Potential of an Indigenous Fungus, *Plectosporium tabacinum*, as a Mycoherbicide for Control of Arrowhead (*Sagittaria trifolia*)

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ABSTRACT

Chung, Y. R., Koo, S. J., Kim, H. T., and Cho, K. Y. 1998. Potential of an indigenous fungus, *Plectosporium tabacinum*, as a mycoherbicide for control of arrowhead (*Sagittaria trifolia*). Plant Dis. 82:657-660.

An isolate of the indigenous fungus *Plectosporium tabacinum* was isolated from arrowhead (*Sagittaria trifolia*) in Yusung, Korea in 1990 and evaluated in laboratory and growth chamber tests as a potential mycoherbicide. The fungus grew comparatively slowly on potato dextrose agar and corn meal agar, attaining a diameter of 65 mm after 12 days at 25°C. Conidia were mass-produced in shake-cultures or in a fermentor using potato dextrose broth containing yeast extract (0.5%, wt/vol) at 25°C. When arrowhead seedlings at the 2- to 3-leaf stage were inoculated with conidial suspensions (2×10^7 conidia/ml) and incubated in a dew chamber for 18 h at 25°C, the plants developed small, brown spots on the leaves and petioles in 2 days, and were blighted completely within 7 days after inoculation. This effect was consistent on arrowhead plants from the 2- to 5-leaf stage. Another arrowhead species, *S. pygmaea*, was as susceptible as *S. trifolia* to the pathogen. Several crops, including rice, barley, and wheat and 34 other common weed species, were immune. In small-scale field tests in paddy fields during the summers of 1992 and 1993, a mean reduction of 71.3% in the number of arrowhead plants was observed following a foliar spray of a conidial suspension (10^7 conidia/ml). These results indicate that *P. tabacinum* has potential as a selective mycoherbicide for arrowhead control.

Additional keywords: bioherbicide, biological control, paddy weed

Arrowhead (*Sagittaria trifolia* L.) is a perennial weed in the family of Alismataceae and propagates by underground tubers. The weed is present throughout east-Asian rice areas and is considered to be difficult to control with herbicides due to herbicide tolerance and season-long emergence (10). In Korea and Japan, this weed has been ranked as the number one or two weed problem in most rice paddy fields (9,10). Yield losses caused by this weed were a 20% reduction in the number of rice ears and a 23% reduction in the weight of husked rice when 20 tubers of the weed were buried per square meter of paddy at transplanting of rice seedlings (9).

In the summer of 1990, a pathogenic fungus was isolated from blighted petioles and leaves of arrowhead and tentatively identified as an *Acremonium* sp. (7). The

disease was severe on both young and old arrowhead plants from June to October in paddy fields in the Yusung area in Korea. Disease symptoms occurred on the leaf, seed, and petiole of arrowhead. Small, brown spots developed initially on areas infected, then spots extended to form large necrotic lesions, resulting in the eventual wilting and blighting of the plant. Infection of the petiole frequently induced defoliation within a few days under hot and humid conditions (7).

From microscopic examination of several isolates and cultural characteristics, the fungus has been reidentified as *Plectosporium tabacinum* (van Beyma) M. E. Palm, W. Gams et Nirenberg by W. Gams, Centraalbureau voor Schimmelcultures, The Nether-

lands (personal communication). This genus is similar to the genus *Acremonium* (11).

The objective of this study was to explore the potential of this fungus as a mycoherbicide for arrowhead under laboratory and growth chamber conditions. Experiments were conducted to determine the effect of temperature on the growth of the fungus; the effects of dew period, temperature, and inoculum concentration on disease development; and the host range of the pathogen. Efficacy in small-scale field trials also was evaluated.

MATERIALS AND METHODS

Isolation, culture, and pathogenicity test. The fungus was isolated from diseased arrowhead petioles and seeds collected from the Yusung area in Korea by rinsing sections of diseased tissue in 0.5% NaOCl for 1 min and placing sections on potato dextrose agar (PDA) supplemented with 100 µg/ml streptomycin sulfate. The plates were incubated for 10 days at 25°C and pieces of mycelium containing abundant conidia from the colonies were transferred to fresh PDA for subsequent experiments. Conidia were collected from 10- to 12-day-old colonies grown on PDA, placed in cryopreservative (10% glycerol) in 1.5-ml plastic cryovials, and stored at -70°C. The isolate YC8-7 has been deposited in the Korean Collection for Type Cultures (KCTC) with the assigned number KCTC 0109BP.

