

Fusarium culmorum isolated from rhizosphere of woolly cupgrass (*Eriochloa villosa*) in Debrecen (East Hungary)

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SUMMARY

Woolly cupgrass (*Eriochloa villosa*) is an East-Asian originated weed species and it has been spreaded worldwide by now. The first occurrence of this species in Hungary was observed and published in 2008 nearby Gesztely village (Borsod-Abaúj-Zemplén county, North-East Hungary) than in the summer of 2011 a significant population was discovered next to Debrecen city (Hajdú-Bihar county, East Hungary). In 2013 this weed was also reported from Szentborbás village, Somogy county (South-West Hungary). These observations of spreading and its biological features (production of stolons and large number of seeds, moreover herbicide tolerance) indicate that woolly cupgrass (*E. villosa*) has a great potential of invasiveness, so it may become a hazardous weed not only in Hungary but in all over the world.

The objective of this study was to identify the fungus which was isolated from woolly cupgrass (*E. villosa*) root residue samples which were collected after maize harvesting on arable land in late autumn, near Debrecen. The identification of the fungus based on morphological characters of colonies and the features of conidia developed on potato dextrose agar (PDA) plates. After the examination of axenic culture we revealed that the fungus from rhizosphere of woolly cupgrass was *Fusarium culmorum*. Pathogenicity and/or endophytic relationship between the fungus and woolly cupgrass is still uncertain so pathogenicity tests and reisolations from plants are in progress.

Keywords: *Fusarium culmorum*, *Eriochloa villosa*, woolly cupgrass, ERBVI, *Fusarium* spp.

ÖSSZEFOGLALÁS

Az ázsiai gyapjúfű (*Eriochloa villosa*) egy Kelet-Ázsiai eredetű gyomnövény, mely gyors terjedése nyomán manapság már a világ számos pontján megtalálható. Magyarországon 2008-ban jelent meg az első cikk a felbukkanásáról Gesztely község határában (Borsod-Abaúj-Zemplén megye), majd 2011 nyarán egy nagyobb populációját találták meg Debrecen (Hajdú-Bihar megye) közelében. 2013-ban ugyancsak fellelhető volt Szentborbás község külterületén, Somogy megyében (Délnyugat-Magyarország). E megfigyelések a hazai terjedéséről és a biológiai alaptulajdonságai (inda-képzés, nagy mennyiségű magtermelés, valamint herbicid-tolerancia) miatt megalapozottan kijelenthető, hogy az ázsiai gyapjúfű (*E. villosa*) nagy inváziós potenciállal rendelkezik, így veszélyes gyomfajként kell rá tekinteni Magyarországon és az egész világon egyaránt.

Jelen vizsgálataink célkitűzése annak a mikroszkópikus gombafajnak a meghatározása volt, melyet a Debrecen közelében elhelyezkedő (a kukorica betakarítását követően a talajon visszamaradt) ázsiai gyapjúfű gyökereinek mintáiból, késő ősszel gyűjtöttünk és izoláltunk. Az izolált kórokozó pontos meghatározása érdekében telepmorfológiáját és az ivartalan spórák (konídiumok) tulajdonságait burgonya dextróz agar (PDA) táptalajon figyeltük meg. Az axénikus tenyésztés eredmények azt mutatják, hogy az ázsiai gyapjúfű minták rhizoszférájából a *Fusarium culmorum* fajt identifikáltuk. A gomba patogénitása és vagy endofiton jellege az ázsiai gyapjúfű növényen még bizonytalan, a mesterséges inokulációs és a vegetatív növényi részekből való izolálhatósági vizsgálataink folyamatban vannak.

Kulcsszavak: *Fusarium culmorum*, *Eriochloa villosa*, ázsiai gyapjúfű, ERBVI, *Fusarium* spp.

INTRODUCTION

The woolly cupgrass (*Eriochloa villosa* [Thunb.] Kunth) belongs to the group of well adaptable weed plant species. The distribution of the plant in the temperate zones of Asia, Caucasus, Russia's Far Eastern region, China, Iran, Japan, Korea, Taiwan and Vietnam is common (Tsvelev, 1984). In several states of the USA and Canada were also released. The species has been found in France, Ukraine, Romania and Hungary (Figure 1).

