

Effect of Red Light Treatment of Seedlings of Pepper, Pumpkin, and Tomato on the Occurrence of *Phytophthora* Damping-off

S.Z. Islam and M. Babadoost

Department of Crop Sciences, University of Illinois, Urbana, IL 61801

Y. Honda

Faculty of Life and Environmental Science, Shimane University, Matsue, Japan

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Abstract. A study was conducted in the greenhouse to investigate the effects of red light (600–700 nm) on the subsequent occurrence of seedling infection of bell pepper, pumpkin, and tomato caused by *Phytophthora capsici*. Three- or 4-week-old seedlings were inoculated with zoospores or transplanted into pots filled with artificially infested soil mix. Red light treatment of seedlings reduced *Phytophthora* damping-off by up to 79%. Only 21% to 36% of red light-treated seedlings became infected, whereas 78% to 100% of the control seedlings, grown either in natural daylight (NDL) or under white light (WL), became infected and died. The height, and fresh and dry weight of seedlings treated with red light were significantly higher than those grown under NDL or WL.

Phytophthora blight, caused by *Phytophthora capsici* Leonian, has become one of the most serious threats to production of cucurbit crops, peppers, and tomatoes nationwide (Babadoost, 2000a, 2000b; Matheron and Matejka, 1995; Roberts et al., 2000). Recently, the incidence of damping-off, foliar blight, and fruit rot on melons, peppers, pumpkins, squashes, and watermelon caused by *P. capsici* has dramatically increased in Illinois (Babadoost, 2000a, 2000b). In the past 3 years, *Phytophthora* blight has been epidemic in processing pumpkin and bell pepper fields in Illinois and caused yield losses up to 100% (Babadoost, 2000b). Infection of the plants in the field may occur at any time during the growing season. Early infections cause seedling damping-off, and later infections cause foliar blight, stem lesions, vine rot, fruit rot, and root and crown rot (Lee et al., 2001; Matheron and Matejka, 1995). Practices such as planting on raised beds, management of field moisture, straw mulching, planting tolerant cultivars, and application of some fungicides may reduce severity of *Phytophthora* diseases of vegetables. No single method is available, however, to provide adequate control of *P. capsici* on vegetables (Hwang and Kim, 1995). Host resistance, if available, would be an ideal biological defense against *P. capsici*. No pumpkin or chili pepper cultivar with measurable resistance to *Phytophthora* blight is available. Resistance to *Phytophthora* blight in a few cultivars of bell pepper has been reported (Boucher and Ashley, 2000), but they

may not be commercially acceptable in all pepper-growing areas.

In nature, plants are exposed to light of different wavelengths, from ultraviolet (UV) to far-red regions. Light of certain wavelengths (i.e., 290–320 nm) may affect certain host-pathogen interactions (Honda, 2000; Kumagai, 1988; Tan, 1978). Light was reported as one of the important factors affecting disease development by inactivating toxins of pathogens in certain plant/pathogen interactions (Akimitsu et al., 1988; Kohmoto et al., 1989). There are several reports of phytoalexins produced by host-parasite interactions in plant tissue that protect the host plant from the pathogen. Ultraviolet irradiation (253 nm) of soybean plants induced resistance against *Phytophthora sojae* by inducing the production of the phytoalexin hydroxyphaseollin (Bridge and Klarman, 1973). Accumulation of phytoalexins in plants receiving short-wave UV radiation (254 nm) was also reported by several investigators (Hadwiger and Schwochow, 1971; Kato et al., 1994; Kodama et al., 1992).

Recently, several investigators reported that red light treatment induces disease resistance in plants (Islam et al., 1998a, 1998b, 1999; Rahman et al., 2001). Light of long wavelengths (red region, 600–700 nm) induced resistance in broadbean (*Vicia faba* L.) against *Botrytis cinerea* (Islam et al., 1998b). They also reported that irradiation of broadbean leaflets with red light resulted in production of an antifungal substance (phytoalexin) in leaf tissue. Red light was also reported to suppress the lesion development of *P. capsici* on detached leaves of eggplant, pepper, pumpkin, and watermelon (Umezue et al., 1999). Induced resistance in plants by red light against *Alter-*

naria tenuissima has also been reported recently (Rahman et al., 2001). However, most of the reports on red light-induced resistance against plant pathogens are from studies carried out on detached leaves. The objective of this study was to investigate whether treatment of seedlings with red light can protect the plants from damping-off caused by *P. capsici*.

