Control of Seedling Blast of Rice with Ultraviolet-Absorbing Vinyl Film

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ABSTRACT

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Almost 99% of 386 monoconidial isolates of *Pyricularia oryzae* were light-dependent for sporulation, whereas only 1% (four isolates) were light-independent. Without fungicides, rice (*Oryza sativa*) seedling blast incidence in the nursery at the four-leaf stage was reduced to less than 10% in an ultraviolet-absorbing vinyl film greenhouse compared with incidence levels in a common agricultural vinyl film greenhouse.

In Japan, almost all seedlings of rice (*Oryza sativa* L.) are grown in the greenhouse and transplanted at the three- to five-leaf stage by machine to paddy fields. Since seedling blast of rice may serve as the primary infection source for leaf blast (5,11), the potential for controlling rice blast disease in the field by limiting inoculum buildup during the seedling stage should be considerable.

Pyricularia oryzae Cavara was reported to sporulate when exposed to continuous near-ultraviolet (UV) radiation (300–400 nm) (3,6) or monochromatic radiation of 340 or 365 nm (8). These observations suggested that by filtering out near-UV wavelengths, sporulation in *P. oryzae* would be inhibited.

In this investigation, we examined the sporulation response of *P. oryzae* to monochromatic radiation and applied this information (2,10) to control the rice blast in the nursery.

MATERIALS AND METHODS

Organisms. Single-spore isolations of *P. oryzae* were made from infected rice seeds or leaf blast lesions of seedlings from a greenhouse using common agricultural vinyl film (CA-vinyl; Clean Ace, Mitsubishi Monsanto Chemical Co., Ltd.) or from a greenhouse using UV-absorbing vinyl film (UVA-vinyl; Hi-S, Nippon Carbide Industries Co., Inc.). Ninety-five isolates were obtained from diseased seeds, 226 from leaf blast lesions in the CA-vinyl greenhouse and 65 from leaf blast lesions in the UVA-vinyl greenhouse.

All isolates (386 total) were grown on potato-dextrose agar (PDA, Difco Laboratories, final pH 5.6) slants (5 ml per test tube 16 mm in diameter) in darkness for 10 days at 25 C or under alternating 12 hr of darkness and 12 hr of light from two 20 W black-light (FL20S·BLB, Toshiba Co.) and daylight (FL20S·D N/L, Toshiba Co.) fluorescent lamps suspended 15 cm apart and 30 cm above the cultures.

Slant cultures were covered with CA-vinyl or UVA-vinyl during light exposure. One isolate that sporulated in the absence of UV wavelengths (UVA-vinyl filter) and another that did not sporulate in the absence of UV wavelengths but was induced to sporulate abundantly under full light spectrum (CA-vinyl filter) were examined for sporulation responses to monochromatic radiation.

Monochromatic irradiation. Monochromatic radiation (300–700 nm) was obtained by a series of interference filters with two 20W black-light and four 20W daylight fluorescent lamps as light sources. Details of irradiation were described previously (1,10). Cultures were irradiated continuously for 10 days at 25 C. Spore suspension was made by adding 5 ml of a 50% sucrose-0.05% Tween 20 solution to each plate and gently scraping the whole surface of the colony with a rubber spatula. Spore concentration was determined with a hemacytometer. The experiements were repeated twice.

Greenhouse experiments. The production of spores on blast-infested seeds was examined by the blotter test (7) under CA-vinyl- or UVA-vinyl-filtered light.

