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STUDIES ON *Diaporthe eres (Phomopsis oblonga)* AS A NEW PATHOGEN OF WATER HYANCITH *(Eichhornia crassipes)* IN ROMANIA

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Abstract

Water hyacinth (Eichhornia crassipes) is a free-floating aquatic weed, known as the worst invasive one in many tropical and subtropical regions worlwide. This weed affect agricultural crops, navigation, irrigation and water quality as well. Sustainable management of water hyacinth is based on chemical, physical and biological means. The aim of this study, conducted at USAMV of Bucharest in 2018, was to identify fungal pathogens of water hyacinth in Romania as candidate for biological control agents and an environmentally safe solution. Diaporthe species are known as saprobes, endophytes or pathogens in many plants. As fungal pathogens, some species are associated with foliar spots, twig canker, shoots blight, wood and fruit rot. We report here the detection, morphological and molecular identification, the pathogenicity and host specificity on water hyacinth of one isolate of D. eres (Phomopsis oblonga). To our knowledge, this is the first report of D. eres a pathogen specific to water hyacinth in Romania.

Key words*: Diaporthe eres (Phomopsis oblonga), new report, water hyacinth.*

INTRODUCTION

Water hyacinth (*Eichhornia crassipes*) is an oceanic macrophytes and one of the worst sea-going weeds in the world. About 20 species are spread across the world in the late nineteenth century and early twentieth century (Wilson et al., 2005).

The genus *Diaporthe* includes more than 900 species, saprobes, endophytes or important as fungal plant pathogens (Uecker, 1988; Rehner & Uecker, 1994; Crous, 2005; Mostert et al, 2000; Rossman et al., 2007; Rossman & Palm-Hernández, 2008).

Phomopsis species (*Diaporthe anamorphs*) are traditionally identified on the basis of the morphological features of fructifications, the characteristics of colonies on artificial culture media and association with the host plant (Brayford, 1990; Mostert et al., 2001a; Chi et al., 2007). The redefinition of the *Phomopsis/ Diaporthe* species is underway, some species being renamed on the basis of a combination of molecular, morphological, cultural and phytopathological data (Udayanga et al., 2011). Several *Phomopsis* species were isolated and characterized as plant pathogens, as endophytes from the living tissues and also as saprophytes from the dead material (Promputtha et al., 2007; Udayanga et al., 2011). Some *Phomopsis* species have been reported as potential herbicides for controlling invasive and destructive weeds (Table 1) due to host specificity, persistence in the environment, their lifestyle and extended spores (Rosskopf et al., 2000a; 2000b; Ortiz-Ribbing & Williams, 2006).

With the trend towards organic farming and the limited use of herbicides, more attention is payed to the use of biological control agents (Ash, 2010; Bailey et al., 2010). Thus, research on biological weed control should address the most urgent and weed control problems where conventional pest management does not work and biocontrol would have potentially signifycant benefits for users (Auld & Morin, 1995; Greaves et al., 1998; Charudattan et al., 1990).

Therefore, pathogens that act on invasive plants should be re-evaluated, identify new ones and categorized as potential biocontrol agents (Charudattan, 1990; Ortiz-Ribbing & Williams, 2006).

Table 1. *Phomopsis* species as biological control agents of weeds

Pathogen	Host/target plant
Phomopsis spp.	*Carthamus lanatus*
P. emicis Shivas	*Emex australis*
P. convolvulus Ormeno	*Convolvulus arvensis*
P. amaranthicola Rosskopf, Charud., Shabana and Benny	*Amaranthus* sp.
P. cirsii Grove	*Cirsium arvense*

In this context, we believe that our results - identifying an isolate with the potential of microbial herbicide for water smile are among the priorities of this field.

MATERIALS AND METHODS

Detection, isolation and identification of Phomopsis oblonga (Diaporthe eres) in water hyacinth (Eichhornia crassipes)

During the observations made on the behavior of the common water hyacinth to different herbicides, a series of symptoms were identified to be caused by phytopathogenic agents.

Leaf fragments with spot symptoms have been superficially disinfected and incubated in a humid chamber as well on artificial culture medium (Potato Glucose Agar). Incubation was performed at 22-24°C.

