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FIRST REPORT OF LOOSE SMUT - *Ustilago syntherismae* (Schweinitz) Peck ON *Digitaria sanguinalis* (L.) Scop. IN BUCHAREST - ROMANIA

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Abstract

There are only two previous citations since the 20th Century of this smut species for Romania. However, this relates to herbarium material collected for the first time in 1957 in Târgu Mureș recorded by K. Vánky (according dataset: The Fungal Collection at the Botanische Staatssammlung München) and to the second collecting in 1978 in Valea lui Mihai-Șimian, Brașov by Negrean G. (according dataset: The Fungal Collection at the Senckenberg Museum für Naturkunde Görlitz). In the present paper the interaction between the phytopathogenic fungus Ustilago syntherismae and the plant at which it infects, Digitaria sanguinalis, has been studied. Aspects related to the phenotypic expression (symptoms) manifested by Digitaria sanguinalis under field conditions and to the morphological characteristics of the spores of the fungus Ustilago syntherismae are presented. The observations took place in the period 2018-2020.Further research is required to investigate the current distribution of the loose smut - Ustilago syntherismae (Schweinitz) Peck on Digitaria sanguinalis (L.) Scop.in Romania.

Key words*: smut, fungus, chlamydospores, Ustilago syntherismae, Digitaria sanguinalis*.

INTRODUCTION

There are about more than 230 species of *Digitaria*, all superficially similar with digitate or sub-digitate inflorescences, in tropical and warm temperate regions, particularly in the Old World (Clayton and Renvoize, 1986).

Digitaria sanguinalis (L.) Scop. is an annual, late spring-and summer-germinating mono-cotyledonous plant (Chirilă, 1989), and the species is a terophyte (Ciocârlan et al., 2004). Tillering initiates after emergence of the fourth leaf. Mature plants cover extensive areas developing a "mulch" or "tuft" 40-60 cm deep (Kissman and Groth, 1993).

During the European Middle Ages, *D. sanguinalis* was cultivated by Slavic peoples in Eastern Europe, where it was cooked in soups and porridges (Nesbitt, 2005).

D. sanguinalis (large crabgrass) is highly nutritious, compared to other grasses, it has a relatively high protein percentage, especially before the plant exhausts itself producing seed. For this reason, it is frequently sown in fields to provide graze for animals. Often large crabgrass are considered as weed when encroaching into newly planted forages (Rouquette et al., 2020), it is also a noxious weed particularly common in maize, in early-sown rice, beet, vegetable crops, orchards and vineyards; it is also found on waste ground, railway embankments, neglected lawns and grassy ridges (Behrendt and Hanf, 1979; Choi et al., 1998).

Originally native to Europe, *D. sanguinalis* is now found in about 45 countries, including Romania.

Reporting pathogens in different areas of Romania is a permanent goal for phytopathological scientific activity in our country (Cristea & Manole, 2014; Cristea & Jurcoane, 2016; Paraschivu et al., 2009; 2010; Zală, 2012). Some pathogens can develop high epidemics in Romania depending on its virulence and the susceptibility of the host plant Mirela Paraschivu et al., 2015). Correct diagnosis of pathogens is the primary requirement in any sound disease management practice (Sundar et al., 2012).

Digitaria sanguinalis is an intermediate host for various microorganisms, large crabgrass itself can be attacked by various pathogens, in this case the *Ustilago syntherismae* fungus. The genus *Ustilago* (Pers.) Roussel (1806 is the

largest genus in the *Ustilaginales*, with approximately 300 species, and most species parasitize monocotyledonous hosts (Boekhout et. a., 2011). The genus *Ustilago* contain about 170 described species of fungi that cause smut on cultivated or spontaneous grasses (Vánky, 2012). Some smut fungi may attack only the stems, flowers, anthers, or ovules of their hosts and no other part and no other host. Smut fungi are important pathogens of grasses. Typically, smut fungi infect the inflorescence of their host plant (Otilia Cotuna et al., 2018). The main symptom of loose smut is the "smutted" grasses heads, which contain masses of black spores where the grain would normally be.

