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Evaluation of Control Efficacy of Biocontrol Agent, *Epicoccosorus nematosporus* on *Eleocharis kuroguwai* in the Field

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This study was conducted to determine the efficacy of *Epicoccosorus nematosporus* for the control of *Eleocharis kuroguwai* and to evaluate the meteorological factors which affect weeding efficacy in field conditions for three years (1996-1998). The best time to control *E. kuroguwai* with *E. nematosporus* as a biological control agent in the field was in July, when temperature ranged from 20.4 to 23.4°C; the surface wetness duration was 12.6-16.1 hours, and application time of 6:00 p.m. and 8:00 p.m.; and weeding efficacy was 81-90%. On 10 June 1996 in Milyang area, where the field experiments were performed, mean temperature was 15.6°C with 11.3 hours of dew duration. Meanwhile, on 20 Aug. 1996 the temperature was 21.3°C with 15.4 hours of dew duration. During these periods, the weeding efficacy was recorded at 61.8 and 60.8%, respectively. Time required for complete plant death was 25.8 and 25.6 days at application times 10 June and 20 Aug., respectively. At the time of application on 7, 18, and 27 July 1996, mean temperature was 20.4-23.4°C with 12.6-16.5 hours of dew duration. The weeding efficacies of these periods were very high with 81.4-90.8%. Three years of field observations from 1996 to 1998 showed that infection in the field can occur at any time through the summer season, although total infection rates vary between months and between years. In 1996, plant infection rapidly increased from 56% on 30 June, 82.4% on 15 July, 94.6% on 15 August, and 92.8% on 15 September under favorable meteorological conditions such as minimum temperature of 17.6°C and maximum temperature of 27.1°C, with 86% relative humidity and 977.5 mm of rainfall during *E. kuroguwai* growing season. However, in 1997, the disease incidence was very low because of unfavorable weather conditions brought about by the hot temperature and the low amount of rainfall at 321.5 mm. Disease progress was slow from 24.4% on 30 June to 49.2% at the end of the growing season.

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Results of previous studies indicated that *Epicoccosorus nematosporus* might have a potential as a mycoherbicide for controlling the rice field weed, *Eleocharis kuroguwai* (Hong et al., 1991, 1992). *E. kuroguwai* is distributed widely and has caused weed problem in rice production areas in Korea (Hong et al., 1995). *E. kuroguwai* is perennial sedge that propagates mainly by terminal tuber or rhizome. It is difficult to control the weeds because the tuber over winters in the soil and sprouts irregularly. The fungus *E. nematosporus* was found to be involved as an epiphytotic on *E. kuroguwai* in Kyeongpook, Sangju region (Hong et al., 1991). With the pathogen, significant reduction in plant height and tuber formation was evident as previously reported (Hong et al., 1995). The optimum inoculum concentration of *E. nematosporus* and application frequency and timing for effective control of *E. kuroguwai* were studied (Hong et al., 1996). The effects of dew conditions on the formation of appressoria and lesion which decisively affected the weeding efficacy of this pathogen were determined (Hong et al., 1997).

It is necessary to determine the suitable temperature and relative humidity to control the target weed with plant pathogen before applying in the field (Hong et al., 2001, 2002a, 2002b, 2002c, 2002d). Nevertheless, under field conditions, dew formation and its duration are difficult to predict (Hong et al., 2002). The lack of field effectiveness of potential agents has been a common problem in different areas of biological control over the past 20 years. Biological control regulates pest populations by manipulating biological control agents, and control efficacy also depends on environment. To identify factors limiting control efficacy, a clear understanding of the ecological structures and the dynamics of plant pathosystems is needed. Constraints on disease development are those factors that retard or prevent the disease (Agrios, 1978; Baker and Cook, 1974; Cowling, 1978; Stevens, 1960; Walker, 1969). These include host resistance or susceptibility to a pathogen, physical and physiological barriers to infection in the host, abundance and distribution of host, age and vigor of host, predisposition of host by environ- mental factors, host effects on environment, and diversity of the host's genetic base.

Time as a factor in disease development influences all components independently, as well as the complex interactions among these components. The complexity of interactions that occur during the development of an epidemic was emphasized by Day (1974). Post-application disease development is also determined by the environment. It has been shown repeatedly that the increase in the rate of disease is a function of the environment. The purpose of this research was to determine the efficacy of *E. nematosporus* for the control of *E. kuroguwai* and to evaluate the ecological and epidemiological factors which affect the weeding efficacy in field conditions.

