagai, 1988). The development of reproductive structures is ecologically very important, because it is related to propagation of plant pathogens in the field. It is, therefore, reasonable to expect that if the UV-B region of the spectrum is increased, pathogenic fungi of plants will vigorously propagate. There have been several reports of attempts to control plant diseases by manipulating the photocontrol of sporulation or the phototropic response in fungi in the greenhouse: in these studies, photosensitivities of fungi were directly utilized. For instance, conidiation in certain pathogenic fungi such as *Alternaria*, *Bipolaris*, and *Botrytis* is caused by UV-B radiation, but nullified by blue light applied together with UV-B radiation (Kumagai, 1978, 1988). Dispersion and lesion development by those fungi were largely reduced by growing plants in greenhouses, where the UV region of the spectrum shorter than 390 nm was eliminated by using UV-absorbing vinyl film (Honda and Nemoto, 1985; Sasaki et al., 1985). Manipulating the phototropic response of conidium germ tubes of *Septoria obesa* Sydow was useful for suppression of brown spot disease in chrysanthemum: elimination of the UV region shorter than 390 nm significantly reduced the negative phototropic response of conidium germ tubes on leaves and suppressed the invasion of the fungus through the stomata (Honda et al., 1992). There have also been some reports that root diseases caused by infection with soil-borne pathogenic fungi, not reached by radiation, were greatly influenced by light. For instance, the photoperiod or solar radiation intensity was found to influence the expression of wilt induced by *Verticillium*

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spp. in various crops (Busch and Edgington, 1967; Jones et al., 1975). Wilt induced by *Verticillium dahliae* Kleb. in watermelon was more pronounced at low than at high solar radiation intensities (Ben-Yephet, 1979). Similarly, severe wilt of carnation caused by *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *dianthi* (Prill. & Delacr.) W.C. Snyder & H.N. Hans. developed at a low intensity of solar radiation (Ben-Yephet and Shtienberg, 1994).

Summer cultivation of spinach is carried out in greenhouses with vinyl film covering in Japan to avoid direct contact with rain drops and rain splashing, which sometimes cause contamination with infested soil capable of inducing soil-borne diseases. Our preliminary work showed that severe damping-off of spinach (*Spinacia oleracea* L. cv. Okame) caused by *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *spinaciae* (Sherb.) W.C. Snyder & H.N. Hans. at high temperatures in summer developed in the greenhouse was suppressed considerably by using UV-absorbing vinyl film which eliminated the UV region of the spectrum shorter than 390 nm (Naito and Honda, 1994).

The objective of the present study was to ascertain if supplementation of visible radiation by UV-B radiation in a growth cabinet would result in effects similar to those described above and to clarify the effects of the interaction of UV-B and visible radiation on the development of damping-off in spinach caused by *F. oxysporum*.

Material and Methods

Spinach was used as the experimental plant. Ten seeds were planted in a Wagner's pot (1/5000 a, Kiya Seisakusho Ltd., Tokyo, Japan) containing 3 L of commercial soil fertilized for garden use (Kureha Chemicals, Japan), inoculated or not inoculated with *F. oxysporum* f. sp. *spinaciae*, a fungus causing severe damping-off in spinach. Several virulent isolates were obtained from wilted spinach plants cultivated in a common agricultural vinyl film greenhouse in summer 1992. The isolates have been stock-cultured on potato-sucrose agar (PSA) slant and one of these isolates, No. 1, was used for this experiment. Spores for inoculation were separated from colonies of *F. oxysporum* grown on PSA plates at 25°C for 7 d, and suspended in distilled water. The spore suspension was filtered through 2-layer cheese cloth to eliminate mycelial fragments. Three hundred ml of spore suspension (10^3 spores ml^{-1} distilled water) was mixed with 3 L of culture soil. Seedlings grown for 7 d in pots in a greenhouse were transferred to a large growth cabinet (Koitotron type KG, Koito Ind., Tokyo, Japan), and further grown for 15 d in visible radiation with or without supplementary UV-B radiation. Pots in the growth cabinet were relocated every day so that the seedlings could receive an equal amount of light. The photoperiod consisted of 12 h of light and 12 h of dark and the temperature was maintained at 30°C during the day and 20°C at night. Visible light (380–780 nm) in the growth cabinet was supplied by a combination of high intensity discharge lamps (Toshiba DR400/T(L), Toshiba, Tokyo, Japan), fluorescent lamps (Toshiba FR80HWA), and tungsten lamps (Toshiba RF220V 200WH). The irradiance of visible radiation was lowered by filtering through white lawn. The supplementary UV-B radiation was supplied byfluorescent lamps (Toshiba FL20SE), and was filtered through cellulose diacetate film (Cactillac Olastic, Baltimore, Ohio, USA) to eliminated UV-C radiation shorter than 290 nm. Fluence rates of photosynthetically active radiation (PAR) and the irradiance of the UV-B region of the spectrum (313±30 nm) were measured with a data logger (LI-1000, Li-Cor, USA) fitted with an LI-190SA sensor (Li-Cor) or an SD 104 UV sensor (Macom Co., UK), respectively.

