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Factors Affecting Sporulation of a Mycoherbicide, *Epicoccosorus nematosporus*, on the Lesion of *Eleocharis kuroguwai*

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Effects of temperature and dew period on sporulation of a mycoherbicide, *Epicoccosorus nematosporus*, on the lesion of its host, *Eleocharis kuroguwai* were determined. Conidia formation was first observed after 10 days on plants incubated for either 12 or 16 h in a dew chamber at 28°C; 16 h dew period resulted in more conidia formation. As the dew period was decreased to less than 8 h, fewer conidia formed. Conidial production was most abundant at 28°C and produced as much as 3.3×10^4 conidia per lesion, while 0.1×10^3 and 2.3×10^3 conidia per lesion were produced at 16°C and 36°C, respectively. Alternating temperature regimes, i.e., 30/15, 30/20, 28/20, and 28/15°C (day/night) were much better than constant temperature, i.e., 30/30, 28/28/, and 20/20°C for sporulation. In the second sporulation, there were as much as 3.1×10^4 conidia per lesion (ca. <50% of the first sporulation). Then, sporulation dropped sharply to 6.2×10^2 conidia per lesion in the third sporulation. Results of this study suggest that temperature combined with dew period is the primary limiting factor in the use of *E. nematosporus* as a mycoherbicide of *E. kuroguwai*.

Keywords : conidial production, *Eleocharis kuroguwai*, *Epicoccosorus nematosporus*, lesion, mycoherbicide.

Previous studies (Hong et al., 1991; 1992) show that *Epicoccosorus nematosporus* is a potential mycoherbicide for water chestnut (*Eleocharis kuroguwai*) in the rice fields. The fungus was found to be epiphytotic on water chestnut in the paddy field, and seemed to be well adapted in rice fields in Korea. Significant reduction in plant height and tuber formation has been described previously (Hong et al., 1995). Optimal inoculum concentration, application frequency, and timing have also been previously studied (Hong et al., 1996; 1997). The importance of environmental conditions, especially on biocontrol agents such as mycoherbicide, has been repeatedly emphasized since the early researches on mycoherbicide (TeBeest et al., 1978; 1985). The effects of dew period and temperature on the primary infections by mycoherbicide have been reported in several field tests (Elwakil et al., 1990; Kirkpatrick et al., 1982; Makowski et al., 1990). When the number of conidia on the primary lesion is low due to unfavorable environmental conditions, control efficacy can be expected from secondary infections, which have a functional relationship between pathogen population and environmental conditions (TeBeest et al., 1895; Walker, 1981). The lack of field effectiveness of potential agents has become a common problem in different areas of biological control over the past 20 years. Identifying the limiting factors and a clear understanding of the ecological structures and the dynamics of plant pathosystems are therefore, necessary. This study was conducted to determine the effects of dew period and temperature on the sporulation of *E. nematosporus* for the control of *Eleocharis kuroguwai*.

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Materials and Methods

Preparation of conidial inoculum. *Epicoccosorus nematosporus* YCSJ-112 (Korean Patent No. 109462) and *Eleocharis kuroguwai* were used for all greenhouse experiments. Aerial mycelia grown on 15-day-old PDA culture were scraped with a spatula, then stored in the growth chamber at 28°C with 4,500 lux fluorescent lamp for 48 h to induce sporulation (Hong et al., 2002). Abundant conidia produced on the surface of PDA were washed with distilled water, collected, and conidial concentration was adjusted as described previously (Hong et al., 1995).

Effect of surface wetness on sporulation. Immediately after inoculation, plants were placed in a dew chamber maintained at 28±1°C without illumination. Four wetness durations were tested: 0, 8, 12 and 16 h. After 15 days, the plants were transferred to the greenhouse bench maintained at 25±5°C. Sporulation was examined as described above.

Effect of temperature on sporulation. Thirty-day-old plants in pots were inoculated with conidial suspension (6.0×10^5 conidia per ml) (Hong et al., 1995). Immediately after inoculation, plants were placed in a dew chamber for 24 h at 28±1°C. Then, the plants were transferred to the growth chambers, with the

temperature set at 16, 21, 28, 32, and 36°C. After 15 days, 30 sporulated lesions on diseased stems were cut 1 cm long, placed in a small vial with 1 ml distilled water, then vigorously shaked with a vortex mixer for 1 minute to separate the conidia from the lesion. Three stems were examined in a pot and a total of three pots were used

Effect of temperature fluctuation on sporulation. After inoculation and dew treatment, the plants were transferred to the growth chamber with a diurnal cycle of 12 h with 4,500 lux of fluorescent light. Temperature fluctuations were 30/30, 20/20, 30/20, 30/15, 28/28, 28/20, or 28/15°C (day/night) for 15 days. Sporulation was examined as described above.

Degree of conidial regeneration. To examine the degree of conidial regeneration, first formed conidia on the lesion were removed with a handy aspirator, and the plants were returned to the growth chamber for another sporulation. Plants were placed in the dew chamber for 12 h at 28°C and transferred to the growth chamber with 14 h of fluorescent light (4,500 lux) illumination for 15 days The procedure was repeated five times at 2-10 days interval. The number of conidia was counted as described above.

