Bull. Fac. Agr. Shimane Univ., 28 : 37−43, December 20, 1994

Control of Damping-Off of Spinach with Ultraviolet-absorbing Vinyl Film

Yoko NAITO and Yuichi HONDA

Abstract Root diseases of damping-off and Fusarium wilt are the most serious problems in the summer cultivation of spinach (*Spinacia oleracea* L.) in a greenhouse. The incidence of root diseases was suppressed to one-third in the greenhouse with ultraviolet-absorbing vinyl film (UVA) as compared with that in the common agricultural vinyl film (CA) greenhouse. Suppression of the disease by UVA was evident in the summer cultivation at high temperatures from June through September. The fungi isolated from the diseased roots of spinach were mainly *Pythium* spp., *Fusarium* spp. and *Rhizoctonia* spp. Isolation frequency of *Pythium* spp. was the highest, followed by *Fusarium* spp., and the occurrence of *Rhizoctonia* spp. was sporadic. Among these fungi responsible for damping-off of spinach, *Fusarium* spp. were suppressed the most in the UVA greenhouse.

Key words: Spinach; disease control; ultraviolet-absorbing vinyl film.

Introduction

Many environmental factors influence on the incidence of plant diseases. It has been reported that certain diseases were suppressed under ultraviolet-absorbing vinyl film (UVA), which cuts off radiation shorter than 390 nm (Honda and Yunoki, 1977). The suppression of the disease by UVA has been reported to be due to the inhibition of sporulation of causal fungi. Diseases of greenhouse vegetables such as gray mold of cucumber (*Cucumis sativus* L.) caused by *Botrytis cinerea* Pers. and Sclerotinia disease of tomato caused by *Sclerotinia sclerotiorum* (Lib.) de Bary were effectively controlled by UVA (Honda *et al.*, 1977; Honda and Yunoki, 1977). Control of brown spot disease by UVA of cultivated chrysanthemum (*Chrysanthemum morifoliorum* Ramat.) caused by *Septoria obesa* Sydow was reported to be due to disturbance of negative phototropism of spore germ tubes, resulting in reduction of fungal invasion (Honda *et al.*, 1992). Thus, some air-borne diseases have been reported to be controlled in the UVA greenhouse. But little is known about the effect of UVA on the development of soil-borne diseases.

We reported here on control of spinach damping-off by UVA and the effect of UVA on the causal fungi involved in damping-off of spinach.

Materials and Methods

Fungal microflora of the greenhouse soil

Before cultivation of spinach, soil samples in CA and UVA greenhouses were collected from the top 15 cm surface soil at five sites where the spinach plants are to

Yoko NAITO: Graduate student of United Graduate School of Agriculture, Tottori University

be cultivated in the greenhouses. Fungi were isolated by the soil plate method with Martin's medium (Martin, 1950). Each sample of ca. 30 mg dry soil was mixed throughly with 20 ml of the melted medium in a Petri dish (9 cm diam.) and incubated for 4 days at 25°C in the dark. Fungal colonies appeared on the plate medium were isolated by their hyphal tips and transferred to potato sucrose agar (PSA) slants for identification. At least 150 isolates from each soil sample were isolated for identification.

Cultivation of spinach in the greenhouses

Spinach (*Spinacia oleracea* L. cv. Okame) was cultivated in a 4.5×9.0 m greenhouse with UVA (Cut-Ace-Clean, Mitsubishi Kasei, Ltd., Tokyo) with a lower limit of transmission at 390 nm from July 11 to August 15. As a control, spinach was grown in a greenhouse with CA (Clean-Ace, Mitsubishi Kasei, Ltd., Tokyo) with a lower limit of transmission at 290 nm.

In CA and UVA greenhouses, naked spinach seeds without pericarp (Takii Seeds Inc., Kyoto) were sown in rows at 5 cm intervals (1 cm deep) in blocks (60×300 cm). During growing period of 35 days, the number of emergence and damping-off of spinach seedlings were counted daily, and the percentages of emergence (total number of emergence/total number of seeds sown) and damping-off (total number of damping-off seedlings/total number of emergence) were calculated. At the end of experiment, the plant height was measured and number of leaves of healthy plants were counted.

Isolation of fungi from diseased seedlings

All of the damping-off seedlings were washed under running tap water for over 2 hr and cut into segments of ca. 5 mm for isolation of fungi. Then, each segment was placed in the center of a water agar plate (WA; 15 ml/9 cm Petri dish). After incubation for 3-7 days at 25°C, hyphal tips growing out from the segment were isolated and transferred to PSA slants for identification (Naiki and Kanoh, 1978).

