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The Optimal Temperature and Dew Duration Affecting the Control of Water Chestnut by *Epicoccosorus nematosporus*

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온도와 습실조건에 따른 올방개 지문무늬병균에 의한 올방개 방제효과

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ABSTRACT: In greenhouse studies, control efficacy of water chestnut (*Eleocharis kuroguwai*) by *Epicoccosorus nematosporus* was affected by temperature and dew condition. The appressoria were formed abundantly in the range of 20~28°C. When stem segments (30 cm long) of the water chestnut were inoculated with the conidial suspension of *E. nematosporus*, the mean conidial number attached amounted to 2,545 conidia. Out of 2545 conidia attached to the stem pieces, 1,733 (68%) conidia formed appressoria. When these stem pieces were treated for 24 hr at 28°C under dew condition, 183.1 (7.2%) lesions were formed 10 days after incubation. The time necessary for the death of the plants was about 25 days. Appressoria were formed at 15~35°C, but decreased rapidly in their numbers at the temperature lower than 10°C or at 35°C. The appressoria formation seemed to be depended on the dew duration, which was effective to the lesion formation and plant mortality. Under dew duration of 16~24 hr with temperature range of 25°C to 30°C, the weed control was increased up to 93.9%. There were no differences between the first and second or third dew treatments. A delay of 2 or 3 days in dew treatment did not increase the mortality of plants. For the use of *E. nematosporus* as a mycoherbicide of water chestnut, a conidial suspension should be applied when dew conditions are kept for 12 hr after inoculation.

***Key words*: dew duration, *Epicoccosorus nematosporus*, temperature, water chestnut.**

Previous studies including pathogenicity, host range (5, 7), and potential application of *E. nematosporus* have revealed that an isolate YCSJ-112 is a potential biological control agent of water chestnut (6, 8). Various greenhouse tests showed that the pathogenicity was genetically stable and specific for the host. This pathogen survived in infected stems of water chestnut in the previous year and has been occurred each year within its host plant (Hong, unpublished). From these primary inoculum sources, the pathogen spreads and builds up gradually to a maximum when the host plant matures from August to September (7). The mean temperature and relative humidity in this period were about the 20°C and more than 85%, respectively. Therefore, the water chestnut was severely infected and killed in these period (7). It is necessary to understand the suitable temperature and relative humidity to control the water chestnut before applying mycoherbicide in the field. Under the field conditions, it is difficult to predict dew formation and duration. Thus, this study was carried out to determine the effects of dew duration on the appressoria and lesion formation which decisively affect the weed control by this pathogen.

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MATERIALS AND METHODS

The general procedures for collecting the tuber of water chestnut, growing the plant, isolating the pathogen YCSJ-112, producing the medium for the inoculum, sporulating the conidia and inoculating methods were similar to the previous studies (6, 7, 8)

Measuring the number of conidia, appressoria and lesions. The shoots of 30 cm long were cut in 1 cm long and placed in small vial with 2 ml of distilled water which was then vigorously shaken by vortex mixer for 1 min to separate the conidia and appressoria attached to the shoot. The number of appressoria on the hemacytometer was counted under light microscope (100×).

Ten shoots were counted for each treated temperature. The suspension containing conidia and appressoria were counted 5 times per each shoot. To enhance lesion development, treated pots in dew chamber were placed in the greenhouse bench which was adjusted to a 15 hr light period with temperature range between 20°C and 30°C. The number of lesions was counted on 10 randomly selected shoots in each pot with 3 replications.

Effect of temperature on the formation of appressoria and lesion. The water chestnut seedlings of 30-day-old were inoculated with a suspension of 6.3×10^5 conidia per milliliter until incipient runoff. Development of the appressoria and lesions were conducted in two dew chambers (four room, Conviron) at the temperature of 10, 15, 20, 24, 28, 32, and 35°C. The inoculated plants were placed in each dew chamber for 24 hr. After 24 hr of dew treatment at each temperature, the number of appressoria formed on the stem segment of 10 shoots was counted under light microscope (100×) with hemacytometer. For the counting of lesion formation from 24 hr of dew treated plants were placed in the greenhouse bench for 20 days about 15 hr of day light period with a temperature range from 20 to 30°C. The number of lesions formed on the shoots was counted from randomly selected shoots in each pot with 3 replications.