Developed colonies were identified both by direct examination, based on morphological characters, and microscopic examination (fructification morphology). Pure culture of the tested isolates are maintained in the collection, on PGA medium.

Confirmation of identification based on morphological characters was accomplished by molecular methods. Thus, cultures of the origin isolate as well as isolates obtained after artificial inoculations were subjected to DNA extraction. Polymerase Chain Reaction (PCR) was conducted with universal ITS1/ITS4 primers. The amplification products were sequenced and the sequences obtained were analyzed using BLAST (the Basic Local Alignment Search Tool (https://blast.ncbi.nl.m. nih. gov/Blast.cgi, NCBI Nation Center for Biotechnology Information).

Pathogenicity test of Phomopsis oblonga (Diaporthe eres).

The specificity and pathogenicity of our *Phomopsis oblonga* (*D. eres*) isolate was analyzed by artificial contamination of water hyacinth (*Eichhornia crassipes*) according to Koch's postulate.

Spores and mycelial suspensions were obtained from pure cultures of the tested isolate. Artificial contaminations were carried out by spraying (26.10.2017). The tested variants were represented by control and artificially contaminated plants (as four plants of water hyacinths/ variant, initially; during the study the plants have been multiplied). The tests were done in basins. Observations were carried out 14-30 days after the date of inoculation, with the presence of characteristic symptoms on the leaves (initially yellowish - brownish or brown spots). One last observation was done 61 days after inoculation. There have been noted, on foliar level:

a. Frequency of attack (F, %), as:

$F\% = (n \times 100)/N$, where: F - frequency of attack; n - number of plants or organs of the attacked plant; N - total number of plants or organs of the attacked plant.

b. Intensity of attack (I, %) or severity of attack:

$I\% = \Sigma (i \times f)/N$ (Al-Waily, 1988), where: I -the intensity of attack rated by scoring; f - number of plants showing the intensity (i); N - total number of plants or organs of the attacked plant. The product (i x f) is calculated for each attack intensity class.

A 5-grade scale was used to measure the severity of the symptoms (Mickenny, 1923): 0 = healthy leaves, no symptoms; 1 = symptoms on 25% of the leaf surface; 2 = symptoms on 50% of the leaf surface; 3 = symptoms on 75% of the leaf surface; 4 = symptoms on the entire surface of the leaves (100%), necrotic tissues, death.

c. Attack rate (AR, %):

$AR\% = (F \times I)/100$, where: F - frequency (%) and I - attack intensity (%).

RESULTS AND DISCUSSIONS

Results on the specificity and pathogenicity of Diaporthe eres (Phomopsis oblonga) in water hyacinth

The specificity and pathogenicity of the *P. oblonga* isolate was tested by artificial inoculation of water hyacinth plants.

Our results highlight the specificity of the analyzed isolate, the presence of the species *Diaporthe eres (Phomopsis oblonga)* being confirmed. The confirmation was based on morphology of colonies and fructifications as well as molecular. Thus, the culture obtained from the reisolations was subjected to molecular identification tests.

A sequence of 527 nucleotides was obtained:

GACCCTTTGTGAACTTATACCTTACTGTTGCCTC
GGCGCTAGCTGGTCCCTCGGGGCCCCTCACCCTC
GGGTGTTGAGACAGCCCGTCGGCGGCCAACCTA
ACTCTTGTTTTTACACTGAAACTCTGAGCACAAA
ACATAAATGAATCAAAACTTTCAACAACGGATC
TCTTGGTTCTGGCATCGATGAAGAACGCAGCGA
AATGCGATAAGTAATGTGAATTGCAGAATTCAG
TGAATCATCGAATCTTTGAACGCACATTGCGCCC
TCTGGTATTCCGGAGGGCATGCCTGTTCGAGCGT
CATTTCAACCCTCAAGCCTGGCTTGGTGATGGG
GCACTGCTTCTTACCCAAGAAGCAGGCCCTGAA
ATTCAGTGGCGAGCTCGCCAGGACCCCGAGCGC
AGTAGTTAAACCCTCGCTCTGGAAGGCCCTGGC
GGTGCCCTGCCGTTAAACCCCCAACTTCTGAAA
ATTTGACCTCGGATCAGGTAGGAATACCCGCTG
AACTTAAGCATATCAATAAGCGGAGGA.