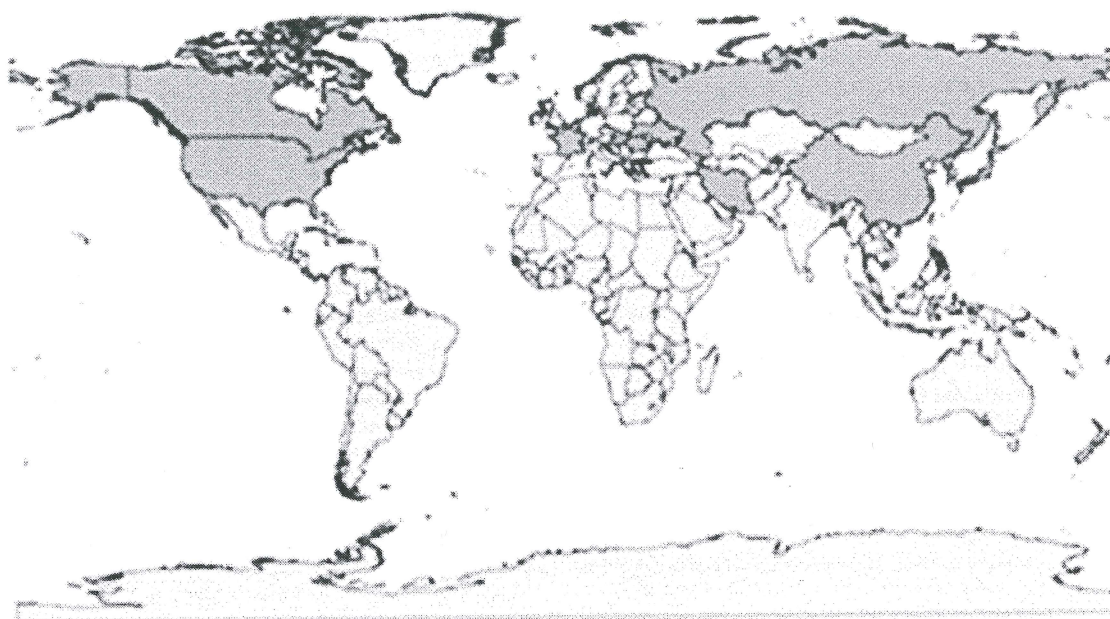
The first report of observation was made by J. Madarász and P. Partosfalvi on the 5th (Hungarian) National Weed Surveys in 2007. Partosfalvi et al. (2008) reported the occurrence of woolly cupgrass near Gesztely village (North-East Hungary) in 2007.


The woolly cupgrass plants were also detected in Debrecen (East Hungary) on agricultural fields by Somogyi et al. (2011).

In 2013 a small population of this weed was reported from close to Szentborbás village (Somogy county, South-West Hungary), too (Takács et al., 2014).

The Hungarian climate provides good conditions for this recently appeared weed species. The most favourable conditions for colonization of woolly cupgrass are 10–11 °C in average annual temperature, 550–600 mm in average annual rainfall, and 5.5–6.5 soil pH (Fárcăşescu et al., 2008). The optimum germination temperature for most of the weeds is between 20–35 °C (Bello et al., 2000). The germination of woolly cupgrass starts in mid-April in Hungary. The mass hatching period is followed by a second minor germination peak. Hence, the emergence fully extended, which makes it difficult to control. The ripening of grain yield starts in August and begin to achieve its distribution by animals and human activities. The woolly cupgrass belongs to T4 group of plants according to the Ujvárosi weed plant-life-type classification system.