Dew conditions. Thirty day old plants inoculated with a conidial suspension (6.3×10^5 conidia per milliliter) with mist sprayer (pressure: 0.1 kg/cm^2) were incubated in a dew chamber (Conviron) for 4, 8, 12, 16, 20 and 24 hr. In a dew chamber, the temperature was maintained in a range of 28°C. At the end of each incubation period, the appressoria and lesion formed on the shoots were counted as decribed above. The plants mortality were rated after 25 days of inoculation.

Effect of dew period and temperature for weed control. Plants were incubated under dew period of 8, 12, 16 and 24 hr combined with the different temperature 15, 20, 25, and 30°C after inoculation with a conidial suspension. The plant mortality was rated in the same method as described above.

Effect of three sequential dew periods of various durations on the plant mortality. As soon as the plant inoculated, they were placed in a dew chamber (28°C) for treatment of 12, 16 or 24 hr and then moved to greenhouse bench (25±6°C) for first dew periods. The second and third dew period of each dew duration were initiated at the interval of 1 and 2 days, respectively. The mortality of the plant was rated in the same method as described above.

Application standard and data statistics. The number of appresoria was counted 24 hr after dew treatment, and each treatment using ten shoots of 30 cm long were replicated with 3 pots. The number of lesions on the shoots was counted with 20 shoots and 3 replications. The mortality of plant was rated after 25 days of inoculation, and 20 shoots of each pot were examined with 3 replications. All data were analyzed statistically and means were seperated by Duncan's new multiple range test for significance at p=0.05.

RESULTS

Effect of applicated conidia on the progress of the plant mortality. When the conidial suspension of the *E. nematosporus* was sprayed on the shoot of water chestnut, the mean of conidia attached to one shoot of 30 cm long amounted to 2,545. Sixty eight percent of appressoria, 1733 in their number was formed after 24 hr under dew treatment. The lesions formed 7.2% after 10 days of inoculation. The time necessary for the death of whole plant seems to go by 25 days after inoculation (Table 1).

Effect of temperature on appressoria and lesion formation. The appressorial formation was significantly increased at the temperature range between 20°C and 28 °C (Table 2). In this temperature range, the number of formed appressoria was recorded in the range of 1,422~1,705 per 30 cm long shoot. The appressoria formation was significantly reduced between 10°C and 35°C. The appressoria were also formed at the temperature of 15~32°C, and were sufficient to affecting the plant mortality.

Table 1. Progress of appressoria, lesion formation and mortality time after being sprayed with a conidial suspension (6.3×10^5 conidia/ml) of *E. nematosporus* on water chestnut

No. of attached conidia per shoot[a]	No. of appressoria per shoot[b]	No. of lesion per shoot[c]	Mortality time (day)[d]
2,545	1,733(68%)	183(7%)	25

[a]The number of conidia was counted after spraying the conidial suspension. Ten shoots were counted for each experiment. The suspension containing conidia and appressoria were counted 5 times per each shoot under light microscope (100×) with Hemacytometer.
[b]The number of appressoria was counted at 24 hr after inoculation with dew condition.
[c]The number of typical lesions which were in size within 3~5 mm was counted after at 10 days of inoculation.
[d]The time necessary to the plant mortality were counted at the 93% of plant death. The plants mortality was rated after 25 days of inoculation and 20 shoots of each pot were examined with 3 replications.

Table 2. Effect of different temperature treatments on appressoria and lesion formation caused by *E. nematosporus* under dew condition

Treated temperature (°C)	Appressoria formed (No./Shoot)[X]	Lesion formed (No./Shoot)[Y]
10	3 a[Z]	0 a
15	290 c	90 c
20	1608 e	115 cd
24	1422 e	104 cd
28	1705 e	120 d
32	810 d	57 b
35	54 b	40 b

[X]The number of conidia was counted after spraying the conidial suspension. Ten shoots were counted for each experiment. The suspension containing conidia and appressoria were counted 5 times per each shoot under 100× microscope with Hemacytometer.

[Y]The number of typical lesions which were in size within 3~5 mm was counted after 10 days of inoculation. The number of lesions was counted on 20 shoots with 3 replications.

[Z]Numbers in each column followed by the same letter are not significantly different by Duncan's new multiple range test (p =0.05).

Also, the formation of 50 or more lesions per shoot sufficiently affected the plant mortality.

