Effect of Temperature on Lesion Enlargement and Sporulation of Pyricularia oryzae in Rice Leaves

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ABSTRACT

Blast lesions on rice leaves expanded faster but reached a smaller final size at high temp regimes of 32 C continuously, 32/25 C day/night, or 32/20 C day/night in a 12-h thermoperiod than at 25 C or 25/16 C day/night. At 16 C, or 20/16 C day/night, the enlargement rate was slow and constant during the 25 days of observation. The potential for conidium formation reached a peak value earlier at higher temp than at median and lower temp. The maximum potential occurred at the median temp. Sporulation proceeded for more than 20 days at each thermal treatment.

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Pyricularia oryzae Cav., the pathogen of rice blast disease, is temperate and tropical in distribution (3, 15). Epidemics of the disease appear to be determined mainly by the disease proneness of the host, the amount of inoculum, temp, and the length of periods when the plant surfaces are wet (2, 4, 14). The potential for pathogen sporulation in lesions was investigated under field conditions in northern Japan (6). The effects of controlled temp on lesion enlargement and the sporulation potential of the fungus in lesions were examined. Sporulation potential is defined as the capacity of a fungus to produce conidia under optimum conditions per unit of time.

MATERIALS AND METHODS.—Rice (*Oryza sativa* L. 'Otori') and an isolate of *Pyricularia oryzae*, Hoku 373 of race JN-1, were used. This isolate can be obtained from the National Institute of Agricultural Sciences in Japan. Rice plants were grown under flooded conditions in plastic pots 15 cm in diam × 20 cm high, containing 3.5 kg soil, 5 g $(NH_4)_2SO_4$, 5 g $Ca(H_2PO_4)_2$, and 3 g KCl. Nine pots with three plants each were prepared for each treatment. The 10th leaf on the main culm (10/0-leaf) was inoculated immediately after expansion by using a punch method (12). The fungal isolate was grown at 28 C for 9 days and illuminated by near-ultraviolet light with a light intensity of 1.5×10^{-6} cal/cm^2/min (measured with a Kipp & Zonen's compensated thermopile) for 2 days after brushing off aerial mycelia with sterile distilled water. Spores produced were harvested in water. A 20-ml suspension of spores was adjusted to approximately 10^5 cells/ml. The spore suspension was poured through 1 g of powdered cellulose on filter paper in a funnel, and 0.3 g of carboxymethyl cellulose was added to make a paste (6). The spore paste was placed on four pinched sites (each 2.5 mm in diam and 5 cm apart) per leaf. Inoculated plants were sprayed with water incubated at 28 C in a moist chamber for approximately 20 h, and placed in a greenhouse at approximately 25 C until lesions began to develop. Growth cabinets consisted of glass boxes of walk-in type with thermoregulators used to maintain temp of 16 ± 3, 20 ± 2, 25 ± 1, and 32 ± 2 C under natural light. For experiments with alternating day/night temp, inoculated plants were incubated after initiation of lesion development in separate growth cabinets with day (12 h) and night (12 h) temp of 20(±3) and 16(±2) C (20/16 C), 25(±1.5)/16(±1) C, 32(±1.5)/20(±1) C, or 32(±1.5)/25(±1.5) C. The thermal level was changed at both 0600 and 1800 hours. Average light intensity during experiments ranged from 0.17 to 0.83 cal/cm^2/min.

The length of 12 lesions was measured every 2 to 4 days for about 25 days. Three 10/0-leaves were removed at each time of measurement to evaluate sporulation potential of the pathogen. The detached leaves were wiped with wet cotton. Lesions with healthy tissue were cut from the leaf. Each was placed against the inner wall of a small glass tube 1.2 cm in diam and 2.7 or 5.0 cm high, containing 0.2 ml of sterile distilled water. Either three or six tubes containing samples were held in larger tubes 2.8 cm in diam and 5.5 cm high with 5 ml of water, lined inside with moistened filter paper and seven larger tubes were held in a dish 9 cm in diam and 7 cm high, with 50 ml water. The inner wall was lined with moistened filter paper and Parafilm was spread over the upper part, and overlain with a glass cover. The moistened container was kept at 28 C in darkness for 15 h. Conidia were counted by use of a hemocytometer (6). Both series of experiments were repeated three times in different years, and representative results are shown.