Materials and Methods

Inoculum production. For mass production of conidia for field inoculation, 30 ml of the mycelial fragment suspension of 10^5 cfu/ml was uniformly plated on oatmeal agar in a tray of 30 cm × 45 cm. The tray was incubated at 28°C for 5 days, and then aerial mycelia were removed with a plastic spatula. The tray was illuminated for sporulation with 4,500 lux light intensity for 48 hours at 28°C in a growth chamber. Conidia produced on the surface of oatmeal agar were collected with 200 ml distilled water, and the concentration of conidial suspension was adjusted to 6.0×10^5 conidia/ml prior to use.

Inoculation. Conidial suspension used as an inoculum was applied at the rate of 1 L/plot (= 500 L/ha) using a manually compressed air sprayer (Dia sprayer, Model No. 7200) with a fine mist adjustment. Inoculation was performed just before sunset in a calm day to keep moisture condition.

Examination of germ tube and appressorial formation. The methods for counting the germ tube and appressoria were described above. Ten shoots were examined for each plot, counting the germ tube and appressoria five times per each shoot.

Experimental design. Field experiments were conducted at the Yeongnam National Agricultural Experiment Station located in Milyang, Korea. In 1996-1998, plots, 2 m × 2 m in size, separated each by 0.5 m border line, were planted with sprouted 100 tubers by presoaking at 25°C for 7 days. Treatments were arranged in a completely randomized block design with four replications. Plots were hand-weeded and cultivated as usual.

Assessment of the plant mortality. The number of diseased shoots in a plot measuring 50 × 50 cm was counted at 30 days after inoculation. Time required for plant death was also recorded.

Examination of tuber in underground. The underground tubers were contained in the soil of 50 × 50 × 30 cm plots. Three places in each plot were collected and counted with four replications 2 months after inoculation.

Effects of meteorological factors on infections and weeding efficacy. The conidial inoculum was applied in the field plots on 10 June; 7, 18, and 27 July; and 20 Aug. 1995. The average temperature during dew duration was recorded using an agricultural meteorological equipment (Hitachi, Model, AMR 17204). Duration of plant surface wetness was recorded during the first initial date from incipient dew of the inoculation date to end of dew the following day.

Weeding efficacy of conidia suspension for three years in the field. The conidial suspension of *E. nematosporus* was applied for 3 years from 1996 to 1998. Plots measuring 2 × 2 m, separated by 0.5 m in which germinated tubers were planted 3 cm deep were planted approximately 100 tubers per plot. Plots were weeded except water chestnut, and cultivated as paddy field. Treatments were randomized completely and each treatment had four replications. Applications of treatments were made with 1 L/plot (500 L/ha) using the fine mist adjustment of a manually compressed air sprayer. Temperature and relative humidity of the micro field climate were measured with the meteorological observation equipment. The weeding progress was monitored in the course of time by counting the percent of plant mortality.

Statistical analysis. Analysis of variance was done using the ANOVA procedure of Statistical Analysis System (SAS Software Co.). All data were analyzed statistically, and treatment means were separated by Duncan's new multiple range test for significance at $p = 0.05$.

Results and Discussion

Effect of application frequency on weeding efficacy. Two or three applications for 2-5 days killed 85.2-95.6% of stems of water chestnut, and resulted in fewer production of tubers compared to those with one time application, where

Table 1. Application frequency and system to properly control *E. kuroguwai* when sprayed with the conidial suspension of *E. nematosporus*

Frequency/Day[a]	Required time for mortality (day)[b]	Plant mortality (%)[c]	No. of tuber per plot
1/1	22.2 a	72.9 c	18.3 b[d]
2/1	17.8 b	85.2 b	16.5 b
2/3	15.2 c	95.6 a	8.6 c
2/4	17.6 b	91.0 ab	10.2 bc
3/5	17.9 b	93.7 a	11.8 bc
Untreated control			119.4 a

[a] Each frequency system was applied by doing: 2/1 = first application at 8:00 a.m. and second application at 8:00 p.m.; 2/3 = firstly application at 8:00 p.m. the first day and second application at 8:00 p.m. the third day; 2/4 = applied at 8:00 p.m. the first day and at 8:00 p.m. the fourth day; 3/5 = applied at 8:00 p.m. the first, third, and fifth day.

[b,c] The required time to plant mortality was recorded during days when mortality occurred.