Results and Discussion

Effect of surface wetness on sporulation. Conidia formation was first observed on plants after 10 days of incubation for either 12 or 16 h in a dew chamber at 28°C (Table 1); 16 h dew period resulted in more conidia formation. As dew period was decreased to less than 8 h, fewer conidia formed. At least 12 h of dew period were required for normal sporulation from lesion. This dew condition is unusual in rice fields in Korea. Hence, the fungus would be highly dependent on external dew period for inoculum production.

Effect of temperature on sporulation. Conidial production was most abundant at 28°C and produced as much as 3.3×10^4 conidia per lesion, while 0.1×10^3 and 2.3×10^3 conidia per lesion were produced at 16°C and 36°C, respectively (Table 2). The effect of temperature on disease development was comparable with the results of the study by Hannusch et al. (1996). Temperatures of 15-25°C were optimal for lesion formation for *Sclerotinia sclerotiorum* and lesions were not formed at 30°C (Hannusch et al., 1996; Abawi et al., 1975; Boland et al., 1987). In this study, however, optimal temperature for the sporulation was 28°C; even at temperature of as high as 32°C, conidia were still produced at 1.1×10^4 per lesion.

Effect of temperature fluctuation on sporulation. For sporulation, alternating temperature regimes, i.e., 30/15, 30/20, 28/20, and 28/15°C (day/night) were much better than constant temperature, i.e., 30/30, 28/28/, and 20/20°C (Table 3). Nothern jointvetch anthracnose develops rapidly over a wide range of temperature similar to other anthracnose diseases (Lauritzen et al., 1933; Leonard et al., 1976; Nutman et al., 1960). However, it fails to sporulate at constant temperature, i.e., few conidia are formed at 30/30 and 20/20°C (day/night). A similar result showed that anthracnose does not develop under constant temperatures of 28/28 or 30/30°C (day/night), but develops at different day and night temperatures (Tu, 1982). Significant diurnal temperature fluctuation may increase the secondary infec-

Table 1. Effect of surface wetness on conidia formation on the lesion caused by inoculation with conidia suspension of *Epicoccosorus nematosporus*

Duration of surface wetness (h)	No. of conidia per lesion ($\times10^3$ conidia)
0[a]	0.0 x[b]
8	29.6 y
12	45.6 z
16	48.3 z

[a] Dry which serves as control.
[b] Values are the average of four replications in each of three experiments. Data were analyzed statistically and means were separated by Duncan's new multiple range test for significance at p=0.05.

Table 2. Effect of temperature during incubation for conidia formation on the lesion inoculated with conidial suspension of *Epicoccosorus nematosporus*

Temperature (°C)	No. of conidia per lesion ($\times10^3$ conidia)
16	2.3 x[a]
21	12.7 y
28	33.3 z
32	11.0 y
36	0.1 x

[a] Values are the average conidial number from 30 lesions in each of three replications. Data were analyzed statistically and means were separated by Duncan's new multiple range test for significance at p= 0.05.

Table 3. Effect of different day/night temperatures on the development of conidia on lesion inoculated with conidia suspension of *Epicoccosorus nematosporus*

Average temperature of day/night (°C)	No. of conidia per lesion ($\times10^3$ conidia)
30/30	16.2 vw[a]
30/20	43.1 y
30/15	57.9 z
28/28	24.3 w
28/20	35.5 x
28/15	44.6 y
20/20	11.0 v

[a] Values are the average of four replications in each of three experiments. Data were analyzed statistically and means were separated by Duncan's new multiple range test for significance at p=0.05.

Table 4. Effect of surface wetness frequency on the regeneration of conidia of *Epicoccosorus nematosporus* after separation from the conidiogenous cells

No. of conidia per lesion ($\times 10^4$ conidia)				
1st	2nd	3rd	4th	5th
6.0 z[a]	3.1 y	0.06 x	0.00 x	0.00 x

[a] Values are the average of four replications in each of three experiments. Data were analyzed statistically and means were separated by Duncan's new multiple range test for significance at p=0.05.

tion of water chestnut by the fungus or could hasten plant mortality.

Degree of conidial regeneration. In a second sporulation, there were as much as 3.1×10^4 conidia per lesion (ca. <50% of the first) (Table 4). Then, sporulation dropped sharply to 6.2×10^2/lesion in the third sporulation. This result suggests that dew period is the primary limiting factor in using *E. nematosporus* as a mycoherbicide of *E. kuroguwai*. *E. nematosporus* needs a much longer dew period for sporulation compared with other mycoherbicides such as *Colletotrichum gloeosporioides* f. sp. *aeschynomene* (TeBeest et al., 1985), *Colletotrichum coccodes* (Anderson et al., 1985), *Fusarium* sp. (Boyette et al., 1985) and *Alternaria cassiae* (Walker et al., 1986).

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