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SLIME MOULD - SWEET POTATO ASSOCIATION ON SANDY SOILS OF ROMANIA

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Abstract

Myxomycetes are naturally occurring organisms with habitats from tropical to temperate area with preference for humid and diverse ecosystems. Yet, an association of myxomycetes - sweet potato in Romania, on sandy soils has been detected in 2020. Sweet potatoes are cultivated at Dabuleni Research Station in mixture of forest top soil, sand and peat under greenhouse conditions. Relative humidity and temperatures are high throughout the season with highest values at the end of summer, favouring myxomycetes organism to complete its life cycle. Several stages of plasmodium and sporangia were observed in the field. The crop was not affected by the colonization of myxomycetes. Morphological identification lead to a species of Stemonitidales. This is the first report in Romania of myxomycetes-crop association.

Key words*: myxomycete, slime moulds, Protista, ecology.*

INTRODUCTION

Slime moulds were previously considered fungi, but presently have joined the Protozoa group, otherwise named paraphyletic kingdom Protista. Once classified as fungi, they have been booted out of that kingdom due to their lack of chitin and their feeding by engulfing food (Glime, 2019). They are now considered Protista due to their motile stages that look and behave like protozoa. The slime moulds are comprised of more than 1000 species from all seven continents (Glime, 2019). The fascinating life cycle of myxomycetes involves two main stages, one consisting of uninucleate amoebae and the second a multinucleate plasmodium, which under favourable conditions develops into spore-containing fruiting bodies (Rikkinen et al., 2019). When slime mould spores germinate, amoeba-like cells form. These are typically haploid, can move and feed on bacteria. If these amoebae encounter the correct mating type, they can mate to form zygotes that develop into plasmodia. The protoplasm within the plasmodium can stream at speeds up to 1.35 mm per second, the fastest rate known for any organism (Glime, 2019). The motile amoebae feed on bacteria and divide until they give rise to the plasmodium. The plasmodium, which also moves, feeds by engulfing surface bacteria, fungal spores, yeasts and algae. The plasmodia have many nuclei with no dividing cell membranes and can form a plasmodial mass that may be several meters in size. The amoebae can avoid adverse conditions by transforming into dormant microcysts, and the plasmodium can convert into a hard, dormant sclerotium which sometimes resembles the slime left by a slug. When this sclerotium once again becomes moist, it returns to the active plasmodium state. Microcysts and sclerotia can withstand drought and cold, and spores may remain viable for years or even decades (Rikkinen et al., 2019). When food becomes limiting, the plasmodium moves to the surface and begins to form its rigid fruiting bodies – sporangia. It is this stage that caused us to originally think they were fungi, but it lacks the chitin that is present in fungi. The life cycle is completed when these sporangia produce spores, usually by meiosis, for the next generation of amoebae (Alexopoulos, 1959). Myxomycetes are ubiquitous in most vegetated,