Isolates of *Pythium* spp. were grown on grass leaf culture in the dark at 24°C for 4 days to induce formation of reproductive structures. Species were identified according to Waterhouse's key (Waterhouse, 1967). *Fusarium* spp. were identified according to Matuo (1980) and *Rhizoctonia* spp. were identified by staining the nuclei with HCl-Geimsa (Sneh *et al.*, 1991).

Artificial inoculation

A vilurent isolate of *Fusarium oxysporum* Schlecht. emend. Snyd. & Hans. isolated from a damping-off seedling was used for artificial inoculation in order to confirm the suppressive effect of UVA on spinach seedling damping-off. Spores from colonies cultivated on PSA plates for 10 days at 25°C was suspended in sterilized distilled water. Spore suspension was filtered through 2 layers of tissue paper to eliminate large mycelial fragments. Spore concentrations were adjusted to 10^3, 10^4, 10^5 and 10^6 spores/ml. The spore suspension (300 ml) was mixed with processed soil for gardening (3000 ml, Kureha-Engeibaido, Kureha-Chemicals Co., Ltd.) in a

Wagner's pot (1/5000 a, Kiya Seisakusho Ltd., Tokyo). The same amount of sterilized distilled water was mixed with the soil as a control. Three pots per treatment were used. Ten seeds were sown in each pot and all pots were kept in a glasshouse for 7 days until most seedlings emerged in the control pots. Then, pots were transferred to CA or UVA greenhouse and plants were grown for 33 days. During the experiment, the damping-off seedlings of spinach were counted and were sampled for re-isolation of the fungus.

Table 1. Fungal microflora of greenhouse soil [1].

	CA[2]	UVA[3]
Total number of isolates (/g dry soil)	8550	8977
Percentage of species isolated		
Trichoderma spp.	46.1%	34.4%
Penicillium spp.	18.2	30.6
Fusarium spp.	18.8	23.8
Aspergillus spp.	11.5	2.5
Pythium spp.	3.6	1.9
Others	1.8	6.8

[1] Soil fungi were isolated by soil plate method with Martin's medium.
[2] Soil samples from CA greenhouse.
[3] Soil samples from UVA greenhouse.

Results

Fungal microflora of the greenhouse soil

Total numbers of fungal isolates obtained from CA and UVA greenhouse soil samples were 8550/g dry soil and 8977/g dry soil, respectively (Table 1). Soil fungal microfloras before the experiment was practically the same for both of CA and UVA greenhouses. Fungi isolated from each greenhouse soil were consisted mainly of *Trichoderma* spp., *Penicillium* spp., *Fusarium* spp., *Aspergillus* spp. and *Pythium* spp. were also isolated, but *Rhizoctonia* spp. were not isolated by this method.

Greenhouse experiments

In summer cultivation (July 11-August 15), the percentage of damping-off seedlings in CA greenhouse increased steadily through the growing period and amounted to 86% at the end of the experiment (Fig. 1). On the contrary, number of damping-off seedlings in UVA greenhouse increased slowly and the percentage of damping-off seedlings remained 29% at the end of the experiment. Percentages of emergence were 62% in the CA greenhouse and 85% in the UVA greenhouse.

The fungi isolated most frequently from the diseased plants were *Pythium* spp., *Fusarium* spp. and *Rhizoctonia* spp. in both of CA and UVA greenhouses (Table 2). Frequency of isolation was highest with *Pythium* spp., followed by *Fusarium* spp. and *Rhizoctonia* spp. in this order. Other than these species *Penicillium* spp., *Alternaria*

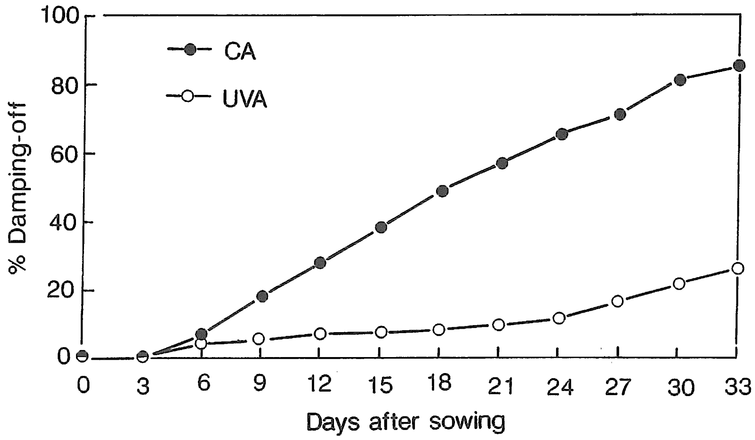

Fig. 1. Effect of common agricultural vinyl film (CA) and ultraviolet-absorbing vinyl film (UVA) on the development of spinach damping-off.

spp. and unidentified species of fungi were isolated. Most isolates of *Pythium* spp. were identified as *Pythium aphanidermatum* (Edson) Fitzpatrick and most isolates of *Fusarium* spp. were mainly *Fusarium oxysporum* Schlecht. emend. Snyd. and Hans. *Rhizoctonia* spp. were revealed to be consisted of bi-nucleate *Rhizoctonia* spp. and multi-nucleated *Rhizoctonia* spp. of 71% and 29% of 42 isolates in total, respectively, from both greenhouses.