Loose smut of *Digitaria sanguinalis* (L.) Scop.is caused by *Ustilago syntherismae* (Schweinitz) Peck (1874) synonyms *Caeoma* (*Uredo*) *syntherismae* Schwein; *Sorosporium syntherismae* (Schwein.) Farlow; *Sphacelotheca digitariae-pedicellaris* Mishra; *Ustilago belgiana* Zundel; *Ustilago destruens* var. *digitariae* Sacc.; *Ustilago digitariicola* Speg.; *Ustilago rabenhorstiana* Kühn.

Caeoma syntherismae is a fungal species that was first discovered by botanist and mycologist Lewis David von Schweinitz (1832), now known as *Ustilago syntherismae* due to Peck (1874).

Although Europe is the continent of which the smut fungus mycota is the best known of all continents (Vánky, 2005), *U. syntherismae* is a specie was rarely been collected in Romania.

The presence of *U. syntherismae* has been reported for the first time, most likely in Pennsylvania-USA; since 1875 in Malaga, Spain (Thuemen, 1880); since 1985 in Switzerland; since 1992 in India; since 1999 in Zimbabwe; since 2001 in Zambia, and more recently, this year in Slovenia (according dataset: University of Minnesota Bell Museum; Brown University Herbarium; Botanische Staatssammlung München; The New York Botanical Garden; Zogg, 1985; Ogris, 2021).There are only two previous citations since the 20th Century of this smut species for Romania. However, this refers to the herbarium material collected for the first time in 1957 in Târgu Mureș by K. Vánky (according dataset: The Fungal Collection at the Botanische Staatssammlung München) and to the second collecting in 1978 in Valea lui Mihai-Șimian, Brașov by G. Negrean (according dataset: The Fungal Collection at the Senckenberg Museum für Naturkunde Görlitz).

There is approximate 1450 known "classical" smut fungi (those possessing ustilospores) which are classified into two classes, eight orders, 18 families and 73 genera (Vánky, 2001).

Systematic classification of the fungus *Ustilago syntherismae*: superkingdom clade *Eukaryota*; *Opisthokonta*; kingdom *Fungi*; subkingdom *Dikarya*; phylum *Basidiomycota*; subphylum *Ustilagino-mycotina*; class *Ustilaginomycetes*; order *Ustilaginales*; family *Ustilaginaceae*; genus *Ustilago* (Schoch et al., 2020)

MATERIALS AND METHODS

The research was carried out on a spontaneous population of *Digitaria sanguinalis* in the area adjacent to Drumul Cooperativei Street, Bucharest.

Visual observation is the fastest method to identify loose smut based on symptoms shown by infected large crabgrass plants. This method involves a high degree of subjectivity, depending largely on the diagnosing person's level of knowledge.

Scouting for *Ustilago syntherismae* attack has a particular importance in large crabgrass to establishing the attack value during the vegetation season.

The attack value is represented by frequency (F%), intensity (I%) and attack degree (AD%).

Frequency is the percentage of plant attacked out of 100 examined large crabgrass plants.

Intensity indicates the degree to which the large crabgrass inflorescence plant is attacked under examination. Intensity was noted directly in percentage.

The attack degree present severity of loose smut in the crop and was calculated using the frequency (disease incidence) and intensity(severity).

Attack degree was calculated using the formula:

$$AD\ (\%) = \frac{F\ (\%) \text{x}\ I\ (\%)}{100}$$

The period analysed in this study was 2018-2020. Observations were made under natural contamination. For scouting optimization and for the observation of the climatic conditions

necessary for the appearance and development of the disease, precipitation, wind speed and temperatures were taken into account (Mirela Paraschivu et al., 2020).
The simple microscopic preparation (Figure 1) was performed with the help of a scalpel and consisted in detaching a small amount of the powdery, blackish mass of chlamydospores from the attacked inflorescences, placing it in the drop of water on the blade and lamella coating (Constantinescu, 1974).

