한국식물병리학회지 13(3) : 167-171(1997)
Korean J. Plant Pathol. 13(3) : 167-171(1997)

Potential Application of *Epicoccosorus nematosporus* for the Control of Water chestnut

Yeon Kyu, Hong*, Jae Min, Cho, Jae Youl, Uhm[1] and Kil Rim, Ryu
Yeongnam Agricultural Experiment Station, Rural Development Administration, Milyang 627-130, Korea.
[1]Department of Agricultural Biology, College of Agriculture, Kyeongpook National University, Taegu 702-701, Korea

올방개 지문무늬병균의 효과적 처리방법에 의한 올방개 제초효과

홍연규* · 조재민 · 엄재열[1] · 류길림
농촌진흥청 영남농업시험장, [1]경북대학교 농과대학 농생물학과

ABSTRACT : To find optimum application methods of *Epicoccosorus nematosporus* for control of water chestnut, five different concentration of conidial suspensions (10^3 conidia/m*l* to 10^7 conidia/m*l*) of the fungus were applied 1 to 4 times on 10~40 days old seedlings of water chestnut in greenhouse. Inoculum levels equal to or greater than 10^5 conidia/m*l* killed significantly more shoots (82.6%~92.1%) and suppressed significantly underground tuber formation compared to inoculum concentration less than 10^4 conidia/m*l*. When the conidial suspension of 6. 3×10^5 conidia/m*l* of *E. nematosporus* was sprayed 2 times in 7 days interval, percentage of the killed plants was up to 98.7%. Numbers of reshoots and tubers were also suppressed significantly compared to one time application. The percents of killed shoots were similar between 10-day-old and 20-day-old seedlings, and significantly higher than those treated on 30- or 40-day-old seedlings. The fungus treated on 20-day-old seedlings was the most effective because there was high number of reshoots from 10-day-old seedlings. Therefore, optimal application conditions for *E. nematosporus* is 2~3 times of application in 7 days interval with 10^5 conidia/m*l* on 20-day-old seedling age.

***Key words*: *Epicoccosorus nematosporus*, water chestnut, inoculum concentration, application frequency, seedling age**

Water chestnut (*Eleocharis kuroguwai*) is widely distributed and has been a severe weed problem in rice production areas (1, 5-8). At present, there is no effective way to control this perennial sedge except hand weeding (4). Recent study shows that *Epicoccosorus nematosporus* casual fungus of fingerprint stem blight disease, is a potential biological control agent on the water chestnut (3). In nature, infected shoots of the water chestnut from the previous year serve as the primary inoculum sources (Hong, unpublished). From these primary inoculum sources, the fungus spreads and builds up rather slowly till the host plant matures from August to September. Therefore natural disease progression can't effectively kill water chestnut especially in their early stages where the the most demage was done to rice (6). To enhance weeding efficiency of mycoherbicide agent, factors including inoculum concentration, application frequency and timing should be considered before applying the fungus to the field (2, 10, 14). Objective of these studies were to find the optimal inoculum concentration of *E. nematosporus* and application frequency and application timing for effective control of water chestnut.

*Corresponding author.

MATERIALS AND METHODS

General greenhouse procedures. An isolate (YCSJ-112) of *E. nematosporus* was isolated from the naturally infected water chestnut in rice field of Sangju, Korea in 1992. The inoculum was produced on oatmeal agar. Seedlings of the water chestnut were obtained from tubers as previously described (3). Briefly, tubers were rinced with tap water and placed on moist filter paper disks in petri dish and then kept for 5 to 7 days at 28°C. Five germinated tubers were planted 3 to 5 cm deep in each 1/5000 plastic pot containing rice field soil. Except seedling age experiment, seedlings were inoculated with a fungus when they were 10 to 25 cm tall (20 to 30-day-old). After inoculation, the seedlings were covered with polyethylene film and transferred to controlled environment with dew facility (90~95 RH, and 20~32°C) for 24 h. After 24 h, they were then returned to the greenhouse bench. Greenhouse conditions were day light (15 h photoperiod), a temperature range from 20 to 32°C, and a relative humidity range from 60 to 80%.

Inoculum concentration. The seedlings were sprayed until runoff with the each of solution containing 10^3, 10^4, 10^5, 10^6 and 10^7 conidia/m*l* suspended in 0.5% dextrose in water. Treated plants were placed in a dark dew facility for 24 h and then were returned to the greenhouse. The number of plants infected and killed were examined 20~30 days after inoculation. The plant reshooted and underground tuber formation in a whole pot was examined 3 months after inoculation.