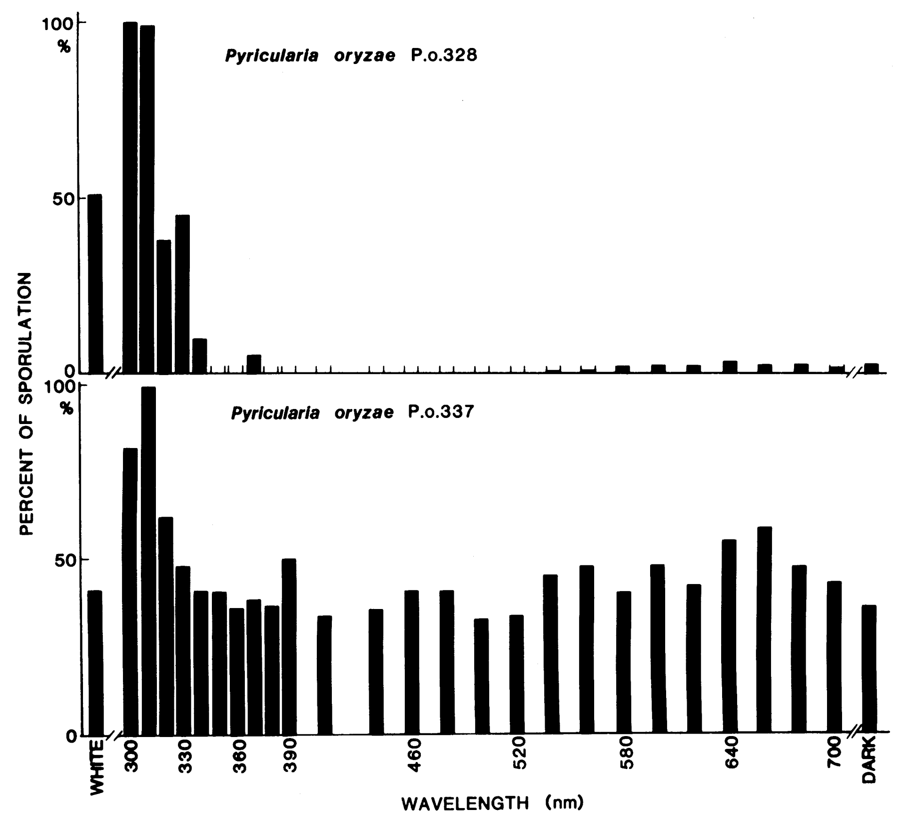

Fig. 1. Effect of monochromatic radiation on sporulation of two strains of *Pyricularia oryzae*. Colonies were irradiated continuously for 10 days under different interference filters (300–700 nm) with suitable glass filters to cut high-order transmission at 25 C. One culture was used for each treatment.

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Five hundred seeds, 25 seeds for each of 20 Pyrex petri dishes (90 mm in diameter) were placed on three layers of moistened blotter paper. Ten dishes were placed in each greenhouse of CA-vinyl and UVA-vinyl for 5 days, and spore production on the seeds was determined with a stereoscopic microscope.

Seeds were sown in paper pots (1.5 × 1.5 cm, 760 pots to a nursery box, three seeds for each pot) filled with the artificial nursery soil on 5 September. Blast-infested seeds were sown in 25 pots at the center of the box as the primary infection source, and seeds disinfected by a 12-hr fungicide dip in a mixture of benomyl (500 mg a.i./L) and thiram (500 mg a.i./L) at ambient temperature were sown in the rest. After an incubation at 31 C in a moist chamber for 2 days, the nursery boxes with germinated seeds were transferred to the CA-vinyl or the UVA-vinyl greenhouse. Temperatures of both greenhouses usually fluctuated between 10 and 26 C. Development of seedling blast was recorded by counting lesions on leaves of 33 plants for each nursery box and three boxes for each vinyl greenhouse.

The experiment was repeated in April to May. In this test, all plants of five nursery boxes for each vinyl greenhouse were examined for lesions on the leaves at the final nursery stage 35 days after sowing, and the numbers of lesions for each paper pot at each location were averaged.

RESULTS

Of 386 isolates of *P. oryzae* obtained from infested seeds and lesions on leaves, four isolates sporulated moderately and the rest failed to sporulate in darkness. More than 96% of isolates sporulated abundantly under alternating light and darkness with the CA-vinyl cover. In contrast, only 10 isolates sporulated moderately and the rest of the isolates did not sporulate under alternating light and darkness with the UVA-vinyl cover.

Of 65 monoconidial isolates from leaf blast lesions in the UVA-vinyl greenhouse, only two isolates were induced to form a small number of spores by UVA-vinyl-filtered light. This result indicated the possibility that the UVA-vinyl filtering system did not favor the UV-independent strains to become dominant in the rice blast population.