Pathogenicity of 21 isolates of *P. tabacinum* was tested by inoculation of a conidial suspension (10^6 conidia/ml) on arrowhead seedlings (2- to 3-leaf stage). Immediately after inoculation, the plants were placed in the dark in a dew chamber for 12 h at 25°C and then transferred to a growth chamber at 25°C. The plants were

Table 1. Radial growth of *Plectosporium tabacinum* isolate YC8-7 on various media after 11 days of incubation at different temperatures

Temperature (C)	Colony diameter (mm) ^y			
	LBA ^z	PDA	CZA	CMA
18	43 a	43 a	41 a	41 a
20	52 b	51 b	53 b	51 b
25	66 c	65 c	65 c	65 c
30	69 c	68 c	64 c	69 c

^y Mean values of two experiments with three replications per treatment. Means followed by the same letter in a column are not significantly different according to Duncan's multiple range test ($P = 0.05$).

^z LBA, lima bean agar; PDA, potato dextrose agar; CZA, Czapek agar; CMA, cornmeal agar.

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This research was supported in part by Non-Directed Research Fund, Korea Research Foundation, 1993.

Accepted for publication 28 February 1998.

Publication no. D-1998-0403-01R

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monitored for disease development for a week. Among tested isolates, the most virulent two isolates, YC8-7 and YC8-16, were evaluated in further tests. Inoculum of each isolate was produced in liquid shake culture or in fermentors in potato-dextrose broth (PDB) supplemented with yeast extract (0.5%, wt/vol). The fungal culture was filtered through three layers of cheesecloth to collect conidia and the conidial suspension was adjusted to 10^7 conidia/ml with distilled water in all experiments, except as indicated for the inoculum level study.

Effect of temperature on radial growth. For measuring radial growth of *P. tabacinum*, a mycelial disc (5-mm-diameter) taken from an actively growing culture of the fungus (isolate YC8-7) was placed mycelia-side down in the center of a petri plate containing lima bean agar (LBA), PDA, Czapek agar (CZA), or cornmeal agar (CMA). All agar media were products of Difco Laboratories, Detroit. Three replicate plates were incubated at 18, 20, 25, or 30°C. Colony diameter was measured after 11 days and the experiment was repeated twice.

Plant preparation. Tubers of mature arrowhead plants were collected from naturally occurring field populations in the fall of 1990 from a rice paddy at the Korea Research Institute of Chemical Technology (KRICT), Taejeon, Korea, and stored at 4°C. The tubers were planted in styrofoam pots (surface area 75 cm²) filled with a sandy loam soil collected from the rice field, and grown until the growth stage appropriate for experiments was obtained. Plants for the host range tests were selected and prepared according to the method developed by the Screening and Toxicology Center, KRICT, established for herbicides screening tests (5,15). Most plants were grown from seeds sown in plastic pots (20 × 15 × 6 cm) filled with sterilized sandy loam soil, and grown in a greenhouse maintained at 28 and 20°C, day and night, respectively. Bindweed was grown from tuberous root sections. At the

time of inoculation, the growth stage of grass plants was between the second and third leaf, and that of the broadleaf plants was between the first and second true leaf.

Effect of inoculum concentration, dew period, and growth stage on disease. Arrowhead seedlings were inoculated with conidial suspensions of 6×10^3 to 6×10^8 conidia/ml and placed in the dark in a dew chamber for 20 h at 25°C. Control plants were sprayed with distilled water. Following the dew treatment, the plants were

placed in a greenhouse and mortality was recorded after 4 days.