Application frequency. The seedlings of sprayed 1 to 4 time in 7 days interval until runoff with conidial suspension containing 6.3×10^5 conidia/m*l* in 0.5% dextrose in water. Treated plants were placed in a dark dew facility for 24 h and were then returned to the greenhouse The number of plants infected and killed were examined 20~30 days after inoculation. The new sprout and underground tuber formation was examined 3 months after inoculation.

Age of seedling. Ten uniformly germinated tubers were planted in a 1/5000 wagnor pot. The stems grown from tubers were thinned at 10th, 20th, 30th and 40th days and leaved 100 stems per pot. The stems after sprout were then marked at the upper part with white correction pen to distinguish from those of original shoot from reshooted plants. The seedlings were sprayed with the conidial suspension of 2.3×10^5 conidia/m*l* in 0.5% dextrose in water. Conditions for dew treatment and disease development were the same as described above.

Evaluation standard and data statistics. Disease incidence was examined 20~30 days inoculation and was determined by the number of infected and killed shoots per pot. The plants showing lesions but still standing were considered as infected while plants showing advanced lesions and fell down were considered as 'killed'. Reshooted plants were counted at every 10 days intervals till 3 months after inoculation All experiments were replicated 4 to 5 pots. All data were analyzed statistically and means were sep erated by Duncan's new multiple range test for significance at p=0.05.

RESULTS

Inoculum concentration. Untreated control showed no symptom but those treated ones showed symptoms in 5~6 days after the inoculations. The initial pinpoint symptoms expanded and the stems became necrotic at the upper part of plants within 20 days. The percentage of killed plants were increased when the inoculum concentrations increased from 10^3 to 10^7 conidia/m*l* (Table 1). Inoculum levels equal to or greater than 10^5 conidia/m*l* killed more than 80% of the stems and produced small quantity of tubers compared to those treated with the conidial concentration of 10^4/m*l* where only 54% of plants killed. Number of tubers in the pot also decreased as the inoculum concentration increased. Untreated control had as many as 61.7 tubers per pot while those treated ones had only 2 to 24 tubers per pot Inoculum levels equal to or greater than 10^5 conidia/m*l* had significantly fewer tubers than those pots treated with 10^3 or 10^4 conidia/m*l*.

Table 1. Varied weeding effects by different conidial concentration of *E. snematosporus*

Conidial concentration (conidia/m*l*)[a]	Plants killed (%)[b]	Tuber formation (No./pot)[c]
10^3	15.3 b[e]	24 c
10^4	53.7 c	17 b
10^5	82.6 d	3 a
10^6	90.2 e	3 a
10^7	92.1 e	2 a
control[d]	0.0 a	62 d

[a] 20-day-old water chestnut seedlings (100 shoots/pot) were inoculated with conidial suspension in 0.5% dextrose in distilled water.

[b] Plants killed were recorded 30 days after inoculation.

[c] The nember of tubers was examined 3 months after inoculation.

[d] Control was sprayed with 0.5% dextrose in distilled water without conidia.

[e] Means followed by the same letter are not srgnificantly different at P=0.05 according to the Duncan's new multiple range test.

Table 2. Weeding effects by the different application frequencies of the conidial suspension of *E. nematosporus*

Application frequency[a]	Plants killed (%)[b]	Plant reshooted (No./pot)[c]	Tuber formation (No./pot)[d]
1	84.5 b	27.7 c	18 b
2	93.8 c	10.9 b	3 a
3	98.7 d	6.1 a	2 a
4	98.1 d	7.5 a	1 a
control[e]	0.0 a	127.0 d	119 c

[a] Water chestnut seedlings of 20-day-old were inoculated with conidial suspension containing 6.3×10^5 conidia/m*l* in 0.5% dextrose in water, and were applied 7 days interval since then.

[b] Killed plants was recorded 30 days after inoculation.

[c,d] New sprouts and the number of tubers were examined 3 months after inoculation.

[e] Control were sprayed with 0.5% dextrose in distilled water without conidia.

Table 3. Effect of water chestnut seedling age on the weeding efficiency when sprayed with the conidial suspension of *E. nematosporus*

Seedling age (day)[a]	Plants killed (%)[b]	Plant reshooted (No./pot)[c]	Tuber formation (No./pot)[d]
10	92.7 b	82.4 c	33 b
20	90.6 b	18.4 a	8 a
30	70.4 a	26.1 a	16 a
40	65.1 a	51.6 b	43 b

[a] Water chestnut seedlings of 10, 20, 30, 40-day-old were inoculated with conidial suspension containing 2. 3×10^5 conidia/m*l* in 0.5% dextrose in water.