One (P.o.337) of 10 isolates that sporulated in the absence of UV wavelengths and another (P.o.328) from a group of isolates that did not sporulate in the absence of UV wavelengths but was induced to sporulate abundantly under full light spectrum had different sporulation responses to monochromatic radiation (Fig. 1). Isolate P.o.328 formed few spores in darkness and was induced to sporulate by UV radiation shorter than 330 nm. UV radiation of 300 and 310 nm was especially effective for inducing sporulation by this isolate. Isolate P.o.337 sporulated moderately in darkness but sporulated profusely under continuous irradiation of monochromatic UV wavelength (300 or 310 nm). Both isolates sporulated moderately under continuous white light. Isolate P.o.328 was placed in the UV-induction group and isolate P.o.337, in the UV-acceleration group.

Although sporulation was moderate in both isolates, it can also be regarded that both were inhibited by 50% under white light from daylight and black-light fluorescent lamps (Fig. 1). Sporulation reduction in isolate P.o.328 may be ascribed to the wavelengths of 380–520 nm, which showed discernible inhibitive

Table 1. Effect of ultraviolet-absorbing vinyl film on development of blast of rice plant seedlings caused by *Pyricularia oryzae*

Film	Number of lesions[a] at each date: 25 Sept.	2 Oct.	5 Oct.	9 Oct.	12 Oct.	16 Oct
UVA vinyl[b]	0	0.0	0.1	0.2	0.7	1.6
CA vinyl[c]	0	1.2	1.2	3.4	8.2	12.4
Ratio of lesions (UVA/CA)	...	0.00	0.08	0.06	0.09	0.13

[a] Average number of type 4 lesions (susceptible type) per plant on 99 plants.
[b] Ultraviolet-absorbing vinyl.
[c] Common agricultural vinyl.

