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FIRST EVALUATION OF *USTILAGO PHRYGICA* FOR THE BIOLOGICAL CONTROL OF *TAENIATHERUM CAPUT-MEDUSAE* (TRITICEAE)

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Summary: *Taeniatherum caput-medusae* (L.) Nevski, medusahead ryegrass, is an invasive weed in the U.S. with origins in the Mediterranean region extending to central Asia. Currently, it infests millions of hectares of land, primarily in the western U.S. This study examines biological control, through the use of plant pathogens, as a possible management strategy. The smut *Ustilago phrygica* collected in Turkey, Cyprus and Bulgaria in 2002 on medusahead induces severe symptoms where seeds are replaced by the sori, thus breaking the life cycle of this annual plant. We describe a field and laboratory host range test with 20 plants each of medusahead plus closely related cultivated cereals of economic importance (wheat, oat, barley, and rye). Preliminary results showed that medusahead plants became infected with 10 mg teliospores of *U. phrygica* under greenhouse and field conditions exhibiting typical smut symptoms for this plant. No infection was observed on any of the cultivated cereals or on non treated controls. The close taxonomic relationships to wheat, barley and rye make biological control of medusahead complicated for testing specificity. The results of infection with *U. phrygica* encourage testing it on a wider range of grasses, evaluating the impact of the fungus, and optimising conditions for infection.

Première évaluation de *Ustilago phrygica* en vue d'une lutte biologique contre *Taeniatherum caput-medusae* (Triticeae)

Résumé : *Taeniatherum caput-medusae* (L.) Nevski, l'orge à tête de méduse, est une plante envahissante aux Etats-Unis, dont l'origine se situe autour du bassin méditerranéen jusqu'à l'Asie centrale. Actuellement, elle occupe plusieurs

millions d'hectares de prairies, principalement dans l'ouest des USA. Cette étude s'intéresse à la lutte biologique au travers de l'utilisation d'agents pathogènes, en vue d'une éventuelle stratégie de gestion de cette plante. Le charbon *Ustilago phrygica* récolté en Turquie, Chypre et Bulgarie en 2002 sur l'orge à tête de méduse engendre des symptômes forts, transformant les graines en ergots, entraînant la rupture du cycle biologique de cette graminée annuelle. Nous décrivons un test de spécificité mené au champ et en serre sur 20 plantes par espèces végétales d'orge à tête de méduse et de céréales cultivées, telles que le blé, l'orge, le seigle et l'avoine. Les résultats préliminaires montrent que l'orge cible est inoculée avec succès avec 10 mg de teliospores d' *U. phrygica* dans les deux conditions, extériorisant des symptômes classiques de charbon. Aucune infection n'a été observée sur aucune des céréales cultivées, ni sur les témoins non traités. La prochaine étape consistera à inoculer le champignon à une plus grande gamme d'espèces de graminées. La proximité phylogénétique de l'orge cible avec les céréales cultivées à haute valeur ajoutée, rend les tests de lutte biologique difficiles à mener. Cependant, ces premiers résultats d'infection expérimentale nous encourage vers une poursuite de l'étude.

INTRODUCTION

Medusahead ryegrass, *Taeniatherum caput-medusae* (L.) Nevski (previously misidentified as *Elymus caput-medusae* L.), is a member of the Triticeae tribe of the grass family. Medusahead is one of the 25,000 non-native plant species in the U.S. causing damages and losses adding up to more than $27 billion annually. Medusahead was first collected in the U.S. in Oregon in 1887, and was probably initially brought through dispersal of seed by imported animals. It is now invasive across millions of hectares of large semi-arid areas of intermountain rangelands in the western U.S., in which it is considered a noxious weed (e.g. Colorado, California, Oregon, Nevada, and Utah). It occurs at more than 60% in monoculture in western rangelands over 188,000 hectares in 1992 (Pellant & Hall 1994). Its rapid spread has presented a serious problem to wildlife and range managers. Medusahead has substantially impacted ecosystem functioning in a way that ensures its persistence. Medusahead is a slender annual grass, 5–60 cm high, which is predominantly self-pollinated. Medusahead grows in areas where extended periods of

intense cold are lacking. This grass has its origins in areas bordering the Mediterranean sea extending eastward to central Asia (Frederiksen 1986). The center of origin is the southwest Asia, specifically Turkey, Iran, and Iraq (Humphries 1978). All genera from the subtribe Triticinae, in which *Taeniatherum* belongs, are introduced to North America. Different strategies that include burning, grazing, plant competition, restoration of natives, and chemicals are being used for control. Prior to our study, no biocontrol strategies have been previously studied. As attempts to control this annual weed have generally resulted in failure (Horton 1991), our study based on foreign exploration tends to find and characterize biological agents to be used for controlling the weed population. There are currently no natural enemies for the biocontrol of medusahead available in North America.