Figure 1: Distribution of woolly cupgrass worldwide



 distribution based on published sources

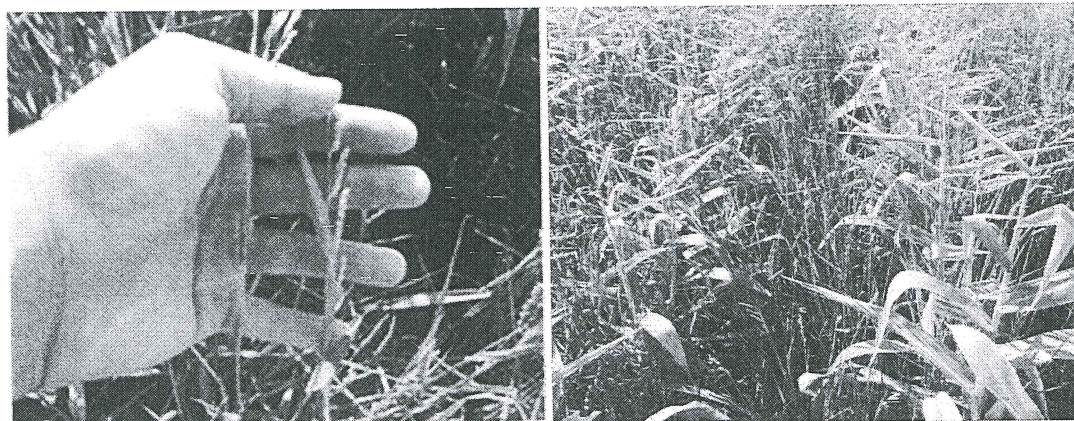
Woolly cupgrass gets its name from the hairs covering many parts of the plant. It is a 30–200 cm high annual grass with dark green colour and densely hairy leaves that have a velvety feel. Only one edge of the leaf blade is typically crinkled, and the flowering branches are also one-sided (Figure 2). The woolly cupgrass impairs corn, sunflower, soybean cultures and stubble-fields.

According to the Systematic Mycology and Microbiology Laboratory Fungal Database (Farr and Rossman, 2016) there were 5 different fungal disease reported from woolly cupgrass (*E. villosa*), viz. *Thanatephorus cucumeris* (A.B. Frank) Donk from China (as '*Corticium sasakii*' Shirai/ H. Matsumoto) and Taiwan, *Pyricularia oryzae* Cavara from Japan, *Tolyposporium bullatum* J. Schröt. from China, *Uromyces eriochloae* (Syd. & P. Syd.) Syd., P. Syd. & E. J. Butler from China and Japan, moreover *Uromyces setariae-italicae* Yoshino from Japan and Taiwan.

After we examined the morphological features of the colonies and conidia (asexual spores) the fungus proved as a *Fusarium* species on *E. villosa*.

Most of the species of *Fusarium* genus are plant pathogens. These fungi are usually soilborne, but they can be also dispersed by wind and rain. The different strains can occur in soil, on plant surfaces as saprotrophic microorganisms or insider plant pathogens as endophytes. The members of *Fusarium* genus are widespread all over the world and they can grow on numerous substrates (Leslie and Summerell, 2006). *Fusarium* species are able to overwinter mostly on infected plant debris as mycelia, or by chlamydospores. The penetration to healthy plants can occur on root tips (directly or through wounds) or at the time of the formation of lateral roots (Agrios, 2005).

Figure 2: Woolly cupgrass inflorescence branch, infesting a maize field



The diseases what the *Fusarium* species can cause on cultivated or weedy plants have wide range, which includes pre- and postemergence damping-off, head blight or foot rot symptoms. *F. culmorum* was also discovered and named as a common casual agent of different diseases on strawberries (Cieslinski and Lis, 1989), leeks (Koike et al., 2003), sorghums (Patil and Padule, 2000), hops (Sabo et al., 2002) and carnations (Broadhurst, 1990).

MATERIAL AND METHODS

The woolly cupgrass root samples infested with mycelia were collected on 2015. 11. 13. next to Debrecen (GPS coordinates: 47;26;29.20 21;34;19.72) at Hajdú-Bihar County (East Hungary) (Figure 3).

Figure 3: Woolly cupgrass (*Eriochloa villosa*) with white, dense mycelia on the roots



First roots of woolly cupgrass (*E. villosa*) were washed from soil with tapwater for 5 minutes. For isolation the species from infested plant debris were cut into 3 cm pieces and the surface of roots were sterilized in 1% chlorogen ($C_6H_5SO_2NCINa.xH_2O$) (Neomagnol) solution for 5 minutes and rinsed 3 times in sterile distilled water. After the surface-sterilization the samples were placed into moist chambers (9 cm Petri dishes containing filter papers and moistened by sterile tapwater) for 7 days.

When mycelia appeared the fungus were transferred to potato dextrose agar (PDA) plates and incubated at 20 °C.