terrestrial ecosystems. Their diversity tends to be higher in communities with higher diversity and biomass of the vascular plants, which support the microorganism substrates upon which the amoebae and plasmodia feed. They are most diverse in tropical and temperate forests, and least in boreal forests, arctic or alpine ecosystems. In tropical forests, myxomycetes seem to produce fruiting bodies more readily in relatively dry habitats (Rikkinen et al., 2019). Schnittler et al. (2015) concluded that corticolous Myxomycetes are some of the most drought-tolerant organisms in that habitat. They are opportunistic, permitted by their ability to survive in a dormant state for decades and to complete their life cycles in a few days of appropriate conditions (Glime, 2019). Identification of species can be difficult for a number of reasons. Not only are there different colour phases during the development of the sporangia, but there are different sexual strategies within currently perceived species (Clark & Haskins, 2015; Steven L. Stephenson & Schnittler, 2017). Within *Trichia varia* "species" there are three distinct sexual biospecies that are reproductively isolated from each other with distinct genotypes but equal phenotypes (Feng & Schnittler, 2015). The class Myxomycetes is comprised of sporocarpic Eumycetozoa with fruiting bodies containing numerous spores and usually a persistent peridium around the sporotheca (taxa with evanescent peridia lack the covering in mature fruiting bodies). The subclass Columellomycetidae comprises the dark-spored myxomycetes with a capillitium connected to a true columella. The superorder Stemonitidia have spores appearing usually darken mass, with order Stemonitales having an epihypothallically developed stalk that usually extends into a true columella (extension of the stalk inside the sporotheca), and an evanescent peridium (a usually persistent covering that surrounds the spore-containing sporothecae of fruiting bodies). The central synapomorphy of the newly circumscribed order Stemonitidales is the fugacious peridium, i.e., the mature sporothecae of these myxomycetes lack peridia. The family Stemonitidaceae is characterized by a branching and anastomosing capillitium arising from the columella and with capillitial threads usually forming a surface net. The genus *Stemonitis* unites the myxomycetes that produce cylindrical sporothecae and have a richly branched and anastomosed capillitium that forms a pronounced surface net. Identification of extant myxomycete species is based almost entirely on morphological characteristics of sporocarps and spores. The genus *Stemonitis* currently includes around 20 accepted species. Many of them are common worldwide, but also morphologically plastic (Strelow et al., 2020).
The present study is a first report of a Stemonitidales member in association with sweet potato crop in a greenhouse at the Dabuleni Research Station, in the south of Romania. Dabuleni Plain is characterized by a landscape of dunes, sandy soils, poor in organic matter prolonged heat, heavy rainfall falling at long intervals of time, long periods of drought, thin and discontinuous snow, low fertility of sandy soils and deflation (Simulescu & Zamfir, 2015).

MATERIALS AND METHODS

Collecting place and agro-technical data
Dabuleni city (43 ° 48′ 18.4 ″ N, 24 ° 05′ 33.4 ″ E) is located in the south of Oltenia, 8 km-close to the Danube, in an area with sandy soil, also called "Sahara Olteniei". The relief of the region consists of plains and meadows covered with sand dunes. It has a continental climate with slight Mediterranean influence. Thus, the region has a period of severe drought in July-September and a regular amount of rainfall in May and June. The average annual temperature is 11°C. Precipitation reaches an annual average of 548 mm, is unevenly distributed throughout the year and can have significant variations from one year to another. The wind in the area causes temperature drops in winter, melting snow in spring and scattering sand in spring and summer. Winds still play an important role in shaping the current relief due to the steppe regime, with low rainfall, especially in dry years. Semi-consolidated and mobile dunes are continuously subjected to the action of winds. The woody vegetation specific to the region is represented by acacia (*Robinia pseudoacacia*), and in the ditches on the bank of the Danube white poplar (*Populus alba*), willow (*Salix alba*) and oak (*Quercus robur*). Nearby Research & Development Center for Agricultural Plants on Sands Dabuleni were planted protective curtains of *Robinia*

pseudoacacia to protect vegetable crops from the action of spring-summer winds. The sandy soils in the area are characterized by a high content of coarse sand (50-70%), little clay and dust (2-8%). Sweet potato tubers were planted in a mixture of forest top soil, sand and peat (1:1:1) at the end of March, in greenhouse. A soil sterilizer (Basamid) with nematocidal, insecticidal, fungicidal and herbicidal action was applied 25 days before. To stimulate the herbicidal action and to retain the sterilizing gas for as long as possible in the soil, the treated soil surface was covered with PE foil until the end of March. After four days sweet potato tubers were planted. In greenhouses, registered average values of parameters were: air temperature 20-28°C, soil temperature 15-25°C and relative humidity 65-75%. To keep temperature below 30°C, ventilation and irrigation with sprinklers were applied.