The only report of pathogens attacking medusahead collected in its native range is the fungus *Fusarium arthrosporioides* isolated from the crown of medusahead in Greece (Siegwart et al. 2003). The authors reported in experimental conditions the effect of the pathogen on medusahead, and on cultivated cereals, which stopped further studies with this pathogen for biological control purpose because it was not found to be specific to medusahead. In North America, the effects of five soil fungi, endemic to the western U.S., were evaluated for disease reaction, root dry weights, and shoot dry weights of five grass species on several weeds including medusahead (Grey et al. 1995). The authors reported that medusahead was susceptible to crown rot (*Fusarium culmorum*). From the literature, a few plant pathogens are listed on medusahead such as *Tilletia bornmuelleri, Puccinia graminis*, and *Ustilago phrygica* (Braun 1995; Vánky 1994). All of them were recently collected from medusahead in Eurasia (Widmer & Sforza 2004). The objective of this study is to focus on the pathogenicity and the specificity of *Ustilago phrygica* (Ustilaginales) by starting a preliminary field and laboratory host range test with local medusahead plants plus closely related cultivated cereals of economic importance.

MATERIAL AND METHODS

Collected pathogens. Symptomatic whole plants were collected in the field in Erzurum province, Turkey in September 2002 at 1,800 meters. The plants were maintained individually in a plastic bag for transportation until they could be transferrerd to a quarantine for identification and spore harvest. Identification of *U. phrygica* was performed by Dr. Kalman Vánky, Tubingen, Germany.

Plant material: Five plant species including medusahead ryegrass and 4 cultivated cereals that included wheat *(Triticum aestivum* cv Kirik), rye (*Secale cereale*), barley (*Hordeum vulgare* cv Tokak), and oat (*Avena sativa* cv Gez) were selected. All seeds and cultivars originated from Turkey.

Smut inoculation: Inoculations with *U. phrygica* are based upon modifications previouslty described (Jones & Dhitaphichit 1991). After Removing the hulls of the seeds and surface-sterilizing for 5 minutes in 5% NaOCl, the seeds were rinsed twice in distilled water. Fifty seeds of each plant species were mixed with approximately 10, 25 or 40 mg of teliospores, 0.83 mg of carboxymethylcellulose, and 1.67 ml of sterile water containing 0.001% Tween. The seeds were vacuum-infiltrated for 5 minutes, then dried 1 hour on filter paper. The treated seeds were placed in 1.5% water Agar solidified in sterile plastic Petri dishes at 25 ^{o}C. After 7 days, the seedlings were transplanted into pots (11x9.5 cm in diameter) with commercial soil and stored in a greenhouse until further use. Non inoculated controls were treated in the same manner except without the teliospores.

Experimental field plot: A 6-square meter experimental garden was designed 60 km southwest of Erzurum (Turkey), 39°34'929" N and 40°54'210"E, in the area where *U. phrygica* was originally collected. Tested plants were transplanted in five single rows with 0.2 m between plants and 0.4 m between rows. The plants were observed until they died. Additional rows contained the controls. Each plant species had 20 individuals treated with *U. phrygica* plus five non treated controls. A total of 125 plants were transferred to the open garden. Plants were watering weekly until the end of June. A similar experiment was conducted in the Atatürk University (Erzurum, Turkey) greenhouse.

RESULTS

Field survey and symptom description

Ustilago phrygica-infected medusahead plants were collected from May to September in 2002 in Turkey, Cyprus, and Bulgaria. Infection was sparse with two to three infected plants together and then no infection occurring for a few meters. Typical symptoms of *U. phrygica* infection included sori usually comprising the whole spike leaving intact stunted and deformed awns, slightly bullate, subepidermal which was long-

remaining and covered by the epidermis that ruptures disclosing the blackish-brown, powdery spore mass. During flowering, the seeds were replaced by the sori, thus effectively breaking the life cycle of this annual plant. The *U. phrygica* used in this study originated from Turkey.