Effect of dew period duration. The number of appressoria formed was highly abundant at the treatment of 24 hr dew duration and the lowest at the 4 hr dew treatment (Table 3). The number of appressoria were significantly increased from 12 hr to 24 hr treatment relative to 4 hr and 8 hr treatments. The number of lesions which sufficiently affected the plant mortality were ranged from 8.3 to 121 per shoot. The percentage of mortality was directly influenced by the formation of the appressoria and lesion. Dew duration of 12~24 hr caused the percentage of mortality about 79.1~91.5%.

Effect of dew period and temperature on plant mortality. Number of lesion formations in inoculated plants in each treatment were increased during the duration of 15~25 days under various temperatures (Table 4). Low levels of plant mortality was represented at the 8 hr dew period under the all treated temperature. High levels of plant mortality were achieved in the dew treatment of 16 hr in the temperature range of 25~30°C. The highest mean plant mortality was occurred in 25°C of 24 hr dew period treatment.

Effect of three sequential dew periods of various durations on the plant mortality. A single dew period more than 12 hr was necessary for the mortality rate of 63.4~89.9% (Fig. 1). The maximum rate of mortality was obtained with the immediate dew treatment after inoculation. However, successive dew treatment of 2 or 3 times could not enhance the mortality of the plants. As a consequence, the initial dew period over 12~16 hr had the greatest effect on the mortality rate of plants.

Table 3. Effect of dew periods on appressoria, lesion formation, and shoot blight in water chestnut inoculated with a conidial suspension of *E. nematosporus*

Dew periods (hr)	Appressoria formed (No./Shoot)[W]	Lesion formed (No./Shoot)[X]	Plant mortality (%)[Y]
4	2 a[Z]	0 a	0.0 a
8	156 b	3 a	6.9 a
12	1445 c	110 c	79.1 b
16	1478 c	83 c	84.6 b
20	1497 c	121 c	90.8 b
24	1583 c	93 c	91.5 b

[W]The number of appressoria was counted at 24 hr after inoculation with dew condition. The suspension containing conidia and appressoria was counted 5 times per each shoot under 100× of microscope with Hemacytometer.

[X]The number of typical lesions which were in size within 3~5 mm was counted after 10 days of inoculation.

[Y]The time necessary for the plant mortality was counted at the 93% of plant death. The plants mortality was rated after 25 days of inoculation and 20 shoots of each pot were examined with 3 replications.

[Z]Numbers in each column followed by the same letter are not significantly different by Duncan's new multiple range test (p =0.05).

Table 4. Mortality of water chestnut after inoculation with conidia suspension of *E. nematosporus* at different temperaure and dew period. The requirng time of the plant mortality were counted at the 93% of plant death. The plants mortality were rated 25 days after inoculation

Dew period (hr)	Percent of plant mortality[X]			
	15	20	25	30 (°C)
8	1.5 a[Y]	3.4 a	10.4 a	8.9 a
12	12.4 b	58.5 b	57.1 b	60.5 b
16	13.6 bc	64.8 bc	91.0 c	88.2 c
24	15.2 c	71.5 c	93.9 c	90.4 c

[X]The requirng time of the plant mortality were counted at the 93% of plant death. The plants mortality were rated at the 25 days after inoculation and 20 shoots of each pot were examined with 3 replications.

[Y]Numbers in each column followed by the same letter are not significantly different by Duncan's multiple range test (p=0.05).

DISCUSSION

The optimum dew periods for disease development on plants vary widely with respect to species or isolates (1, 17). An understanding of the effects of varying dew

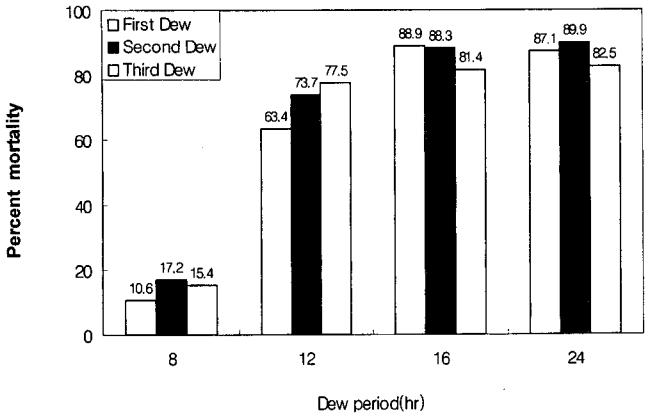

Fig. 1. Effects of dry period on mortality of water chestnut plants after being inoculated with *E. nematosporus*. The first dew periods were initiated immediately after inoculation, the second were 1 day later, and the third were 2 days later.