Total chlorophyll content (mg g^{-1} fresh weight) in the aboveground plant parts was determined according to the method of Arnon (1949). Absorbance at 645 and 663 nm was measured with a spectrophotometer (UV-300, Shimadzu Ltd. Co., Kyoto, Japan). UV-absorbing substance was extracted with 70% (v/v) methanol containing 1% (v/v) HCl at room temperature for 3 h, and its contents (the absorbance at 330 nm g^{-1} fresh weight) were determined.

The existence of *F. oxysporum* on plant roots showing damping-off was usually examined as follows. Plant roots were washed in running tap water for 2 h and cut into segments of ca. 5 mm in length. Each segment was placed in the center of a water agar plate (9 cm in diam) and incubated for 3–7 d at 25°C. *Fusarium oxysporum* was identified on the basis of the morphology of micro- and macroconidia. The invasion of *F. oxysporum* through the roots of unwilted plants with or without root browning, the results of which are shown in Fig. 2, was examined as follows. The plant roots washed with water were dipped in 70% ethanol for 10 sec and sterilized with 0.5% NaClO for 1 min, then rinsed twice with sterilized water. Each root segment was then incubated on a water agar plate for 3–7 d at 25°C.

Results

When experimental plants were grown for 15 d in a growth cabinet in the soil inoculated with a virulent isolate of *F. oxysporum*, damping-off developed in plants in visible radiation with or without supplementary UV-B radiation. This was not the case for plants grown without inoculation of plant pathogen.

Figure 1 shows the effects on development of damping-off in spinach of UV-B radiation supplemented with low or high irradiance level of visible radiation. The irradiance of UV-B radiation was 1.0 Wm^{-2}. Irradiance of low and high levels of visible radiation was 90 and 135 $\mu mol\ m^{-2}\ sec^{-1}$, respectively. The disease incidence in plants grown at the low irradiance level of visible radiation supplemented with UV-B radiation was over 80%, while that at the high level was ca. 70%. The incidence of disease (%) was obtained as follows: the number of seedlings with damping-off / number of total seedlings × 100. The values at low and high levels of visible radiation were 2.1 and 1.6 times higher, respectively, than those of control without UV-B supplement. The control values at low and high levels were almost the