Myxomycetes isolation and characterization

Sample collection and field observations were carried out at 2 sampling points - greenhouses, from late May until mid-September. In addition to direct observation of sweet potato crop - myxomycete association, samples were collected at different stages of life cycle in Petri plates, kept at 5°C until laboratory further manipulation. The fruiting bodies and microscopic structures were examined by light microscopy. Sporotheca and spores were mounted in water with Tween80 (0.005%) for observation. Observation and measurement of sporocarp morphological characteristics were carried out with Olympus SZ61 stereomicroscope with an attached Olympus SP350 camera. Sporotheca and spores were observed using optical Motic B series microscope with an attached microQ UCMOS series Toup Up camera. ToupView software was used for image manipulation and measurements.

RESULTS AND DISCUSSIONS

In May 2020, the first author observed and collected an unidentified myxomycete on sweet potato crop in greenhouse at Dabuleni Research Station. The specimen was sent to the correspondent author, who identified it as a member of Stemonitidales. During August and September, several observations were made in the field and multiple samples were collected at different stages of the life cycle.

The specimen was observed in small clusters on living plants of sweet potato - shoots, leaves and petioles. Different stages of plasmodium and fruiting bodies were observed (Figure 1). Initially, the plasmodium is slender-veined and transparent (Figure 1, a), followed by a phase in which the extensive reticulum is withdrawn into a dense, cream-coloured strands forming a coarse network. The veins appear thick and opaque. The veins condense further to form a coralloid mass. The formation of the white coralloid structure (Figure 1, b) marks the change from assimilative to the reproductive phase (Indira, 1971).

Figure 1. From left to right: a) early stage of plasmodium just prior to fruiting, condensing into thick veins; b) prefructification coralloid plasmodium (fruiting creamy white knobs, the sporangial initials - sporotheca formation) typical for *Stemonitis* with a clustered fruiting body; c) coralloid stage on leaves; d) mature plasmodium on soil

Next, it condenses and forms a thick uniformly flattened mass at the surface of which appear minute knobs, the sporangial initials (Figure 1, b). The coralloid stage (Figure 1) may last for varying periods for 10-15 day, depending on the conditions in the greenhouse. During the observations temperature ranges between 28-30°C, micro-sprinkler irrigation was applied every two days and relative humidity was 75%. In the present study no intermediate phases between the initial sporocarps and the fully formed ones were detected. Intermediate phases are described in detail elsewhere (Dai et al., 2020; Indira, 1971). Yet, coralloid plasmodium was detected at different stages up to decay (dessication) possibly due to stress caused by the environmental factors (Figure 2). Dark-grey sclerotia were also observed in collected samples (Figure 2, e).

Figure 2. Plasmodium phases from up to bottom, left to right: a) plasmodium development: coralloid form of mature plasmodium on debris; b) coralloid form of mature plasmodium on petiole of sweet potato; c) decaying coralloid form of mature plasmodium on debris; d) decaying coralloid form of mature plasmodium on sweet potato leaves; e) sclerotium formation

The fruiting body (Figure 3) is represented by reddish-brown sporangia (sporocarps), in clusters supported on slender stalks. Fructification sporocarps, aggregated, stipitate, in clusters of approximately 50, dark brown, acute or sub-acute apex. Sporocarps tufted, 10-15 mm total height, in clusters of approximately 25. Sporotheca dark brown with a reddish tinge with height of 2.26-2.89 mm. Sporocarp with solid, black, not hollow stalk with height of 1.31-1.42 mm.

Stemonitida is a morphologically homogeneous order, characterized by a unique feature - the stalk is internally secreted, slender, regular and smooth and it extends itself into the spore mass (Fiore-Donno et al., 2012; Kalyanasundaram & Paramasivan, 1993).

The typical stalk, the dark spore colour and the absence of lime deposits make members of the Stemonitida easily recognizable when collected in the field.

In the traditional classification Stemonitida includes only one family and 16 genera (Poulain et al, 2011).