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

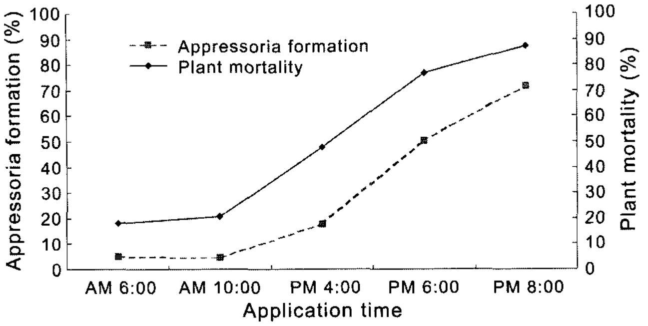

Fig. 1. Optimum application time in a day when inoculated with conidial suspension of *E. nematosporus.* This experiment was conducted on 15 July 1996 when the maximum and minimum temperatures were 30.1°C and 21.5°C, respectively, recorded during the day time; and the maximum and minimum temperatures were 25.6°C and 19.8°C, respectively, during dew duration. An 8:00 p.m. application was recorded as 13.6 hours of dew duration.

18.3 tubers were produced (Table 1). The plant mortality (%) was highest in the plot with two applications of the inoculum for 3 days, 95.6%, followed by 2/4, 2/1, 3/5, and 1/1. The number of tubers in the plot also decreased as number of application increased. Untreated control plots had as many as 119.4 tubers per plot, while two applications in a 2- to 5-day-interval had only 8.6-16.5 tubers per plot. This result suggests that at least two applications in 2-4 days are needed for the effective control of water chestnut. For effective control, established shoots, re-shoots, as well as underground tuber formation must be considered. One of the difficulties encountered in attempting to kill the weeds is their ability to regenerate, such as water chestnut and bindweed (Ormeno-Nunez et al., 1988; Reuveni et al., 1986). Therefore, the shoots must be severely infected and must die before regeneration occurs. Theoretically, high mortality will be achieved when all the underground tubers have sprouted at the time of fungal application. Percentage of appressorial formation was increased to 70.2 and 87.8% when applied at 6 p.m. and at 8 p.m., respectively (Fig. 1), but decreased to 5.0 and 17.8% when applied at 6 a.m. and 4 p.m., respectively. Weeding efficacy was high when applied at the time of dew initiation at 8 p.m. Under field conditions, a long period of dew is required to obtain satisfactory control (Makowski, 1993). Therefore, the timing of field application for effective control with *E. nematosporus* is crucial. When hot, dry conditions prevail after field application of *E. nematosporus*, infections are low, and disease development is slow, giving poor control of water chestnut. However, when applications are properly timed, optimum conditions can be achieved in the field (Makowski et al., 1989; Mortensen, 1988).

Effects of meteorological factors on infections and weeding efficacy. On 10 June in Milyang area, where the field experiment was performed, mean temperature was 15.6°C with 11.3 hours of dew duration, while on 20 Aug., the temperature was 21.3°C with 15.4 hours of dew duration (Table 2). During these periods, the weeding efficiency was recorded at 61.8 and 60.8%, respectively. Time required for complete plant death was 25.8 and 25.6 days on 10 June and 20 Aug., respectively. At application times 7, 18, and 27 July, mean temperature was 20.4-23.4°C, with 12.6-16.5 hours of dew duration. The weeding efficiencies of these periods were very high at 81.4-90.8%. On 7 July, percent appressorial formation was 71.2%, and the time required for complete plant death was 19 days. Underground tuber formation was significantly reduced to 16.8 tubers per plot. Meteorological condition of these

Table 2. Optimum meteorological condition which affects the weeding efficacy on water chestnut when sprayed with the conidial suspension of *E. nematosporus* in the field

Application time (Date/Month)	Meteorological condition		Percent of appressoria formation[c]	Required time to mortality (day)[d]	Plant mortality (%)[e]	Suppressiveness to tubers (No. per plot)[f]
	Avg. Temp. (°C)[a]	Durable surface wetness time[b]				
10 June	15.6	11.3	35.4 b	25.8 a	61.8 c	40.6 a[z]
7 July	20.4	12.6	71.2 a	19.0 b	90.8 a	16.8 b
18 July	21.6	16.5	70.2 a	17.4 b	88.6 a	15.0 b
27 July	23.4	16.1	73.6 a	24.4 a	81.4 b	18.0 b
20 August	21.3	15.4	71.6 a	25.6 a	60.8 c	33.4 a