Material and Methods

Phytophthora capsici was isolated from an infected pumpkin seedling (*Cucurbita moschata* L.) collected from Pekin, Ill., and the culture was maintained on lima bean agar (LBA; Difco Lab., Detroit) slants. Sporangial suspensions were prepared from 5- to 6-day-old culture plates of *P. capsici* grown on LBA at 24 °C under continuous white fluorescent light. Ten milliliters of sterilized-distilled water (SDW) were added to each plate and the sporangia were dislodged using a soft brush. The suspension was then incubated at 20 °C for 1 h to allow the sporangia to release their zoospores. Zoospores were separated from the empty sporangia by passing the suspension through a four-layered facial tissue (Kleenex). The concentration of zoospores was adjusted to 20×10^4 zoospores/mL water using a hemacytometer (no. 3120, Hausser Scientific Co., Horsham, Pa.).

Inoculum for artificial soil infestation was prepared by growing *P. capsici* on oatmeal-V8-juice broth (V8JB) substrate in 1-L conical flasks. The substrate, consisting of 200 g oatmeal and 120 mL V8-juice broth per liter, was autoclaved for 30 min and inoculated with mycelial plugs, 7 mm in diameter, taken from the margin of a young colony of *P. capsici* grown on LBA. The flasks were then incubated at 24 °C in darkness. After 6 weeks, the colonized oatmeal was added to steamed soil mix (1 soil : 1 sand : 1 peat) and mixed thoroughly. The inoculum potential of the soil mix was 600 colony forming units (cfu) per gram of soil, which was determined by the dilution-plate-count method.

Seeds of bell pepper (*Capsicum annum* L.; Hybrid SPP 6112, Sakata Co., Morgan Hill, Calif.), pumpkin (cv. Dickinson), and tomato (*Lycopersicon esculentum* Mill.; Hybrid XTM 6217, Sakata Co.) were sown in 10-cm-diameter plastic pots (one seed per pot) containing steamed soil mix and were grown on a greenhouse bench under continuous red light irradiation (600–700 nm) from fluorescent tubes (FL20S-R-F, National Co., Japan). The fluorescent tubes provided red light with an intensity of $287 \mu\text{W}\cdot\text{cm}^{-2}$, which was a supplement to natural daylight. Control seedlings were kept under continuous white light (WL; FL20SS-D/18, Mitsubishi, Tokyo; 380–740 nm; $473 \mu\text{W}\cdot\text{cm}^{-2}$) or under natural daylight (NDL) in the same greenhouse. Three- or 4-week-old seedlings were transferred from red light to NDL and inoculation was accomplished by adding the suspension of motile zoospores over the surface of the soil in each pot (5 mL/seedling/pot). Control seedlings either grown in WL or NDL were also inoculated using the same procedure. An additional

set of control seedlings were inoculated with SDW only. Seedlings were watered before inoculation to keep the soil wet. After inoculation, the pots were placed in plastic trays containing water that kept the soil moist for at least 12 h. The seedlings were then placed on a greenhouse bench, in a completely randomized design, and watered twice daily. Pumpkin and tomato seedlings were also inoculated by transplanting the seedlings into artificially infested soils in pots. Each pot contained one seedling and 14 pots were used for each treatment. Beginning the fourth day after inoculation, seedlings were evaluated for damping-off symptoms every day for 15 successive days. The seedlings were also evaluated on the 20th day after inoculation. Height, and fresh and dry weights of seedlings were also measured. Dry weight was determined after drying the seedlings at 70 °C for 24 h. Each experiment was repeated two or three times. Since the results of the experiments were not significantly different from each other, data from one experiment are presented. Data were analyzed using analysis of variance procedures (SAS Institute, Cary, N.C.).