To investigate the effect of dew period on disease development, arrowhead seedlings (2- to 3-leaf stage) were sprayed with conidial suspensions of the isolate YC8-7 containing 2×10^7 conidia/ml and placed in the dew chamber in the dark at 100% relative humidity (RH) for 4, 8, 18, or 24 h at 25°C before removal to greenhouse benches maintained at 28 and 20°C (day-night). The plants were monitored for dis-

Fig. 1. Effect of dew period length on mortality of arrowhead seedlings (2- to 3-leaf stage) inoculated with *Plectosporium tabacinum* isolates YC8-7 or YC8-16. Means followed by the same letter for a dew period are not significantly different according to Duncan's multiple range test ($P = 0.05$).

Fig. 2. Effect of *Plectosporium tabacinum* isolate YC8-7 on mortality of arrowhead at various stages of growth. The plants were inoculated with a conidial suspension (2×10^7 conidia/ml) and disease development was assessed 4 days after inoculation. Bars represent the standard error of the mean. LS = leaf stage.

Table 2. Effect of inoculum concentration of *Plectosporium tabacinum* isolates YC8-7 and YC8-16 on mortality of arrowhead seedlings placed in a growth chamber for 4 days after incubating for 20 h in a dew chamber at 25°C

Conidia/ml	Percent mortality ^z	
	YC 8-7	YC 8-16
6×10^3	37.5 a	0.0 a
6×10^4	80.0 b	4.3 a
6×10^5	92.5 b	20.8 a
6×10^6	95.0 b	29.3 a
6×10^7	100.0 b	70.8 b
6×10^8	100.0 b	82.0 b

^z Mean values of two experiments with three replications per treatment. Means followed by the same letter in a column are not significantly different according to Duncan's multiple range test ($P = 0.05$).

ease development for 4 days. Development of the disease on arrowhead at various stages of growth was observed for 12 days after inoculation. The experiments consisted of three replicates and were repeated two times.

Host-range tests. Seedlings of test plants were sprayed with suspensions containing 2×10^7 conidia/ml of isolate YC8-7 and incubated in the dark in a dew chamber for 20 h at 25°C. One noninoculated pot of each species was included as a control. Plant responses were considered either immune (no visible reaction) or susceptible

on a scale of 1 to 3, where 1 = small lesion, 2 = partial blight, and 3 = complete blight and death, rated by visual observation after 10 days of incubation in the greenhouse.

Field studies. In 1992 and 1993, the pathogen was tested under field conditions in the Chinju area, Korea. Twenty arrowhead tubers and 15 rice seedlings (25 days old) of the cultivar "Dongjin" were planted simultaneously in 2.0-m² paddy fields on 22 May 1992 and 25 May 1993 during the normal transplanting season for rice. All plots were constructed with polyvinyl chloride plate enclosures (1.4 × 1.4 × 0.3 m

high). On 23 July 1992 and 20 July 1993, 20 arrowhead plants in each plot were sprayed with 1 liter of a conidial suspension of *P. tabacinum* (10^7 conidia/ml). After 2 weeks, the number of remaining arrowhead plants was counted. Treated and untreated control plots were replicated four times and Duncan's multiple range test was used to separate means ($P = 0.05$).

RESULTS

The fungus grew fastest at 30°C on LBA, PDA, and CMA, with mean colony diameters of 69, 68, and 69 mm, respectively. Growth was reduced significantly at 18°C on all media compared to other incubation temperatures (Table 1). Abundant conidia were produced on PDA and CMA at 25 to 30°C.

The isolate YC8-7 was more virulent than YC8-16, with a concentration of 6×10^4 conidia/ml for YC8-7 showing a similar efficacy as 6×10^8 conidia/ml of YC8-16 when incubated for 20 h in a dew chamber at 25°C (Table 2). The isolate YC8-7 killed all seedlings at a conidial concentration of 6×10^7 conidia/ml and above. At a dew temperature of 25°C, mortality of arrowhead seedlings for isolate YC8-7 was 65, 92, 97, and 98% after 4, 8, 18, and 24 h of dew, respectively (Fig. 1). Mortality for YC8-16 was lower than YC8-7 for a dew period of less than 18 h.