[a] The temperature was averaged within the dew duration.
[b] Durable surface wetness time was recorded from 7:00 p.m. of the application date to 10:00 a.m. of the next date.
[c] The number of conidia and appressoria of the collected ten shoots per each plot were counted at the end of dew period. The suspension containing conidia and appressoria were counted five times per each shoot by the methods of measuring the appressoria.
[d] The percent plant mortality and the required time to plant mortality were recorded in those days when mortality occurred.
[e] Underground newly-formed brownish tubers were counted at the three locations randomly selected in a plot measuring 50 × 50 × 30 cm at the end of September.
[f] Numbers in each column followed by the same letter are not significantly different by Duncan's multiple range test ($p = 0.05$).

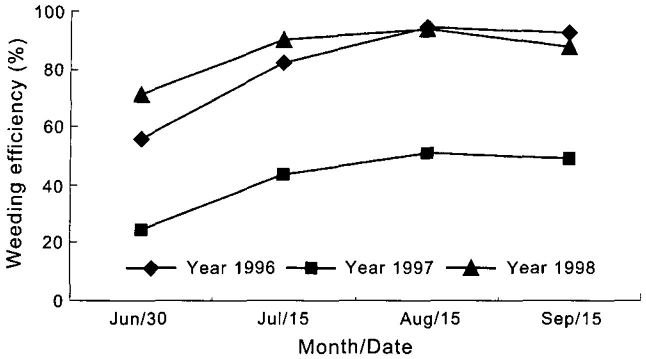

Fig. 2. Comparison of the weeding efficiency for 3 years weeds inoculated with conidial suspension of *E. nematosporus* in field condition.

periods was the most suitable for conidial germing and appressoria formation, and consequently affects the increase in weeding efficacy and suppressiveness of underground tubers. Although meteorological condition was suitable to disease development, late August was not suitable for control effects. This is because the water chestnut seedlings were too old to penetrate the fungus, and disease development was slower than the young plants under 30-day-old seedling age. In addition, most of the water chestnuts were beginning to form underground tubers during this time. It may be advantageous to apply mycoherbicide agent initially for complete shoot kill, and then again as new shoots begin to emerge from surviving tubers (Britton, 1993). In the field, the water chestnuts were formed initially from early August to late September (Hong et al., 1996). The final objective of control of the water chestnut was not only to suppress the upper part of seedlings for the current year, but also to suppress the underground tubers for the following year's population. To kill the water chestnut propagated by the tubers, the most suitable time to apply is before the beginning of tuber formation.

Weeding efficacy of FSBD for 3 years in the field. In 1996, the plants were inoculated with the conidia suspension of *E. nematosporus* on 15 June. Plant infection rapidly increased from 56% on 30 June, 82.4 on 15 July, 94.6% on 15 August, and 92.8% on 15 September under favorable meteorological condition with minimum temperature of 17.6°C, maximum temperature of 27.1°C, and 86% of relative humidity with 977.5 mm of rainfall during the water chestnut growing season (Fig. 2). However, in 1997, the disease incidence was very low because of unfavorable weather condition brought about by the hot temperature and the low amount of rainfall at 321.5 mm. Disease progress was slow from 24.4% on 30 June to 49.2% at the end of the growing season. In 1998, because of the more favorable weather condition than that in 1997, from June to September, disease development progressed rapidly from 71.4% of initial plant mortality to 87.8% of plant mortality at the end of the growing season. During these periods, the temperature was recorded at a maximum of 28.7°C and minimum of 18.5°C, with 498.7 mm of rainfall. These studies clearly establish that infection can occur at any time through the summer, given suitable temperature of over 12 hours of dew period and sufficient rainfall. Hong et al. (1995) reported that the first symptoms on water chestnuts in paddy rice field in Korea appeared in late July or early August. Heavy infections were observed in 20-30-day-old water chestnut, which usually occurs in mid-July. When hot, dry conditions prevail after field application of *E. nematosporus*, infections are low, and disease development is slow, giving poor control of water chestnut. However, when applications are properly timed, optimum conditions can be achieved in the field (Makowski et al., 1989; Mortensen, 1988). Environmental factors, such as temperature, moisture, light, nutrient levels, and pH may apparently influence predisposition and, thus, may act as constraints to disease development (Hong et al., 1997, 2001, 2002a, 2002b). Environmental factors also directly affect the interactions of host and pathogen, and their presence, absence, amount, and duration may contribute to disease control (Colhoun, 1973, 1979).

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