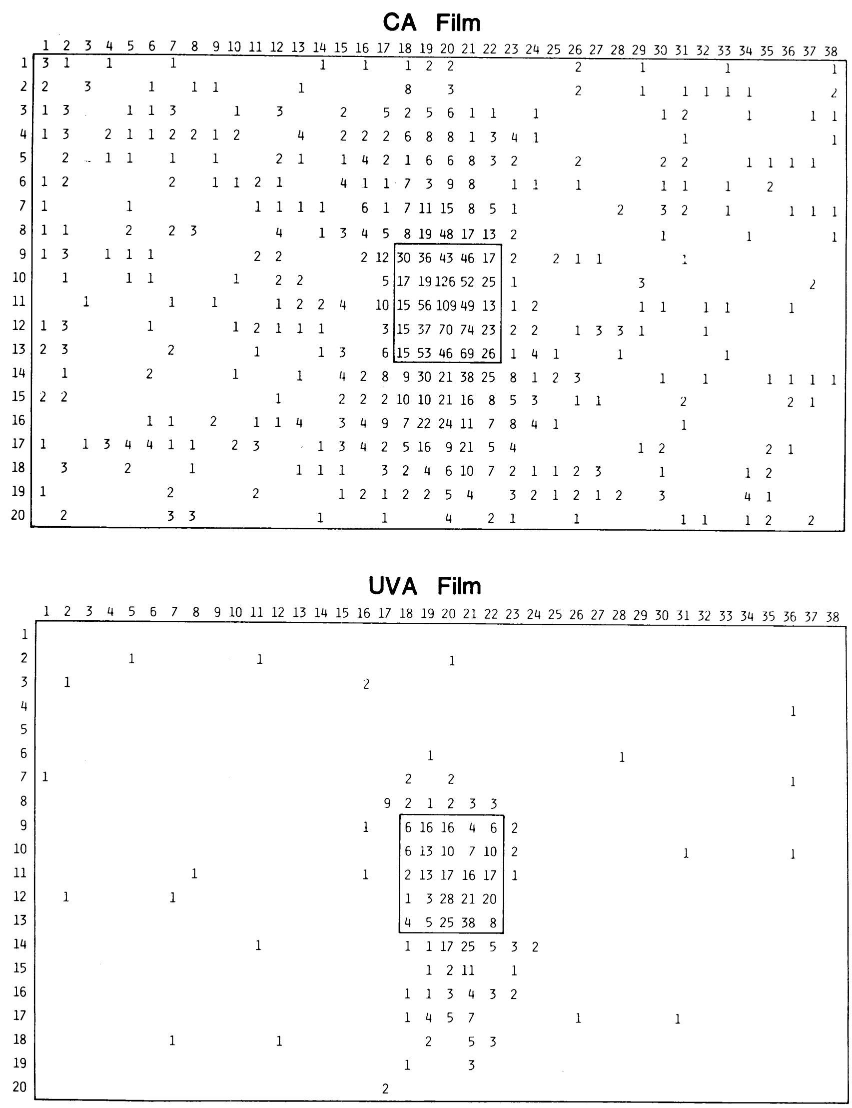

Fig. 2. Distribution of leaf blast lesions from infested seeds sown at the center (within the frame) of the nursery box 35 days after sowing in common agricultural (CA) vinyl and ultraviolet-absorbing (UVA) vinyl greenhouses. Each figure represents the average number of type 4 lesions per three seedlings in one pot. Five nursery boxes were examined for each vinyl greenhouse, and numbers of lesions were averaged for five paper pots of the same place in five nursery boxes (one pot per box).

activity to sporulation. In isolate P.o.337, however, no monochromatic radiation was inhibitory to sporulation. A possible explanation for sporulation decline in isolate P.o.337 under white light is that an irradiation dosage of UV wavelengths from black-light lamps would have been excessive for this isolate (4).

In the study on production of spores by blast-infested seeds, 63.8% of the infested seeds formed spores in the CA-vinyl greenhouse compared with 1.3% in the UVA-vinyl greenhouse. Blast of rice seedlings in the nursery boxes was first observed 27 days after sowing in the CA-vinyl greenhouse; 3 days elapsed before disease was seen in the UVA-vinyl greenhouse. The number of type 4 (susceptible) lesions (9) per plant increased from 1.2 on 2 October to 12.4 on 16 October in the CA-vinyl greenhouse (Table 1). In contrast, the number of lesions increased slowly, with only 1.6 lesions per plant on 16 October, in the UVA-vinyl greenhouse.

Secondary infection from infested seeds sown at the center of the nursery box was restricted to the vicinity of the infection source, and the number of lesions per plant remained at a low level in the UVA-vinyl greenhouse (Fig. 2). In contrast, secondary infection covered the entire nursery box, and the number of lesions in plants around the infested seeds reached more than 16 per plant, which frequently led to plant death. The number of lesions on plants from infested seeds was also greater in the CA-vinyl greenhouse.

DISCUSSION

Two strains of *P. oryzae* with different responses to light for sporulation were obtained: strain P.o.328 requires light for sporulation and strain P.o.337 sporulates in darkness with sporulation enhanced by UV radiation 310 nm and shorter. The light-dependent strains seem to predominate in the field population of *P. oryzae*. Isolation frequency of light-independent strains was about 1%. Inhibition of sporulation appears to be the basis for effective suppression of rice blast in the nursery stage in the UVA-vinyl greenhouse.

Blast lesions on rice seedlings remain for more than 30 days after transplanting in the paddy field (5). As temperature rises above 20 C, conidia will form abundantly on the lesions and serve as the primary infection source in the field. In Japan, most rice seedlings are grown in nursery boxes in the vinyl greenhouse until the three- to five-leaf stage, then transplanted to the paddy field by machine. Transplanting seed-transmitted rice blast will result in an early outbreak of leaf blast in the field (11). Through the use of UVA-vinyl as the covering material of the greenhouse, seedling blast at the four-leaf stage, when the seedlings were transplanted, was reduced to less than 10% compared with diseased seedlings in the CA-vinyl greenhouse. These results suggested an excellent potential for controlling a field disease by controlling inoculum buildup during the seedling stage in the nursery with UVA-vinyl and without fungicidal applications.

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