Arrowhead in the 1- to 2- or 3- to 5-leaf stages was highly susceptible to *P. tabacinum*, and susceptibility decreased with plant maturity (Fig. 2). The mortality of arrowhead in the 6- to 8-leaf stage was 50% 12 days after inoculation.

Only arrowhead plants in the family of Alismataceae were highly susceptible to *P. tabacinum* (Table 3). Small, restricted lesions developed on seedlings of Japanese morning-glory and Jimson-weed. No reaction occurred on other plant species tested.

In field experiments in both 1992 and 1993, the number of arrowhead stands in plots treated with *P. tabacinum* was significantly lower than that of non-treated plots (Table 4). The isolate YC8-7 reduced

Table 3. Reaction of various crop and weed species to *Plectosporium tabacinum* isolate YC8-7

Family, scientific name	Common name	Disease reaction ^a
Alismataceae		
<i>Sagittaria pygmaea</i> Miq.	Arrowhead	S-3
<i>S. trifolia</i> L.	Arrowhead	S-3
Amaranthaceae		
<i>Amaranthus lividus</i> L.	Pigweed	I
Chenopodiaceae		
<i>Chenopodium album</i> L.	Lamb's-quarters	I
Commelinaceae		
<i>Commelina communis</i> L.	Asiatic dayflower	I
Compositae		
<i>Bidens pilosa</i> L.	Beggar-ticks	I
<i>Xanthium strumarium</i> L.	Cocklebur	I
Convolvulaceae		
<i>Calystegia japonica</i> Choisy	Bindweed	I
<i>Ipomoea nil</i> (L.) Roth	Japanese morning-glory	S-1
Cruciferae		
<i>Brassica napus</i> L.	Rape	I
<i>Raphanus sativus</i> L.	Radish	I
<i>Lepidium sativum</i> L.	Garden cress	I
Cyperaceae		
<i>Cyperus difformis</i> L.	Small-flowered umbrella sedge	I
<i>Cyperus serotinus</i> Rottb.	Flat sedge	I
<i>Eleocharis kuroguwai</i> Ohwi	Water nutsedge	I
<i>Scirpus juncoides</i> Roxb.	Bulrush	I
Gramineae		
<i>Agropyron smithii</i> Rydb.	Wheat grass	I
<i>Avena fatua</i> L.	Red oat	I
<i>Avena sativa</i> L.	Oat	I
<i>Digitaria sanguinalis</i> (L.) Scop.	Large crabgrass	I
<i>Echinochloa crus-galli</i> P. Beau.	Barnyard grass	I
<i>Eleusine indica</i> (L.) Gaertn.	Goose grass	I
<i>Eragrostis ferruginea</i> P. Beauv.	Love grass	I
<i>Hordeum sativum</i> Jessen	Barley	I
<i>Oryza sativa</i> L.	Rice	I
<i>Panicum dichotomiflorum</i> Michx.	Fall panicum	I
<i>Setaria viridis</i> L.	Green foxtail	I
<i>Sorghum bicolor</i> Moench	Grain sorghum	I
<i>Triticum aestivum</i> L.	Wheat	I
<i>Zea mays</i> L.	Corn	I
Malvaceae		
<i>Abutilon theophrasti</i> Medik.	Velvet-leaf	I
<i>Gossypium hirsutum</i> L.	Cotton	I
<i>Sida spinosa</i> L.	Prickly sida	I
Leguminosae		
<i>Aeschynomene indica</i> L.	Indian joint-vetch	I
<i>Glycine max</i> L.	Soybean	I
<i>Trifolium repens</i> L.	White clover	I
Polygonaceae		
<i>Polygonum hydropiper</i> L.	Smartweed	I
<i>Rumex japonicus</i> Houtt	Dock	I
Pontederiaceae		
<i>Monochoria vaginalis</i> Presl.	Monochoria	I
Solanaceae		
<i>Datura stramonium</i> L.	Jimson-weed	S-2
<i>Solanum nigrum</i> L.	Black nightshade	I

^a S = susceptible on a scale of 1 to 3, where 1 = small lesion, 2 = partial blight, and 3 = complete blight and plant death; I = immune.