RESULTS.—*Effect of constant temperatures on lesion enlargement and potential for sporulation.*—Exposure of plants to 32 C caused lesions to expand rapidly for 8 days and to approach a maximum length of 25 mm shortly thereafter (Fig. 1). A swift cessation of lesion enlargement then took place. At 25 C lesions expanded almost as rapidly as those at 32 C for 8 days and approached a maximum length of 35 mm during the next 12 days. Lesions expanded slower at 20 C than at 25 or 32 C and grew to a length of 25 mm in 20 days. At 16 C the rate of expansion was slow and constant over the 20-day period when a length of 20 mm was obtained. The margins of lesions became dark purple in color at 4, 7, 9, and 11 days at 32, 25, 20, and 16 C, respectively.

Sporulation potential is indicated in Fig. 2. The maximum potential was detected on the 3rd day at 32 C, on the 3rd day to the 9th day at 25 C, and on the 9th day at 20 C. No pronounced maximum peak occurred at 16 C. The maximum number of conidia produced on the peak day for 20 C was nearly double that for peak days at 32 or

25 C. Sporulation potential decreased much more rapidly with time in the higher temp regime than at the lower temp. The highest potential for accumulative spore production was detected in lesions expanding at 20 C. Peak days for conidial production coincided with the commencement of necrosis at the various temp. Leaves 11/0 to 14/0 required 7 days to expand at 32 and 25 C, 15 days at 20 C and no growth beyond expansion of leaf 11/0 occurred during the experiment at 16 C.