Results

Pepper. Symptoms of *Phytophthora* infection began to appear in red light-treated pepper seedlings 12 d after zoospore inoculation, and 36% of seedlings became infected and died (Fig. 1). In contrast, symptoms on control seedlings (grown under WL or NDL) first appeared 5 d after inoculation and within 8 d of inoculation, 100% of the seedlings were infected, stem-girdled, and died.

Pumpkin. In the zoospore-inoculated experiment, seedlings treated with red light showed symptoms 12 d after inoculation. Only 21% of the seedlings became infected (Fig. 2). In comparison, control seedlings grown under either WL or NDL became symptomatic 5 d after inoculation and within 15 d after inoculation, 78% of seedlings were infected and died. In the infested soil experiment, 28% of red light-treated and 78% of control seedlings grown under NDL became infected and died (Fig. 3).

Tomato. In red light-treated tomato seedlings, symptoms started 4 d after zoospore inoculation, and 28% of the seedlings were infected and died 15 d after inoculation (Fig. 4). In contrast, symptoms on control seedlings started 4 d after inoculation and within 7 d 100% of the seedlings became infected, stem-girdled, and died. In the infested soil experiment, only 21% of red light-treated transplants were found infected and developed symptoms, while 80% of the control transplants grown under NDL became infected and died 15 d after inoculation (Fig. 5).

In all of the red light treatments, no more seedling damping-off occurred 15 d after inoculation of plants.

The height, and fresh and dry weights of red light-treated seedlings were significantly higher than those of seedlings grown under WL or NDL (Table 1). The average height of red light-treated seedlings of pepper, pump-

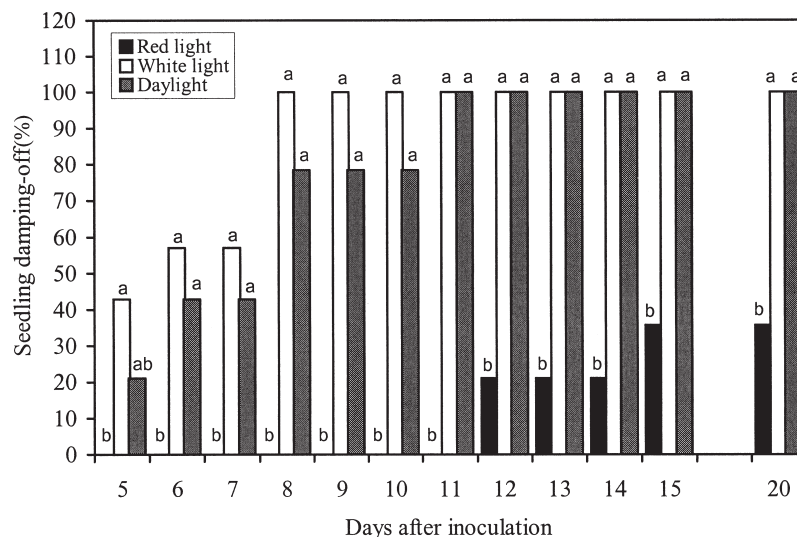


Fig. 1. Effect of light treatment on the occurrence of damping-off in 4-week-old pepper seedlings inoculated with *Phytophthora capsici* zoospores. Within each day, the columns with a letter in common are not significantly different from each other ($P = 0.05$, Fisher's Protected LSD).