[b] Killed plants were recorded 30 days after inoculation.

[c,d] Plant reshooted and the number of tubers were examined 3 months after inoculation.

Application frequency. More number of shoots were killed and fewer reshoots and tubers formed as the number of applications increased. In the untreated control pot, the number of reshoots were increased significantly as many as 127 per pot as well as the number of underground tubers which resulted in the number of tubers as 119.4 per pot. About 84.5% of plant were killed after the first inoculation (Table 2). Which also had 27.7% of reshooted plants and had 18.3 tuber per pot. At the time of the initial pinpoint symptoms were showed up, the second treatment was applied. Second treatment killed 93.8% of shoots and restricted the reshooted plant within 10.9% and underground tubers formed only 3 per pot.

Seedling age. While plants in all age of the seedling were infected by *E. nematosporus*, the older the seedlings were aged the slower the initial symptoms were appeared and the fewer the plant killed (Table 3). When the seedling were aged 20 days the fungus killed 90.6% of shoots, suppressed the new sprout, and reduced the tuber formation by infection of *E. nematosporus*. While with 30 or 40- day- old seedlings the fungus killed 70.4% and 65.1%, respectively.

DISCUSSION

All these factors, inoculum concentration, number of application, and seedling age, affected the success of the biological control of water chestnut by fingerprint stem blight disease. High rates of the agent ($>1.1\times10^5$ conidia/m*l*) were more effective than low rate (10^3 & 10^4 conidia/m*l*) because a greater number of the conidia caused the lesions which could girdle the shoot rapidly. In this study, significant differences of killed plants were observed between 10^4 and 10^5 of conidial suspension suggests that at least 10^5 conidial concentration was needed for the effective control of water chestnut. Walker (16) showed that spraying mixtures of containing 2.5×10^5 to 10^6 conidia/m*l* of *Alternaria macrospora* gave the best control of *Spurred anode* (*Anoda cristata*). Although high application rates would achive maximal weed control, excessive rates may be unacceptable in practice because of high costs and dissemination (9). Presently, the production costs including media, electric and labor of 10^5 coniadia/m*l* of *E. nematosporus* will be ₩35,000 per 10a in lab (Hong, unpublish). The cost surely be lowered by commercial mass production. Two or more applications of conidial suspensions of *E. nematosporus* may be required for complete control of water chestnut. To effective control, we have to consider established shoots, reshoots, as well as the underground tuber formation. One of the difficulties encountered in attempting to kill regenerating is the weeds ability of weeds such as water chestnut and bindweed (12, 13). Therefore the shoots must be severely infected and die before regeneration occur. Ormeno-Nutez *et al.* (11) observed regeneration in bindweed seedlings inoculated with *P. convolvulus*. Regeneration is particularly important became underground parts such as tubers collpse lastly. Theoretically high mortality will be achieved when all the underground tubers were shooted at the time of fungal application. The most effective ime to apply conidia was 20 to 30 days after sprout because most of the shoots from the tubers were emerged and which were readily killed by the application of conidial suspension. The greatest emerging time of water chestnut is about the end of June to the middle of July which is the time of 20- to 30-day-old after transplanting the rice in field. In field application with these greenhouse results, the conidial suspension of *E. nematosporus* should be treated concentratedly with 2 or 3 times in this season. It should be considered the environmental factors like temperature, dew and maintainence of conidial viability in field condition in further experiment schedule. Although our goals to establish quantitative relationship among inoculum levels, application frequency and seedling age of water chestnut were not fully achieved. But, this study revealed some of the important factors which to be used as a guideline for future field application.