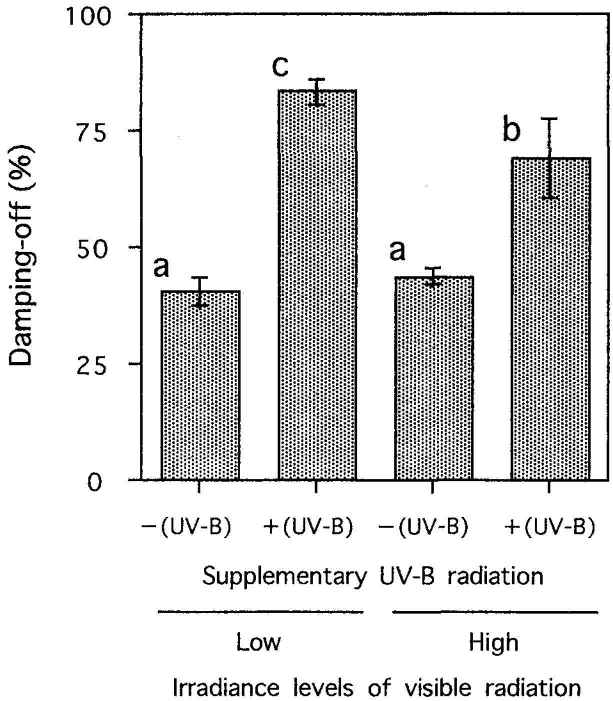

Fig. 1. Effect of supplementation of UV-B radiation to low or high irradiance level of visible radiation on the development of damping-off in spinach. Experimental plants were grown for 15 d at low (90 μmolm^{-2}s^{-1}) or high (135 μmolm^{-2}s^{-1}) irradiance level of visible radiation supplemented with UV-B radiation (1.0 Wm^{-2}). Columns marked with the same letter showed no significant difference at the 0.05 level according to Duncan's new multiple range test. Each value is the mean of six pots. Vertical bars show mean ± SE.

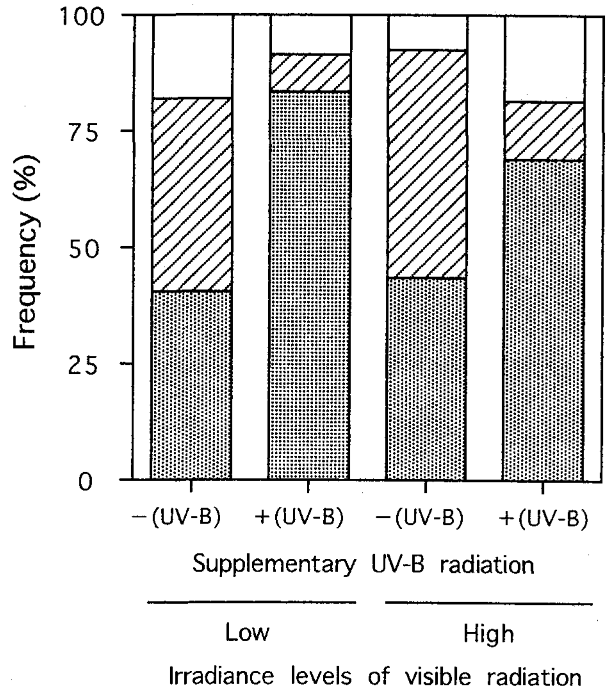

Fig. 2. Degree of infection with *F. oxysporum* in unwilted and damping-off plants grown at low or high irradiance level of visible radiation with or without supplementary UV-B radiation. Supplementary UV-B radiation was 1.0 Wm^{-2}. Experimental plants were grown for 15 d at low (90 μmolm^{-2} s^{-1}) or high (135 μmolm^{-2}s^{-1}) irradiance level of visible radiation supplemented or not supplemented with UV-B radiation. Observations were made at the termination of the experiment. ▩: Damping-off plants with *F. oxysporum*, ▨: Unwilted plants with *F. oxysporum*, □: Unwilted plants without *F. oxysporum*.

same, around 40%. It was thus evident that the occurrence of damping-off caused by *F. oxysporum* was increased by supplementary UV-B radiation. Furthermore, the effect of UV-B radiation was also partially lowered by the elevation of the level of irradiance of visible radiation applied concomitantly with UV-B radiation.

Damping-off was not observed in plants grown without inoculation of pathogenic fungus in any light conditions. On the other hand, *F. oxysporum* was isolated from both the diseased plant roots and the unwilted plant roots (Fig. 2). Here, experimental conditions were the same as those shown in Fig. 1. The ratio of the number of plants infected with pathogen to the total number of plants, namely, the degree (%) of infection, was over 80% irrespective of light conditions. However, the incidences of disease under both low and high levels of visible radiation without supplementary UV-B radiation were significantly lower than those under supplementary UV-B radiation. These results indicated that the pathogen which infected spinach did not necessarily cause damping-off in 15 d.

Table 1 shows the effects of supplementary UV-B radiation on the growth of spinach grown for 15 d at low or high irradiance level of visible radiation. Experimental conditions were the same as those in Fig. 1. Each growth component, such as the number of leaves, plant height and fresh weight of aboveground plant parts, significantly decreased compared with that of the control. However, fresh weight of roots was not greatly influenced and the amount of UV-absorbing substance was greatly increased by supplementary UV-B radiation. On the other hand, with elevation of the irradiance of visible radiation from low to high level, reduction of growth was not greatly influenced within the range of the irradiance level of visible radiation examined here.