A similarity of 100% of our isolate was obtained using BLAST analyse with *D. eres (P. oblonga)* strains.

Symptoms of water hyacinth leaves after artificial infections and *P. oblonga* pathogen isolation are shown in Figure 1.

Figure 1. Symptoms of the *Diaporthe eres* (*Phomopsis oblonga*) on the water hyacinth leaves (photo Al-Gburi)

The pathogenicity of our isolate on the leaves of the water hyacinth was confirmed according to Koch postulate. Leaves with fungal infection were disinfected and incubated on the PGA culture medium. After 3-4 days, new mycelial growths have been observed on the culture medium. Those fragments were transferred to obtain pure cultures.

Suspensions of spores and mycelium were used to inoculate the leaves of water hyacinth plants. From plants that developed symptoms similar to those observed on the plants where the fungus was initially isolated, reisolation was performed.

Based on the morphological characters and molecular tests the presence of *D. eres* species was detected and identified as a specific pathogen to water hyacinth (Figure 2).

Figure 2. Isolation from infected leaves and inoculation in Petri dishes with culture medium (photo Al-Gburi)

Following the artificial infection tests, the potential of the *P. oblonga* isolate as a herbicide was analyzed.

Aspects during the articicial inoculation tests are shown in Figures 3 and 4.

Figure 3. Symptoms with the appearance of brown or black spots on leaves

Figure 4. Spraying the plants of the water hyacinth - artificial contamination with *D. eres* isolate

The effect of the fungi on the leaves was estimated by measuring the effect of the pathogenic fungi on the leaf surface.
The frequency of the leaves with characteristic symptoms was 86.62% and the intensity of the attack was 68.45%.
The frequency of attacked leaves after inoculation was further classified as: 8.45% with the note 1 for the attack intensity; 9.15% with the note 2 for the attack intensity; 14.79% with the note 3 for the attack intensity and 54.22% with the note 4 for the intensity of the attack (Table 2).
There is a high frequency of leaves in classes 3 and 4, classes in which the affected area is 75% and 100%, respectively.
The healthy (13.38%) and infected leaves (86.62%) were counted and sorted according to their attack degree. The attack intensity was 6.97% and the attack rate was 4.65% after 61 days after the artificial contamination.

Tabel 2. Frequency of leaves with symptoms characteristic of *D. eres* (*P. oblonga*) depending on the intensity of the attack (artificial infections)

Frequency (%)	Intensity	
	(note)	Surface attacked (%)
8.45	1	25
9.15	2	50
14.79	3	75
54.22	4	100

We highlight high values of these two indicators, which confirm the high degree of leaf colonization and the expansion of the attack over time.
The rate of attack calculated based on frequency and intensity was 59.29%, a value that we consider very good for a biological control agent. We have noticed the preservation of the herbicide potential of the *P. oblonga* isolate after 61 days on newly emerging leaves compared to the application of a classical herbicide, where this effect is not recorded. This fact constitutes an argument in addition to the orientation of studies in the direction of microbicides.
We believe that our isolate has the potential of a biological control agent. Current studies are carried on to determine the dose and the possibility of a herbicide treatment, reducing the dose of chemical molecules.

We report, for the first time in Romania and worldwide, the potential of the species *Diaporthe eres (Phomopsis oblonga)* as a biological control agent (bio-herbicide) of aquatic weed species like water hyacinth.

CONCLUSIONS

We report for the first time in Romania and globally the potential of *Phomopsis oblonga (D. eres)* as a biological control agent (bio-herbicide) of aquatic weed species of the water hyacinth type.
The use of fungi on water hyacinth plants had a clear effect by reducing the level of plant growth.