Total number of isolates obtained from diseased plants in the UVA greenhouse was half of that in the CA greenhouse. Isolation frequency of *Fusarium* spp. from the diseased plants in the UVA greenhouse significantly decreased as compared with that of the CA greenhouse. Total number of *Pythium* spp. isolates also decreased in the UVA greenhouse. On the contrary, isolation frequency of *Rhizoctonia* spp. was higher in the UVA greenhouse, especially in the later stage of growing period, at that time overgrown plants became dense in the UVA greenhouse in the summer cultivation.

Damping-off by *Pythium* spp. was most serious, and spinach wilt caused by *Fusarium* spp. at 5 leaf-stage and later was next to it in the summer cultivation.

Growth of spinach was significantly accelerated ($P<0.05$) in the UVA greenhouse as indicated by the plant height of 5.6 cm as compared with 2.3 cm in the CA greenhouse at 21 days after sowing.

Artificial Inoculation

Artificial inoculation with a virulent isolate of *F. oxysporum* resulted in severe disease at high inoculum concentrations (Fig. 2). There was no difference in disease incidence between CA and UVA greenhouses at inoculum concentrations higher than 10^5 spores/ml. However, at lower inoculum concentrations than 10^4 spores/ml, the disease incidence became significantly lower in the UVA greenhouse than in the CA greenhouse.

Table 2. Number of fungal isolates from diseased spinach seedlings.

Isolates	CA[1]	UVA[2]
Pythium spp.	556 (71.5)[3]	271 (71.7)
Fusarium spp.	211 (27.1)	67 (17.7)
Rhizoctonia spp.	4 (0.5)	38 (10.1)
Others	7 (0.9)	2 (0.5)
Total	778 (100.0)	378 (100.0)

1) Diseased seedlings from CA greenhouse.
2) Diseased seedlings from UVA greenhouse.
3) Relative isolation frequency is shown in the sector.

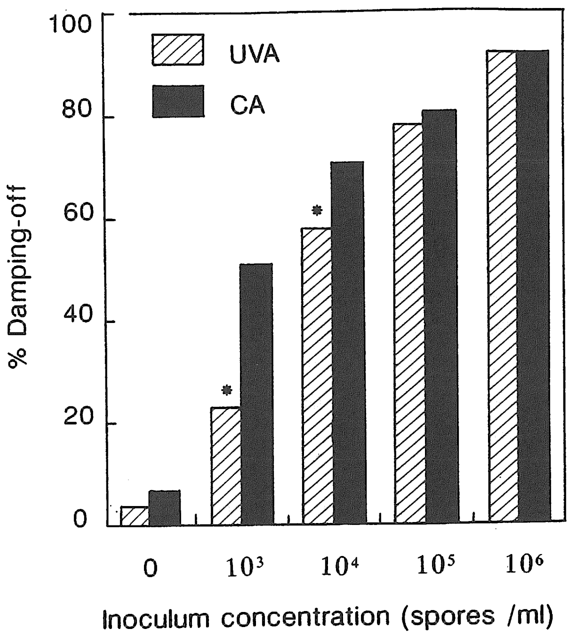

Fig. 2. Suppression of damping-off of spinach by UVA in pot experiment inoculated with spore suspension of *Fusarium oxysporum*. Percentages of damping-off were determined 40 days after inoculation and sowing. The asterisks indicate significant difference ($P<0.05$) between CA and UVA greenhouses by Duncan's new multiple range test.

Discussion

It is difficult to control the soil-borne diseases of spinach, such as damping-off, Fusarium wilt and foot rot. In summer cultivation at high temperatures which are unfavorable for spinach cultivation, it is necessary to take special care for physiological disorder besides disease control. In this study, we revealed that the incidence of soil-borne diseases of spinach in the UVA greenhouse was suppressed to one third as compared with that in the CA greenhouse. Further, the growth of plants in the UVA greenhouse was better than that in the CA greenhouse. Thus, UVA was effective for promoting the growth of spinach and for controlling damping-off. We have conjectured that growth promotioin in the UVA greenhouse resulted in induction of the disease resistance of spinach or the escape of infection.