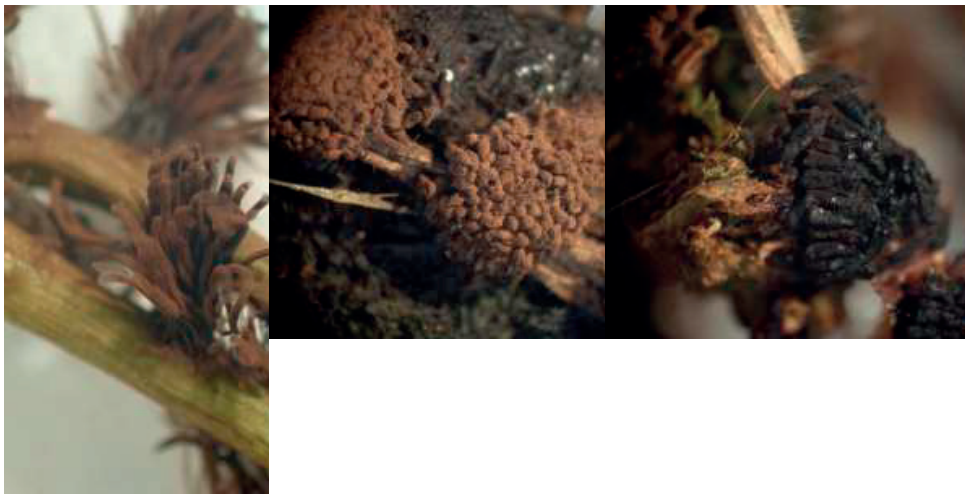

Figure 3. Fruiting body development from left to right: a) gregarious mature stalked sporocarps with powdery spore mass on sweet potato leaves; b) decaying gregarious mature stalked sporocarps on debris; c) dried, decayed sporocarps on debris

The hypothallus was membranous and red brown. The peridium was brown and persistent. The capillitium was densely reticulate, arising from the entire columella. The spores were warted, globose spores of (7.5-) 9 (10) µm diameter, SD = 0.61 µm, pale purple brown in colour (Figure 4).

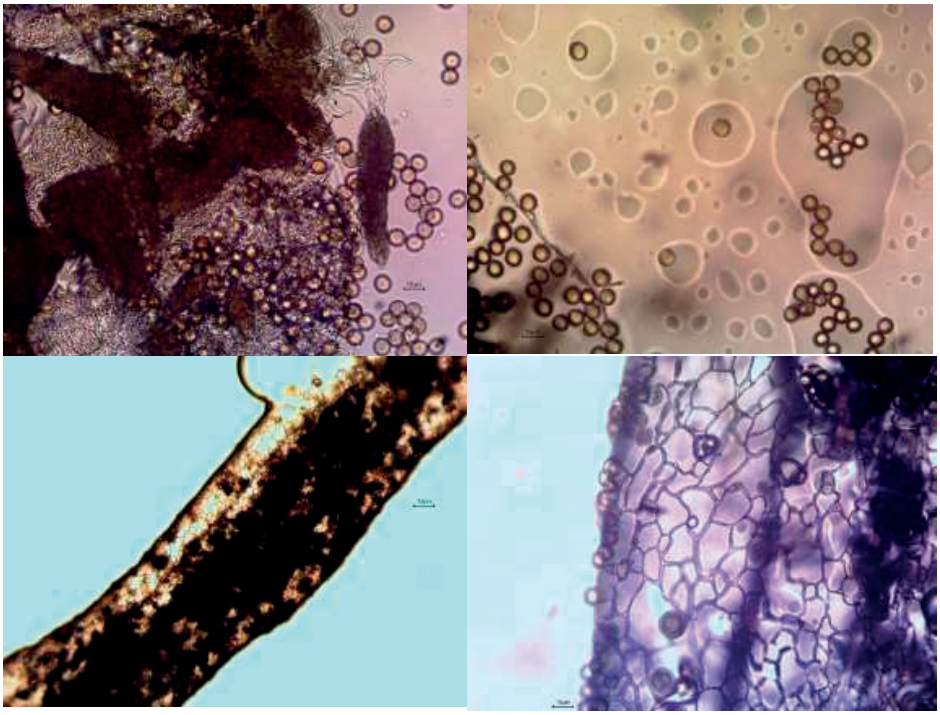

Figure 4. Up: Reticular spores released after crushing sporotheca (x 40); Down from left to right: Capilitium of a sporotheca (10x); Capilitium - the mesh of internal network holding spores (40x)