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REFERENCES

AL-Waily D.S.A. (1988). Studies of early blight of tomato caused by *Alternaria solani*. Master thesis. Agric. Colleg, Unvi of Baghdad.
Ash, G.J. (2010). The science, art and business of successful bioherbicides. *Biol. Control*, *52*, 230–240.
Auld, B.A., Morin, L. (1995). Constraints in the development of bioherbicides. *Weed Technol*., *3*, 638–652.
Bailey, K.L., Boyetchko, S.M., Langle, T. (2010). Social and economic drivers shaping biological control: a Canadian perspective on the factors affecting the development and use of microbial biopesticides. *Biol. Control*, *52,* 222–229.
Brayford, D. (1990). Variation in *Phomopsis* isolates from *Ulmus* species in the British Isles and Italy. *Mycol Res*., *94*. 691–697.
Charudattan, R., Devalerio, J.T., Prange, V.J. (1990). Special problems associated with aquatic weed control. *In* New Directions for Biological Control: Alternatives for suppressing Agricultural Pests and Diseases, 287–303.
Chi, P., Jiang, Z., Xiang, M. (2007). Flora Fungorum Sinicorum. Vol. 34. *Phomopsis*. Science Press, Beijing, China.
Crous, P.W. (2005). Impact of molecular phylogenetics on the taxonomy and diagnostics of fungi. *Bull. OEPP/EPPO*, *35,* 47–51.

Greaves, M.P., Holloway, PJ., Auld, BA. (1998). Formulation of microbial herbicides. In: Burges HD (ed) Formulation of microbial biopesticides, beneficial microorganisms, nematodes and seed treatments. Kluwer, London, 203–234.

Mostert, L., Crous, P.W., Kang, J.C., Phillips, A.J.L. (2001). Species of *Phomopsis* and a *Libertella* sp. occurring on grapevines with specific reference to South Africa: morphological, cultural, molecular and pathological characterization. *Mycologia, 93*, 146–167.

Mostert, L., Crous, P.W., Petrini, O. (2000). Endophytic fungi associated with shoots and leaves of *Vitis vinifera*, with specific reference to the *Phomopsis viticola* complex. *Sydowia*, *52*, 46–58.

Ortiz-Ribbing, L., Williams, M.M. (2006). Conidial germination and germ tube elongation of *Phomopsis amaranthicola* and *Microsphaeropsis amaranthi* on leaf surfaces of seven *Amaranthus* species: implications for biological control. *Biol. Control*, *38*, 356–362.

Promputtha, I., Lumyong, S., Vijaykrishna, D., McKenzie, E.H.C., Hyde, K.D., Jeewon, R. (2007). A phylogenetic evaluation of whether endophytes become saprotrophs at host senescence. *Microb. Ecol*., *53*, 579–590.

Rehner, S.A., Uecker, FA. (1994). Nuclear ribosomal internal transcribed spacer phylogeny and host diversity in the coelomycete *Phomopsis*. *Canadian Journal of Botany*, *72*, 1666–1674.

Rosskopf, E.N., Charudattan, R., DeValerio, J.T., Stall, W.M. (2000). Field evaluation of *Phomopsis amaranthicola*, a biological control agent of *Amaranthus* spp. *Plant Dis.*, *84*, 1225–1230.

Rosskopf, E.N., Charudattan, R., Shabana, Y.M., Benny, G.L. (2000). *Phomopsis amaranthicola*, a new species from *Amaranthus* sp. *Mycologia*, *92*, 114–122.

Rossman, A.Y., Farr, D.F., Castlebury, L.A. (2007). A review of the phylogeny and biology of the *Diaporthales*. *Mycoscience*, *48*. 135–144.

Rossman, A.Y., Palm-Hernández, M.E. (2008). Systematics of plant pathogenic fungi: why it matters. *Plant Dis*., *92,* 1376–1386.

Udayanga, D., Liu, XZ., Cai, L., Hyde, K.D. (2011). The genus *Phomopsis*: biology, applications, species concepts and names of common phytopathogens. *Fungal Diversity*, *50*, 189–225.

Uecker, F.A. (1988). A world list of *Phomopsis* names with notes on nomenclature, morphology and biology. Ed. Berlin-Suttgart: J. Cramer.