There was the report that the pathogenic fungi isolated from damping-off, wilting, foot rot and root rot of spinach were mainly *Fusarium oxysporum* Schlecht. f. sp. *spinaciae* (Sherbakoff) Snyd. and Hans., *Rhizoctonia solani* Kühn., *Pythium* spp., *Aphanomyces* sp., *Phytophthora* sp. and *Fusarium solani* (Mart.) Appel and Wollenw. emend. Snyd. and Hans. in Japan (Naiki and Kanoh, 1978). In our experiment, the pathogenic fungi isolated most frequently from the diseased plants were *P. aphanidermatum* and *F. oxysporum*, followed by bi-nucleate *Rhizoctonia* spp.

Naiki and Morita (1983) showed that the differences of the isolation frequency of the pathogenic fungi and the incidence of the diseased plants were found among the fields which varied in their cropping histories and soil texture. Kodama (1988) reported that the Fusarium wilt of spinach was almost perfectly controlled in a greenhouse covered with another ultraviolet-absorbing vinyl film (Murasaki-Ace, Mitsubishi Kasei Ltd., Tokyo). In our experiment, suppression of damping-off in the UVA greenhouse was significant, but the disease was not perfectly controlled. This difference may be due to the difference in the soil texture and the inoculum potential of the soil. In the artificial inoculation experiment the disease was suppressed in the UVA greenhouse when the inoculum concentrations of *F. oxysporum* were 10^3 and 10^4 spores/ml. The disease incidence, however, did not differ between CA and UVA greenhouses at higher spore concentrations than 10^5 spores/ml. The result indicated that the UVA is effective for controlling damping-off of spinach seedlings only when inoculum potential is low.

References

HONDA, Y., T. TOKI and T. YUNOKI, Control of gray mold of greenhouse cucumber and tomato by inhibiting sporulation. Plant Dis. Rep., **61**: 1041-1044, 1977.

HONDA, Y. and T. YUNOKI, Control of Sclerotinia disease of greenhouse eggplant and cucumber by inhibition of development of apothecia. Ibid., **61**: 1036-1040, 1977.

HONDA, Y., T. KASHIMA and T. KUMAGAI, Suppression of brown spot disease of cultivated chrysanthemum by manipulating phototropic response of conidium germ tubes of *Septoria obese*. J. Phytopathology, **136**: 270-278, 1992.

KODAMA, F., Control of Fusarium wilt of spinach with ultraviolet-cutting vinyl film. A griculturist and Horticulturist, The April number: 66-68, 1988. (In Japanese.)

MARTIN, J. P., Use of acid, rose bengal and streptomycin in the plate method for estimating soil fungi. Soil Sci., **69**: 215-232, 1950.

MATUO, T., *Fusarium* species and identifications. *In* MATUO, T. *et al.* (eds.), *Fusarium Disease of Cultivated Plants*. Zenkoku Noson Kyouiku Kyoukai Publishing Co., Ltd., Tokyo, pp. 17-58, 1980. (In Japanese.)

NAIKI, T. and M. KANOH, Root diseases of spinach in plastic house cropping and their pathogens. Ann. Phytopath. Soc. Japan, **44**: 543-553, 1978.

NAIKI, T. and Y. MORITA, The population of spinach wilt fungus, *Fusarium oxysporum* f. sp. *spinaciae,* and the wilt incidence in soil. Ibid., **49**: 539-544, 1983.

SNEH, B., L. BURPEE and A. OGOSHI, Identification of *Rhizoctonia* species. APS Press, St. Paul, USA, 133 pp., 1991.

WATERHOUSE, G. M., Key of *Pythium* Pringsheim. Mycological Papers, No. 109, Mycol. Inst., Kew, England, 15 pp., 1967.

紫外線除去フィルムによるホウレンソウ立枯症の防除

内藤陽子・本田雄一

摘　　要

ホウレンソウ立枯症の発生は紫外線除去ビニルフィルム（UVA）のハウスでは一般農業用ビニルフィルム（CA）のハウスに比較して，約3分の1に抑制された。この抑制は夏期栽培で顕著であった。罹病部から分離される糸状菌は，主として，*Pythium* spp. と*Fusarium* spp., *Rhizoctonia* spp. であった。これらの内，*Pythium* spp. の分離頻度が一番高く，次いで*Fusarium* spp. が高く，*Rhizoctonia* spp. は散発的であった。立枯症に関与する菌の中では*Fusarium* spp. がUVAフィルムによって最も強く抑制された。