Effect of alternating day and night temperatures on

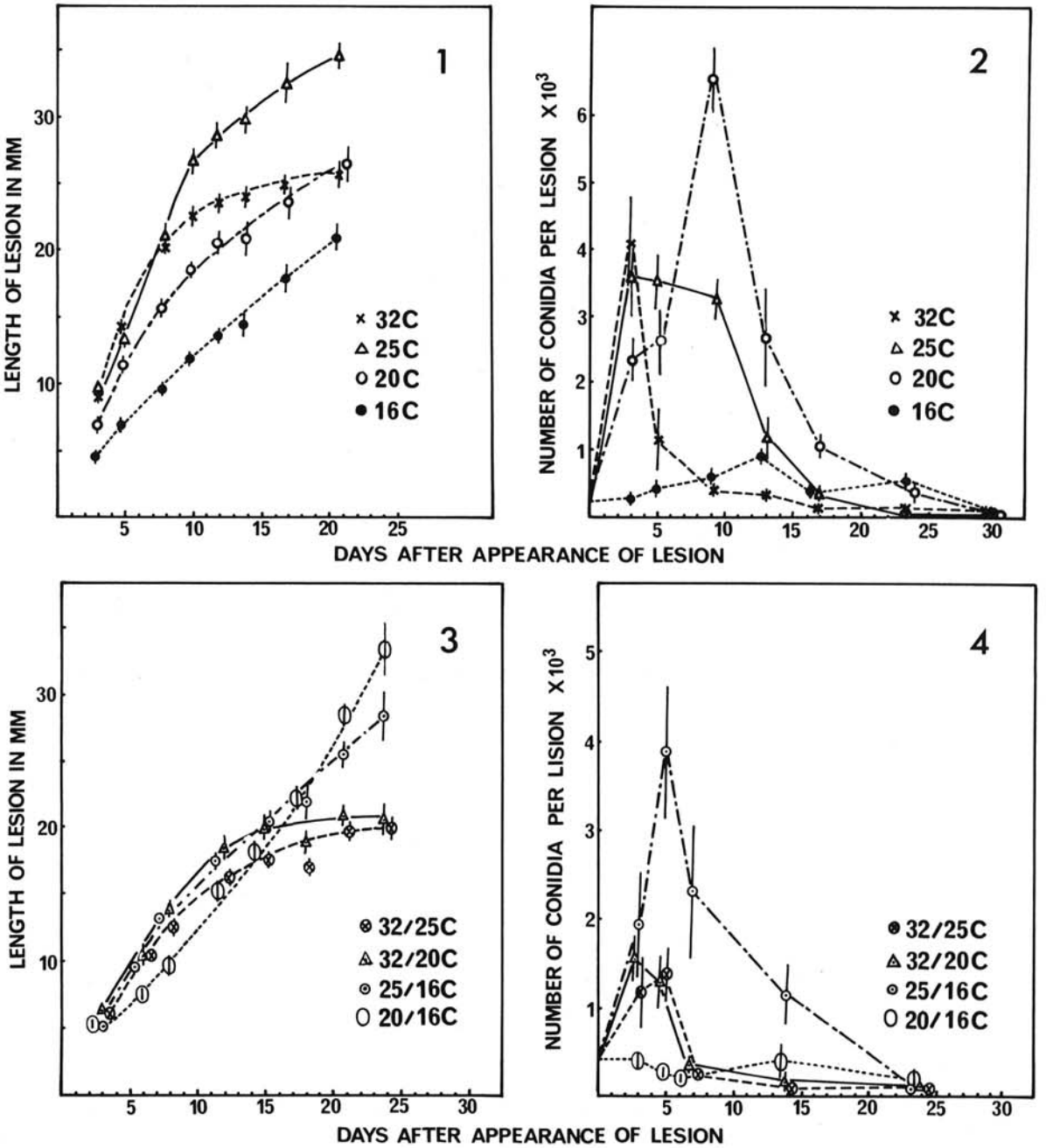

Fig. 1-4. **1)** The effect of constant temp on enlargement of leaf-blast lesions on Otori rice. The length of 12 lesions on the 10th leaves of main culms was measured. All points are an average with the standard errors represented by vertical lines at each point. **2)** Potential for conidium formation of *Pyricularia oryzae,* isolate Hoku 373 of race JN-1, in lesions that developed at constant temp. A new set of 12 lesions was sampled and used for each spore induction period. Spores were induced to form when lesions were placed in a humid atmosphere at 28 C for 15 h. **3)** The effect of alternating day and night temp on enlargement of leaf-blast lesions. The higher temp were employed during the day, and the lower temp at night. **4)** Potential for conidium formation of *Pyricularia oryzae* in lesions that developed at alternating temp.

lesion enlargement and potential for sporulation.—In the higher temp regimes of 32/25 C and 32/20 C treatments, lesion enlargement increased rapidly at first and leveled off by day 15 (Fig. 3). Under the median temp of 25/16 C, the rate of lesion enlargement was rapid initially, but somewhat less rapid from the 15th to the 25th day. Under the lower temp treatment of 20/16 C, the rate of lesion enlargement was less initially than that at higher temp treatments, but the rate remained constant for 25 days.

Maximum potential for sporulation occurred from the 3rd to the 5th day of lesion enlargement in both 32/25 C and 32/20 C treatment, and on the 5th day in 25/16 C treatment (Fig. 4). In 20/16 C treatment, no definite peak of potential for sporulation appeared. The highest potential for accumulative spore production was found in 25/16 C treatment.

DISCUSSION.—Mycelial growth on potato-sucrose agar plates at 16 C fits the equation $y = 1.71x - 2.77$, and at 25 C, $y = 3.51x - 3.33$, where y is a radius of the colony in mm and x is the time in days (R. Yoshino and T. Yamaguchi, *unpublished*). At 16 C lesions expanded according to the equation $y = 0.897x + 2.29$, and at 25 C, $y = -6.0 + 4.9x - 0.15x^2$, where y is length of lesion in mm and x ($x \leqslant 20$) is the time in days. Thus a linear increase was obtained in both mycelial growth on media and lesion expansion at 16 C. At 25 C and higher, cessation of lesion enlargement occurred in the later phase and the final size of lesion was limited. High temp within the tested range restricts lesion size of leaf blast indirectly by affecting development of the host. The rapid senescence of leaves is considered to cause the restriction of lesion enlargement (19).

Outbreaks of leaf blast can occur in Japanese fields following a 5-day period of temp between 15 and 33 C. It is common knowledge in Japan that high temp suppress blast development. Temperatures of 32 C (day) and 20 C (night) appear from late July to early August in Hokkaido, from mid-July to mid-August in Tohoku, and from late June to early July in southern regions (10, 11). In southern Japan, the temp rarely falls below 25 C from mid-July to early September. Therefore, prevailing temp near 30 C would limit the size of the lesions and their sporulating potential. The fungus in lesions sporulates between 12 and 34 C with optimum at 28 C. Hence, sporulation decreases sharply above 28 C (H. Kato, *unpublished*). Therefore, high temp at night curtails inoculum production of *P. oryzae* not only by decreasing the sporulation potential, but by decreasing sporulation itself. Ontogenetic predisposition accelerates the reduction of spore yield per lesion following formation of the panicle primordium during the summer season (5, 6). When temp near 20 C appear during these seasons, it is likely to induce a severe outbreak of blast by increasing the sporulation potential.

That plants predisposed to high temp during early stages of leaf development display resistance to disease is well documented (1, 4, 7, 8, 9, 13, 16, 17, 18). However in our studies, the temp treatments commenced with visible lesions after host penetration and colonization by the pathogen. Hence, high temp during pathogenesis and inoculum production are as important as high temp before infection to restrict the pathogen from causing epidemics of rice blast.

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