Figure 1. Performing the microscopic preparation

Examination of the preparation under a microscope was first done with a small lens (with a magnification of 10x) viewing the entire slide, using the macrometric visa. Next, I chose the most characteristic portion of the preparation, which, after fixing in the center of the microscopic field, will be viewed with a large lens (with a magnification of 40x). To clarify the image I used the micrometric visa. The microscopic preparation was visualized under the Zeiss Primo Star microscope, and to determine the dimensions of the chlamydospores we used the Zen software.
To determine the number of chlamydospores in the soil (at a depth of 1 cm) we used decimal dilutions (Figure 2). In order to prepare soil dilutions, weigh 1 g of soil. The soil is then placed in a test tube with 9 ml of distilled water. Then, the mixture of 1 g of soil + 9 ml of distilled water is stirred well for 5 minutes. By stirring the dilution $1/10=10^{-1}$ is obtained. From the 10^{-1} dilution, take 1 ml of liquid with a sterile graduated pipette and place in another test tube with 9 ml of distilled water. We obtain, by gentle stirring, the dilution $1/100=10^{-2}$. Then proceed as in the previous dilution and obtain dilutions 10^{-3}…10^{-7} (Waksman, 1927).

Figure 2. Decimal dilution until 10^{-1} to 10^{-7}

RESULTS AND DISCUSSIONS

Loose smut of large crabgrass are generally characterized by black, dusty masses of spores. These spores are, in reality, teliospores but are frequently and incorrectly called chlamydospores (Barnes, 1979). However, I prefer the term chlamydospores. *Ustilago syntherismae* is a fungus parasite of meristematic tissues that infects plants by chlamydospores and the infection appear in the plant when spores sprout and infectious hyphae penetrate the base layers of the bud and reach meristematic tissue (Ferreira and Comstock, 1989). For this reason, when the inflorescence appears it is largely affected by loose smut.
The first appearance of symptoms was found to end of May, every year.
Clamydospores in the inflorescence replacing the spikelets with a blackish semi-agglutinated to dusty spore mass, at first hidden by enveloping leaf sheaths.
The intensity of the inflorescence attack varies from one large crabgrass plant to another. If in some large crabgrass plants only a few spikelet are affected, in others finally, only the rachises of spiciform panicle remain intact (Figure 3). This can be attributed to the number of chlamydospores that have infected a plant.
During the three years of research, the main climatic parameters (average monthly temperature and amount of precipitation), from March (the time of the emergence of large crabgrass) to June (appearance of diseased inflorescences), were between 5.3°C (the lowest temperature in March, 2018) and 24.0°C (highest temperature in June 2019).

Figure 3. Typical symptoms of loose smut

The amount of precipitation is important in June, in terms of dislocation of chlamydospores from inflorescences (Figure 4).

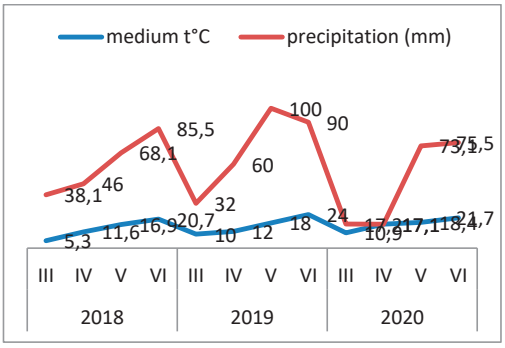

Figure 4. Average monthly temperatures and the volume of precipitation from the emergence of the large crabgrass to the appearance of loose smut (source: https://insse.ro/; http://www.meteoromania.ro; meteoblue.com/ro)

Also wind speed is important in June, in terms of chlamydospore dissemination. In June, the wind speed in Bucharest is, on certain days, between a minimum of 4 km/h and a maximum of 35 km/h, which means that we are dealing with a perceptible to significant wind according to the Beaufort wind force scale. The scale was devised in 1805 by the Irish hydrographer Francis Beaufort (Mather J.R., 2005).