Discussion

Damping-off is a disease caused by infection of plant roots with a soil-borne fungus, *Fusarium* spp., and its development is strongly affected by soil temperature (Naiki and Morita, 1983). In spinach, this disease occurs severely at high temperatures during summer cultivation from July to August. Besides high temperatures, the occurrence of damping-off in spinach is influenced by light conditions: the incidence of this disease was found to be greatly decreased in plants cultivated in greenhouses covered with UV-absorbing vinyl film that eliminated radia-

Table 1. Growth of spinach seedlings under low or high irradiance level of visible radiation with or without supplementary UV-B radiation.

Parameter	Irradiance levels of visible radiation[a)] Low light				High light			
	Supplementary UV-B radiation[b)] −(UV-B)		+(UV-B)		−(UV-B)		+(UV-B)	
Number of leaves	2.90 ±0.18	ab[c)]	2.25 ±0.10	a	3.35 ±0.15	b	2.35 ±0.11	a
Plant height (cm)	3.96 ±0.15	b	2.35 ±0.15	a	4.81 ±0.16	c	2.57 ±0.08	a
Aboveground plant parts FW (g)	0.139±0.010	b	0.068±0.004	a	0.209±0.011	c	0.098±0.003	a
Root FW (g)	0.036±0.001	a	0.030±0.001	a	0.037±0.002	a	0.035±0.001	a
Total chlorophyll content (mg g^{-1} FW)	0.749±0.012	b	0.632±0.010	a	0.723±0.011	b	0.717±0.010	b
UV-absorbing compounds (A_{330} g^{-1} FW)	14.97 ±0.45	a	25.65 ±0.84	b	13.31 ±0.39	a	24.30 ±0.54	b

a) Irradiances of low (Low light) and high (High light) levels of visible radiation were 90 $\mu molm^{-2}s^{-1}$ and 135 $\mu molm^{-2}s^{-1}$, respectively.
b) Supplementary UV-B radiation was 1.0 Wm^{-2}.
c) Each value is the mean of 20 plants (±SE). The means of growth components marked with the same letter showed no significant difference between groups at the 0.01 level according to Scheffe's S test.

tion below ca. 390 nm from solar radiation as compared with the incidence in greenhouses covered with common agricultural vinyl film (Naito and Honda, 1994). Supplementary UV-B radiation without UV-C remarkably enhanced the development of damping-off caused by *F. oxysporum*. This is quite significant because it suggests that the elevation of UV-B radiation due to the depletion of stratospheric ozone could induce an increase in the occurrence of diseases caused by soil-borne pathogenic fungi in addition to diseases caused by air-borne fungi, sporulation of which is directly enhanced by UV-B radiation.

On the other hand, visible radiation applied concomitantly with UV-B radiation alleviated the increase in the incidence of disease induced by supplementary UV-B radiation (Fig. 1). It has been reported that severe wilt caused by *F. oxysporum* in carnations and by *V. dahliae* in watermelons developed at a lower irradiance of solar radiation but not at a higher irradiance (Ben-Yephet and Shtienberg, 1994). These pathogenic fungi mostly attacked plant tissues depleted of carbohydrates under low irradiance of visible light or under shading conditions. In our experiments, each growth component, such as plant height, number of leaves and fresh weight of aboveground plant parts, was suppressed by supplementary UV-B radiation, which was in part alleviated by concomitant application of visible radiation at high light intensity (Table 1). These results suggested the possibility that the increase in the incidence of disease in spinach by supplementary UV-B radiation and its retardation by visible radiation at high intensity might be attributable to their influence on photosynthesis in the aboveground plant parts. However, since the incidence of disease was not significantly influenced by the elevation of the irradiance level of visible radiation within the range examined in this experiment under conditions without supplementary UV-B radiation, factors other than the influence on photosynthesis must be involved. Infection by the pathogen was over 80% irrespective of light conditions, but the incidence of disease under conditions without supplementary UV-B radiation was remarkably lower than that under conditions with UV-B radiation. Furthermore, the fresh weight of roots did not change greatly with light conditions, suggesting the possibility that the rhizosphere environment might not be significantly influenced. It is, therefore, considered that the defense response of spinach to the soil-borne fungus *F. oxysporum* depends to a great extent on the unknown physiological state of the aboveground plant parts influenced by UV-B radiation.

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