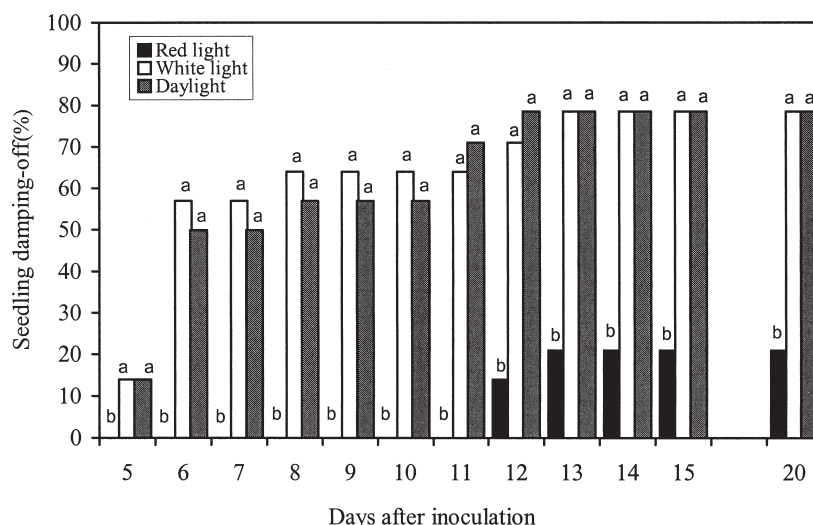


Fig. 2. Effect of light treatment on the occurrence of damping-off in 3-week-old pumpkin seedlings inoculated with *Phytophthora capsici* zoospores. Within each day, the columns with a letter in common are not significantly different from each other ($P = 0.05$, Fisher's Protected LSD).

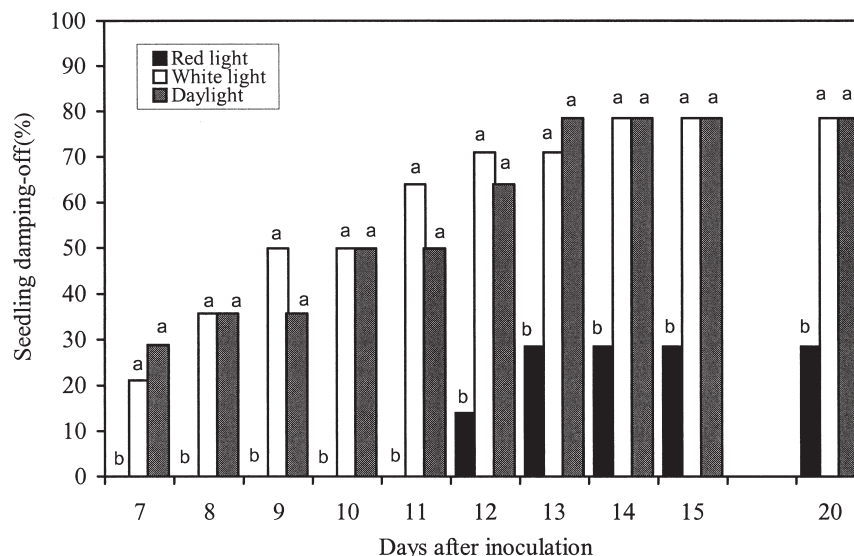


Fig. 3. Effect of light treatment on the occurrence of damping-off in 3-week-old pumpkin seedlings transplanted into the soil infested with *Phytophthora capsici*. Within each day, the columns with a letter in common are not significantly different from each other ($P = 0.05$, Fisher's Protected LSD).

kin, and tomato were 36.1, 33.0, and 37.6 cm, respectively; whereas the average values were 26.4, 25.1, and 26.7 cm for seedlings grown under NDL. The average fresh weight of red light-treated seedlings of pepper, pumpkin, and tomato was 9.8, 25.7, and 10.1 g, respectively; while the average fresh weight of seedlings of pepper, pumpkin, and tomato grown under NDL was 5.8, 19.6, and 6.4 g, respectively. The average dry weight of red light-treated seedlings of pepper, pumpkin, and tomato was 1.5, 3.0, and 1.7 g, respectively, compared to 0.82, 1.9, and 0.87 g, respectively, for NDL. There was no significant difference in height, and fresh and dry weight of seedlings grown under NDL or WL.

Discussion

Red light treatment of seedlings in greenhouse trials significantly reduced the subsequent occurrence of *Phytophthora* damping-off in pepper, pumpkin, and tomato seedlings compared with plants grown under NDL or WL. The results indicate that the red light treatment induces resistance in seedlings against *P. capsici*. These findings agree with the report by Umezu et al. (1999) in which red light treatment induced resistance in detached leaves of pepper, pumpkin, and eggplant against *P. capsici*.