Table 4. Arrowhead stand in field plots^a treated with conidial suspensions of *Plectosporium tabacinum* isolates

Isolate ^b	1992 ^c	1993
YC 8-7	4.0 a	4.3 a
YC 8-16	5.7 a	5.5 a
Untreated check	13.7 b	15.2 b

^a Plots (2 m²) were established within natural paddy fields by planting 20 arrowhead tubers simultaneously with 15 rice seedlings.

^b In each plot, 20 arrowhead plants were sprayed with 1 liter of a conidial suspension of *P. tabacinum* (10^7 conidia/ml).

^c Values are the mean numbers of arrowhead stands from four replications 2 weeks after treatment. Means followed by the same letter in a column are not significantly different according to Duncan's multiple range test ($P = 0.05$).

stands by 70.8 and 71.7% in 1992 and 1993, respectively. The two isolates YC8-7 and YC8-16 did not differ in their ability to reduce arrowhead stands. Diseased arrowhead in treated plots 2 weeks after inoculation was blighted completely, whereas the plants in the untreated plots were healthy and vigorous.

DISCUSSION

The results of the experiments conducted show that *P. tabacinum* has potential as a mycoherbicide for controlling arrowhead. *P. tabacinum* has been recently described as a new name for *Fusarium tabacinum*, the anamorph of *Plectosphaerella cucumerina* (11). This fungus has been known to incite a number of diseases in crop plants including sunflower, cucurbits, tomato, and other hosts (1,12,16). The isolates of *P. tabacinum* used in this study caused necrosis, blight, and wilting of arrowhead within a short period after inoculation of a conidial suspension. The highly virulent isolate YC8-7 consistently induced disease over a wide range of inoculum concentrations with a 10-h dew period. The requirement of long periods of free moisture for infection is generally one of the principal constraints to the development of many fungal pathogens as mycoherbicides (3,4). In the case of this pathogen, however, the disease developed in a relatively short dew period and the application of the fungus in highly humid rice paddy fields could negate this problem.

Arrowhead seedlings of less than 5-leaf stage were susceptible to *P. tabacinum*, and the susceptibility decreased significantly with plant maturity. This suggests that the fungal inoculum should be applied to the weed in the seedling stage for effective control. The fungus proved to be highly host-specific to the *Sagittaria* genus and nonpathogenic to crop or other weed species tested, except two weed species, and therefore could be used as a mycoherbicide (13,15). However, the high specificity of the pathogen to arrowhead plants might be a disadvantage for development as a mycoherbicide, because most chemical herbicides used in rice paddies control more than one species.

Chemical herbicides such as bensulfuron-methyl or pyrazosulfuron-ethyl have

been used for controlling arrowhead as well as many other perennial paddy weeds, but these are known to be ineffective in controlling arrowhead because of its irregular emergence during the rice-growing season (5,10). Under this situation, *P. tabacinum* could be suitable as a mycoherbicide because the fungus could kill continuously emerging arrowhead seedlings during a whole growing season. Alternatively, this fungus can be combined with other chemical herbicides or biological control agents such as *Epicoccossorus nematosporus*, specific only to water nutsedge (*Eleocharis kuroguwai*), to control multiple species of important, hard-to-control perennial weeds in rice paddy fields (2,6,8).

The fungal inoculum was prepared easily by submerged culture, with conidia produced abundantly in common media such as PDB or cornmeal broth at 25 to 30°C. This fact is one of the important components in the cost-effective mass production of inoculum required for developing the pathogen as a commercial mycoherbicide (2). Inoculation of a conidial suspension to arrowhead plants consistently reduced the plant stand during 2 years of field experiments.

For developing this pathogen as a mycoherbicide, various limitations in biological, technological, environmental, and economic aspects should be solved (14). For example, effective methods to lower the cost of mass production and formulation to improve the efficacy of the pathogen are needed. Additional field trials will be conducted to demonstrate consistent field effectiveness.

ACKNOWLEDGMENTS

We thank W. Gams, Centraalbureau voor Schimmelcultures, The Netherlands, for the identification of *P. tabacinum*; and R. Charudattan, Plant Pathology Department, University of Florida, for suggestions and corrections in the preparation of this manuscript.

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