요 약

올방개 지문무늬병균(*E. mematosporus*)의 접종농도, 처리회수, 올방개의 묘령이 제초효과에 미치는 영향을 온실에서 검정하였다. 포자현탁액의 농도를 10^5 conidia/m*l*로 조정하여 분무접종하였을 때 15~20일 이내에 올방개 지상부 줄기의 82.6%~92.1% 정도가 고사하였으나, 그 이하의 농도에서는 제초효과가 53.7% 이하로 급격히 감소하였다. 또한 지상부 줄기의 고사는 지하부 괴경형성에도 영향을 미쳐, 10^5 conidia/m*l*의 농도에서는 무처리나 10^3, 10^4 농도의 처리에 비해 약 6~21배의 괴경형성 억제력을 나타내었다. 올방개 지문무늬병균 분생포자현탁액(6.3×10^5 conidia/m*l*)은 여러번 처리할수록 제초효과가 높았는데 2회 이상 처리했을 때 지상부 줄기의 고사율(93.8%)이 급격히 증가하였고 고사하기까지의 시간도 단축되었으며 신초의 발생율은 1회 처리에 비해 2.5배 무처리에 비해 12배 감소시켰고 지하부 괴경형성도 1회처리에 비해 6배, 무처리에 비해 35배 억제하였다. 올방개의 묘령에 따라서도 제초효율에 차이가 있었는데 신초 형성 후 20일 되었을 때 처리하는 것이 줄기 고사율이 가장 높았고, 신초의 발생율이 현저히 감소하였으며 또한 지하부 괴경형성 억제력도 높게 나타났다. 이상의 결과로 보아 올방개 지문무늬병균의 최적처리의 포자량은 10^5 conidia/m*l* 이상, 1주일 간격으로 2~3회 처리하며 신초형성 20일 후 처리하는 것이 가장 효과적이라 생각된다.

REFERENCES

1. Conway, K. E. 1976. Evaluation of *Cercospora rodmanni* as a biological control of water hyacinths. *Phytopathology* 66 : 914-917.
2. Heiney, D. K. and Templeton, G. E. 1991. Effects of spore concentration, temperature, and dew period on disease of field bindweed caused by *Phoma proboscis. Phytopathology* 81 : 905-909.
3. Hong, Y. K., Cho, J. M., Kim, J. C. and Uhm, J. Y. 1995. Identification, pathogenicity and host range of a potential bioherbicide, *Epicoccosorus nematosporus*, causing fingerprint stem blight on water chestnut, *Eleocharis kuroguwai. Korean J. Plant Pathol.* 12(1) : 58-65.
4. Kim, K. U. and Kwon, S. T. 1985. Bud sprouting and tuberization of *Eleocharis kuroguwai* OHWI. *Kor. Weed Sci.* 5(1) : 43-49.
5. Kim, S. C., Heu, Ho, Bae, S. H. 1976. Study on weed control on paddy field. II. Study on dormancy and germination habits of some perennial weeds on paddy field. *Res. Rep. RDA.* 18(Crop) : 105-109.
6. Kim, S. C., Heu, Ho., Park, R. K. and Jae, S. Y. 1977. Studies on competition between major perennial weeds and rice in transplanted paddy field. *Kor. Crop Sci.* 22(1) : 61-69.
7. Kim, S. C. and Jae, S. Y. 1977. The effects of day length on tuber formation, tuber cutting and soil depth covered on new shoot development of perennial weeds. *Kor. Crop Sci.* 22(1) : 70-79.
8. Ku, Y. C., Oh, Y. J. and Lee, J. H. 1982. Emergence and growth of weeds and their chemical control in paddy field under different water depths. *Kor. Weed Sci.* 2(1) : 47-52.
9. Lawton, M. B. and Burpee, L. L. 1990. Effect of rate and frequency of application of *Typhula phacorrhiza* on biological control of typhula blight of creeping bentgrass. *Phytopathology* 80 : 70-73.
10. Mcrae, C. F., Harrigan, E. K. S., and Brown, J. F. 1984. Effect of temperature, dew period, and inoculum density on blight of safflower caused by *Alternaria carthami. Plant Disease* 68 : 408-410.
11. Ormeno-Nunez, J., Reeleder, F. D., and Watson, A. K. 1988. A foliar disease of field bindweed (*Convolvulus arvensis*) caused by *Phomopsis convolvulus. Plant Disease* 72 : 338-342.
12. Reuveni, M., Tuzun, S., Cole, J. S., Siegel, M. R., and Kuc, J. 1986. The effects of plant age and leaf position on the susceptibility of tobacco to blue mold caused by *Peronospora tabacina. Phytopathology* 76 : 455-458.
13. Swan, D. G. 1983. Regeneration of field bindweed (*Convolvulus arvensis*) seedlings. *Weeds Today* 14 :

3-4.
14. TeeBeest, D. O. and Templeton G. E. 1985. Mycoherbicides: progress in the biological control of weeds. *Plant Disease* 69: 6-10.
15. Walker, L. H. 1981. Factors affecting biological control of Spurred anoda (*Anoda cristata*) with *Alternaria macrospora. Weed Sci.* 29: 505-507.

(Received May 22, 1997)