Seedlings irradiated by white light did not show any disease suppression and there was no significant difference in occurrence of seedling damping-off between the plants grown under WL and NDL. The light intensity of the red light region of a daylight-type white fluorescent tube was measured using a BP-60 filter as described by Islam et al. (1998b); the resulting light intensity was $74 \mu\text{W}\cdot\text{cm}^{-2}$. In this experiment, the intensity of red light provided by the red light fluorescent tubes used for irradiation of seedlings was $287 \mu\text{W}\cdot\text{cm}^{-2}$, indicating that the intensity of red light might be an important factor in inducing resistance in plants. However, further investigations are needed to evaluate the effects of time and period of irradiation and the intensity of red light in inducing resistance in plants. In our study, the seedlings were irradiated continuously by red light from fluorescent tubes. About one-fourth of the sunlight reaching the earth's surface is red light (Honda et al., 1977). So, the supplemented red light from fluorescent tubes during daytime may not be very important and irradiated red light during the night may contribute to inducing resistance against *P. capsici*.

Among the light components, red light (600–700 nm) has been reported to be the most effective wavelength region for photosynthesis in green plants (Thomas et al., 1973). However, we don't know the mechanism of induction of resistance by red light treatment in plants against *P. capsici*. Red light irradiation may induce the production of anti-fungal substance(s) in plants. It has been reported that irradiation of broadbean leaves by red light induced the accumulation of an antifungal substance in leaf tissue. This substance prohibited germination of spores of several fun-

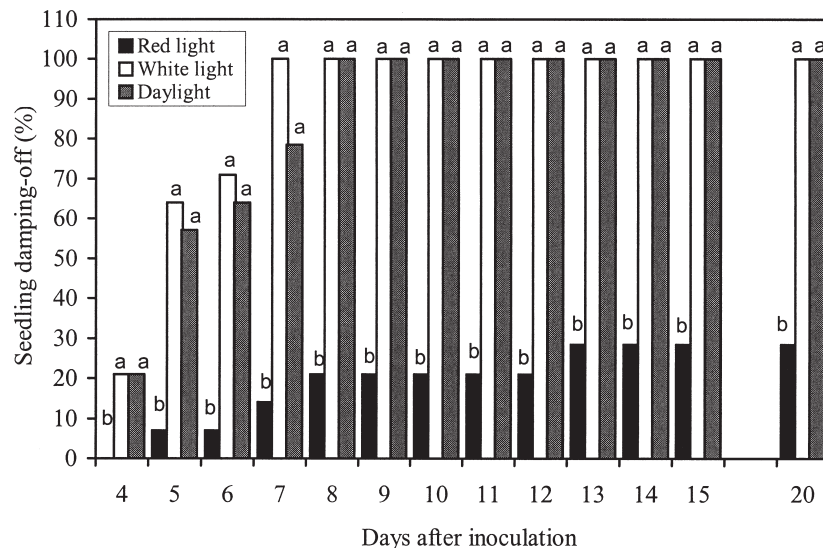


Fig. 4. Effect of light treatment on the occurrence of damping-off in 4-week-old tomato seedlings inoculated with *Phytophthora capsici* zoospores. Within each day, the columns with a letter in common are not significantly different from each other ($P = 0.05$, Fisher's Protected LSD).