Experimental inoculation

No infection of any plants was observed with spore concentrations of 25 or 40 mg (data not shown). Under each condition (open field and greenhouse), one medusahead plant displayed typical smut symptoms. The infected plants showed symptoms approximately 3 months from the inoculation date. Complete results are listed in table 1. Except for a few dead plants, the majority of medusahead plants produced leaves without bolting. No development of smut symptoms on any other cultivated cereals was recorded. In addition, none of the cultivated cereals died during the experiment under both conditions, with 100% of the controls and 96% of inoculated plants bolting with normal heads.

Table 1: Disease evaluation after inoculation of medusahead and cultivated cereals with 10 mg of *Ustilago phrygica* teliospores in greenhouse and open field conditions.

		Hordeum vulgare	Secale cereale	Triticum aestisum	Avena sativa	Taeniatherum caput-medusae
Greenhouse	Treated	0/19	0/18	0/19	0/20	**1**/16
	Controls	0/5	0/5	0/5	0/5	0/5
Open field	Treated	0/20	0/18	0/19	0/20	**1**/16
	Controls	0/5	0/5	0/5	0/5	0/5

Number of symptomatic plants/Number of bolted plants.
For each species 20 plants were used for treated material, and 5 for non treated controls.

DISCUSSION AND CONCLUSIONS

Experimental and field-collected data obtained in 2003 tend to confirm that *U. phrygica* may be specific to *T. caput-medusae*. We showed that wheat, rye, oat, and barley, inoculated with 10 mg of *U. phrygica* teliospores did not become infected with the smut and almost always produced normal seeds. In contrast, one medusahead plant become

infected in each condition proving successful infection, but insufficient for statistical analysis. This also proves that 10 mg of teliospores is sufficient for infection compared to inoculation with 25 mg and 40 mg that resulted in no infection on any species. Regarding the non bolted medusahead plants at the end of this preliminary experiment, we hypothesize that seeds were not stored under cold conditions long enough as it occurs naturally in eastern Turkey from October to April, and were not mature enough for optimal development.

U. phrygica has been studied specifically on *Aegilops* and *Hordeum* species, which are closely related to *Taeniatherum* spp. (Nielsen 1985, 1987). It is reported that a few American natives (*Elymus canadensis, Hordeum jubatum, Hordeum pusillum,* and *Hordeum compressum*) were successfully inoculated artificially with spores originally collected on *T. caput-medusae* in Turkey (Vánky 1994). These results should not be a barrier to study and test *U. phrygica* over the range of species proposed in the test plant list. A study involving *Ustilago tritici* showed that two North American natives, *Hordeum jubatum* and *Elymus canadensis*, were successfully infected under greenhouse conditions, however, *U. tritici* was not found in any field collections of smut from these two grasses in the Canadian area investigated (Nielsen 1993). It is known that pathogens may show an artificial expansion of their host range under greenhouse conditions (Adams 1988; Watson 1985). In that regard, crops and North American natives will be first targeted in future preliminary host range testing.

According to phylogenetic relationships, wheat and rye belong to the same subtribe (Triticineae), and barley to the same tribe as medusahead rye. It is too early to argue that the *U. phrygica* tested in this study is a *formae speciales,* but 2003 results are very promising for testing this smut fungus in the near future over a wider range of plant species including several species from the Triticineae such as *Aegilops* spp., and from the Hordeinae such as *Agropyron* spp. and *Elymus* spp. A list of plants taxonomically related to medusahead for screening of potential control agents was proposed that includes members of different tribes of Pooideae such as Triticeae (*Hordeum* sp., *Elymus* sp., *Agropyron* sp., *Aegilops* sp., etc), Stipeae, Brachypodieae, Poeae, Andropogonae, and Paniceae, plus closely related families like Chloridoideae and Ehrhartoideae. The selected plant species cover a large number of grasses closely related to medusahead, including all the major cultivated cereals present in the U.S. In addition, a number of natives is included that covers representative species in all the western states where medusahead is widespread

and displace populations of these natives. In 2004, a wider range of plant species taken from the most related genera to Taeniatherum will be tested with *U. phrygica*. Both European and North American species will be tested.

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