periods on the infection of water chestnut by *E. nematosporus* could determine the optimum timing of applications and the most suitable formulation of the mycoherbicide to provide a consistent level of the weed control. The lack of optimum dew periods in field condition is very common to a number of potential mycoherbicides but might be overcome by formulation technique (2, 14, 16). On the basis of the results, the optimum conditions for adequate appressoria formation on the water chestnut seedling by *E. nematosporus* are obtained at the temperature range of 20~28°C and dew period of 12 hr. At various combinations of temperature and dew duration for plant mortality, the temperature of 25~30°C and dew duration of 12~24 hr were necessary for maximizing the plant mortality. The low level of diseased plants which were incubated at the temperature of 32°C has an interesting physiological implication; the reduced lesion formation may be of factors in the host (4). Some infection was evident after a 2 hr dew period, and significant reduction in number of plants and dry weight was evident after 4 hr dew period. Reduction of 95~100% in disease incidence and dry weight was reached with an 8 hr dew period (19). Other fungi penetrate their hosts within 9~12 hr after inoculation under unfavorable environments (11, 13). The penetration by appressoria in many plant pathogens has been occurred immediately after formation of infection structures. There are several guides regarding the early stages of pathogenesis. Many pathogenic fungi require a period of free moisture for spore germination and infection (15).

For the disease development, adequate wetness treatment during the infection process was the most important factor (3, 18, 20). Under 18 hr dew period with a temperature range of 20~30°C appeared to be the most favorable condition for the death of seedlings, but a sharp decrease of infection was occurred in the temperature range of 10~35°C (18). Lesion size of *A. helianthi* on sunflower was significantly increased with the 10 sequential dew periods of 10-15 hr than plants treated with two dew periods of 10~15 hr (1). With dew treatment of 1 or 2 days might reduce the conidial viability, and then affected the infection efficacy of the pathogen. Slowdown of lesion development by the fluctuating temperatures were common in field environments, and it might reduce the efficacy of fungi for biological control of the weeds. Similarly, high or alternating temperatures slowed the lesion formation and subsequent disease development of the *Pyricularia oryzae* (9). Like other anthracnose diseases (10, 12), water chestnut fingerprint shoot blight develops rapidly over a wide range of temperature. At a field site of Miryang from June to September, the dew period was about 12 hr and temperature ranged from 19 to 31°C (Hong, unpublished). Thus it is obvious that extending the dew period from 12 hr to 16 hr would be desirable for the use of *E. nematosporus* as a mycoherbicide.

요 약

올방개 지문무늬병균(*E. nematosporus*)의 분생포자 접종시 온도조건과 습실처리 조건이 올방개의 제초효과에 미치는 영향을 온실에서 검정하였다. 지문무늬병균의 분생포자현탁액(6.3×10^5 conidia/ml)을 올방개 줄기(25~30일 정도의 묘)에 분무접종하였을 때 30 cm 길이의 줄기당 평균 2,545개의 분생포자가 부착되었고 28°C에서 24시간 습실처리후 부착기가 형성된 포자의 수는 전체 분생포자 중 68%인 1,733개가 형성되었다. 부착기 형성 후 침입이 성공적으로 이루어진 후 형성된 병반의 수는 30 cm 줄기당 약 183.1개(7.2%)의 병반이 형성되었으며 95%이상의 줄기가 고사하기 까지는 약 25일이 소요되었다. 부착기는 結露條件下에서 온도 20~28°C 범위에서 잘 형성되었다. 결로조건하에서 부착기가 형성될 수 있는 가능한 온도 범위는 15~35°C이었다. 그러나 10°C 이하와 35°C에서는 부착기의 형성이 급격히 감소하였고 이 현상은 고온조건(35°C) 보다 저온(10~15°C)조건에서 더욱 감소하였다. 부착기의 형성은 결로시간에 의해 좌우되며 이는 병반의 형성과 제초효과에 직접적인 영향을 미친다. 28°C 항온조건하에서 12시간이상의 결로조건하에서 충분한 수의 부착기와 병반이 형성되었다. 온도조

건 25°C에서 30°C 범위에서 결로시간을 16시간에서 24시간 동안 처리 하였을 때 올방개에 대한 제초효과는 약 93.9%를 나타내었다. 결로조건을 연속으로 처리하였을 때는 12시간 이상의 결로조건을 2회 이상 처리하여도 1회처리에 비해 제초효과는 더 이상 증가하지 않았다.

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