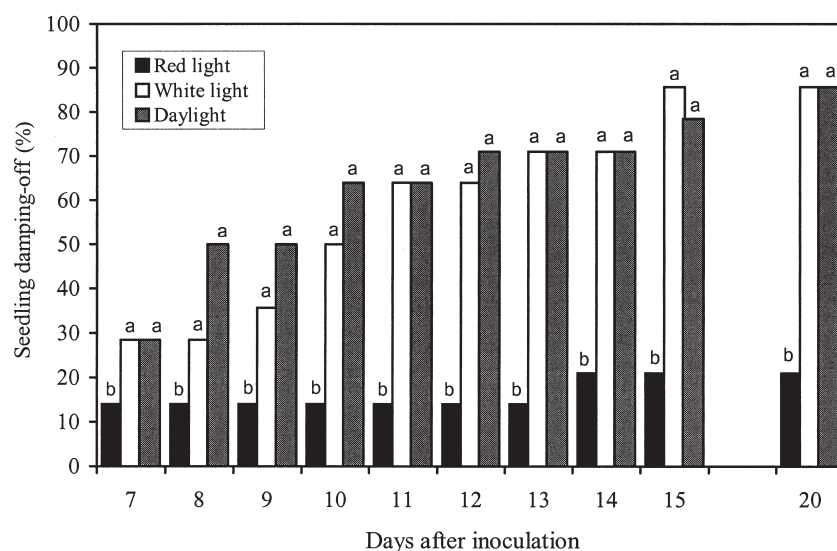


Fig. 5. Effect of light treatment on the occurrence of damping-off in 4-week-old tomato seedlings transplanted into the soil infested with *Phytophthora capsici*. Within each day, the columns with a letter in common are not significantly different from each other ($P = 0.05$, Fisher's Protected LSD).

Table 1. Effect of light treatment on height and fresh and dry weight of vegetable seedlings.

Vegetable ^a	Height (cm)				Fresh weight (g)				Dry weight (g)			
	Red light	White light	Day light	LSD (0.05)	Red light	White light	Day light	LSD (0.05)	Red light	White light	Day light	LSD (0.05)
Pepper	36.1	26.9	26.4	1.70	9.8	6.0	5.8	0.46	1.5	0.79	0.82	0.23
Pumpkin	33.0	25.4	25.1	1.26	25.7	20.0	19.6	1.97	3.0	1.8	1.9	0.18
Tomato	37.6	26.9	26.7	1.50	10.1	6.7	6.4	0.46	1.7	0.83	0.87	0.19

^aHeight, and fresh and dry weights were determined 6 weeks (pepper and tomato) and 4 weeks (pumpkin) after seedling emergence. Each value represents the average of 12 plants.

gal pathogens, including soilborne fungi (Islam et al., 1999). Antifungal substances also have been detected in plants in response to UV light irradiation (Bridge and Klarman, 1973; Hadwiger and Schwochaw 1971; Kato et al., 1994; Kodama et al., 1992).

In the experiments conducted, 78% to 100% of plants grown under either WL or NDL became infected with *P. capsici* and died. On

the other hand, only 21% to 36% of the red light-treated plants became infected, with the remaining plants continuing to grow well. This indicates that infection of pepper, pumpkin, and tomato plants with *P. capsici* in the fields may be reduced by red light treatment of the seedlings prior to transplanting.

In this study, seedlings were inoculated either by growing the plants in infested soil or

by adding a zoospore suspension onto the crown section of the seedlings. Both methods appeared to be reliable with respect to inoculating the plants and evaluating the effect of the red light treatment. The zoospore-inoculation method, however, was simpler and faster than the soil-infestation method. Thus, the zoospores can be used as a rapid and reliable method for evaluation of the effectiveness of the red light treatment for reducing seedling infection with *P. capsici*. However, final evaluation of the red light-induced resistance must be carried out in the field soil highly infested with *P. capsici* under conditions conducive for development of *Phytophthora* damping-off.

In this study, 3- to 4-week-old seedlings were inoculated either by transplanting into infested soil or with a zoospore suspension. Within 2 weeks of inoculation, 78% to 100% of pepper, pumpkin, and tomato plants grown under WL or NDL became infected with *P. capsici* and died, indicating that these plants, and perhaps other hosts of *P. capsici* as well, are susceptible to *P. capsici* during early growth stages. This is similar to the finding by Roberts et al. (2000), who concluded that 6- to 8-week-old tomato plants were susceptible to *P. capsici*, with up to 100% of the plants becoming infected and dying. As a result, the effectiveness of a red light treatment on preventing infection with *P. capsici* can be evaluated in young plants.

Red light treatment promoted seedling growth, significantly increasing height, and fresh and dry weight of plants, compared with those grown under either WL or NDL. The dry weight of red light-treated seedlings was almost twice that of plants grown under WL or NDL. Such rapid growth may be a significant factor in reducing susceptibility of plants to *P. capsici*, as well as for early crop production.

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