Chlamydospores are globose, with a diameter of about 5-9 µm, unicellular, brown, finely equinulate, with a thick wall about 1 µm (Figure 5).

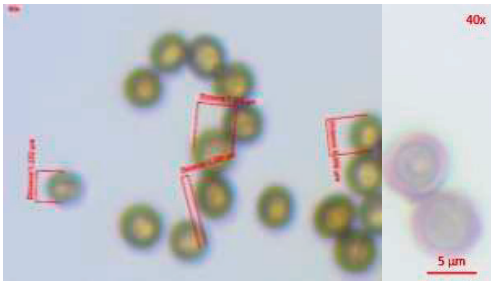

Figure 5. Measurement of some chlamydospores

The number of large crabgrass inflorescences attacked by loose smut increased compared to 2018 (when the average frequency was 47 attacked inflorescences) by 29.79% (61 attacked inflorescences) in 2019 (61 attacked inflorescences) and by 61.71% in 2020 (76 attacked inflorescences). The intensity of the inflorescence attack varied between 74.0% and 83.0. The average degree of attack increased progressively in the three years of research, from 39.01% in 2018 to 61.56% in 2020. Expressed as a percentage, the increase in the degree of attack was 15.72% higher in 2019 and with 57.81% in 2020 (Table 1).

Table 1. Scoring of the loose smut attack on the inflorescences of large crabgrass (%)

Scoring the attack → / Year ↓	F (%)	I (%)	AD (%)
2018	47.0	83.0	39.01
2019	61.0 (+29.79%)	74.0	45.14 (+15,72%)
2020	76.0 (+61.71%)	81.0	61.56 (+57.81%)

The spread of chlamydospores over short distances is achieved with the help of insects and humans. Chlamydospores adhere to the feet of insects and the soles of human footwear. Over long distances the spread is done with the help of the wind. Raindrops have the role of dislocating the chlamydospores from the attacked inflorescences, moving them to the ground, fixing the spores to the soil surface and circulating them in the surface layer of the soil. Chlamydospores also ensure the transmission over the winter, from one vegetative cycle to another, of the *Ustilago synterismae* fungus.

The average number of chlamydospores of *Ustilago synterismae* determined in the soil layer 1 cm deep, at the beginning of the

vegetation start of the large crabgrass plants, was 2046 x 10^{-7}/ml.

I wanted to highlight the changes induced by the loose smut attack in terms of the height of the large crabgrass plants with attacked inflorescences, the number of internodes of these plants and the length of the inflorescences, compared to healthy plants (Figure 6).

Figure 6. Large crabgrass plants (healthy and with inflorescences attacked by loose smut) intended for different measurements

The fungus *Ustilago synterismae* caused a reduction in the height of plants with attacked inflorescences by 16.04% compared to healthy plants; the number of internodes in diseased plants decreased by 20.83% and the length of the attacked inflorescences decreased by 45.08% compared to the length of healthy inflorescences (Table 2).

Table 2. Changes caused by loose smut (%)

Large crabgrass plants	plant height (cm)	number of internodes	inflorescence length (cm)
healthy	21,25	4,8	6,1
with inflores-cences attacked by loose smut	17,84 (-16.04%)	3,8 (-20,83%)	3,35 (-45,08)

CONCLUSIONS

Ustilago syntherismae (Schweinitz) Peck (1874) was rarely collected in Romania.

The attack of the loose smut manifested itself only at the level of the inflorescences of large crabgrass.

The average temperatures recorded during the vegetation period of the large crabgrass plants, which favored the appearance of loose smut, were between 5.3 and 24.0°C.

A high reserve of chlamydospores favors the increase of the frequency and intensity of the loose smut attack at *Digitaria sanguinalis*.

The evolution of loose smut disease in hearths explains the increase in the degree of attack from one year to another.

Further research is required to investigate the current distribution of the loose smut - *Ustilago syntherismae* (Schweinitz) Peck on *Digitaria sanguinalis* (L.) Scop.in Romania.

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