Morphological identification

The identification of fungus on woolly cupgrass roots were based on the morphology of asexual spores (macroconidia) under light microscope and the specifications of the colony. Conidia were photographed by Zeiss AxioCam MRc5 camera. The species was characterized by mycelial growth rate, the pigmentation of the colony and the density of mycelia growing on potato dextrose agar (PDA) plates on the 5th day. We

studied these parameters to delimitate the *Fusarium* species according to Leslie and Summerell (2006).

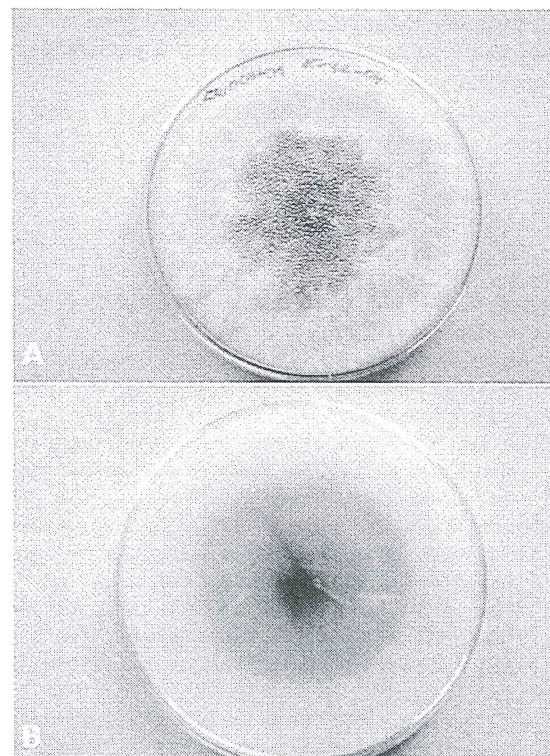
RESULTS AND DISCUSSION

Colony morphology of the fungus on woolly cupgrass roots

The morphology of the colonies on potato dextrose agar (PDA) and the macroconidia on sporodochia were typical of those specifications which were described for *Fusarium culmorum* (Wm. G. Sm.) Sacc. by Leslie and Summerell (2006).

Fusarium culmorum is a rapid growing fungus (fill the plate within 5 days) and produces abundant sporodochia. Colour of colonies showed pale orange first and became dark brownish to reddish later. The examined strains formed red pigments in the agar and dense, white, aerial mycelia (Figure 4).

Figure 4: Colony morphology of *Fusarium culmorum* on PDA plate (A: front, B: reverse) on the 5th days of transfer



Macroconidia

The asexual spores under microscope were relatively short and stout (compared to other *Fusarium* species), and cell walls were thick. The macroconidia were the widest at midpoint, dorsal side were curved, but ventrally almost straight. The foot cells of the spores were rounded and blunt. Number of septa were 3, 4 or 5 (Figure 5). There were no microconidia observed.

It is important to highlight that off-season woolly cupgrass (*E. villosa*) root samples were collected during this survey and we couldn't see any disease symptoms except the white mycelia on the roots, which means that the isolated *Fusarium culmorum* may existed as a saprothrophic microbe in the rhizosphere of the weed.

Figure 5: The short and stout asexual spores (macroconidia) of *Fusarium culmorum*



This observation also indicates the importance of clearing agricultural fields and edges from weeds, because these can serve as reservoirs and may contribute to survive pathogens.

CONCLUSIONS

Eriochloa villosa (wooly cupgrass) is an annual grass weed resulting serious losses, and the control is difficult and expensive. Its biological features (production of stolons and large number of seeds, herbicide tolerance) indicates that the effective control carried out only by combined elements of Integrated Pest Management (IPM) methods (chemical, cultural and mechanical). Until now there is no information about effective biocontrol opportunity against this invasive weed species.

The objective of this survey was to identify the fungus which was occurred on the rhizosphere of wooly cupgrass (*E. villosa*) samples. It was concluded that the identified species was *Fusarium culmorum*. Traditional identification was made on the base of morphological properties of colony and characterisation of asexual spores (macroconidia).

As *F. culmorum* is a common plant pathogen and causes different diseases on important cultivated plants so it can't be used as a potential biocontrol agent against this hazardous weed species.

To our knowledge this is the first report on the occurrence of *Fusarium culmorum* from wooly cupgrass (*E. villosa*) rhizosphere in Hungary. Pathogenicity and/or endophytic relationship between fungus and wooly cupgrass is still uncertain so pathogenicity tests and isolations from plants are in progress.

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