There are only subtle differences between the four genera *Stemonitis*, *Stemonaria*, *Stemonitopsis* and *Symphytocarpus*. Strelow et al., (2020) hypothesized that spore ornamenttation may constitute a more meaningful characteristic than the capillitial network to reveal affinities between species in *Stemonitis* and the genera segregated from it. Their phylogenetic analyses suggest that spore ornamentation is a major, hitherto neglected feature essential to delimitate genera in *Stemonitis* and allied genera. The two *Stemonitis* clades are characterized by spores either warted or reticulated (Strelow et al., 2020).

Our specimen presented warted spores, therefore preliminary analysis indicates it as a member of this genus. Also, several species were discarded according to ornamentation and size of spores: *S. lignicola* (Poulain et al., 2011), *S. fusca* and *S. typhina* (Dai et al., 2020), *S. herbatica* (Indira, 1969), *S. planusis* (Bo & Yu, 2017). Ground litter (consisting mostly of dead leaves) on the forest floor is one of the primary substrates for myxomycetes (Martin & Alexopoulos, 1969; Stephenson & Stempen, 1994).

Studies on this substrate have indicated that ground litter in both temperate and tropical forests supports a diverse assemblage of these organisms (Harkönen, 1981; Stephenson, 1989; Stephenson & Stempen, 1994). Presumably, when a leaf dies and falls to the forest floor, it acquires airborne spores of myxomycetes (Rojas, 2020).

Sweet potato crop includes adding peat in the substrate composition each year. It comes easy to speculate that the most probable route for the detected specimen of Stemonitidales in the greenhouse, was via top soil forest used in the substrate mixture. Another way of entrance would have been anemophily from the nearby forests. In any case, the important fact is that this organism was able to colonize beside debris, living plants of sweet potato, completing its life cycle by producing fruiting bodies.

Myxomycetes species are known to prefer certain substrates for fruiting as *Dictydium cancellatum* sporangia always can be found on rotten pine logs, *Physarum cinereum* fruits on living St. Augustine grass and *P. bivalve* prefers living herbaceous plants (Townsend et al., 2005).

It comes at hand to speculate that Myxomycete specimen completed its life cycle due to combined factors of water input (micro-sprinkling irrigation), infiltration rates, high temperature and relative humidity. These might have been similar to its natural habitat (hot and humid forests nearby). It is noteworthy to mention that plants thrive in association with the Myxomycete, the organism did not attack the plant, but only used it as physical support, which indeed might have as effect a lower photosynthetic surface. Several water showers were used to "wash away" the "invading" organism.

The presence of fruiting structures (sporangia) is considered as dependent on the arrival of rain after a prolonged warm period, making their presence most common in autumn in temperate regions (De Holanda et al., 2016).

So, the agro technical method against the colonization of the myxomycetae might have act as propitious to complete its life cycle - sporocarps with spores which theoretically germinate and a new cycle is on the road. Myxomycete perform important functions in many different terrestrial ecosystems. At the amoebae stage feed mainly on bacteria but their plasmodia feed on a wider variety of organisms, including fungal fruiting bodies and spores, algae and possibly lichens. Some appear to produce the enzymes necessary to break down dead plant tissues. Soil nutrients are largely immobilised in microbial biomass. Nutrients are released through the feeding activities of bacterivores like myxomycetes, making them responsible for mediating the flow of nutrients first to the surrounding substrates and then to higher trophic levels, including plants and animals (Stephenson & Rojas, 2017).

Eliasson & Lundqvist (1979) noted that species with large plasmodia typically are rare under arid conditions. This would suggest that the slime molds on bryophytes are the larger species in most habitats because of the moisture-holding capacity of the bryophytes. Yet, *Didymium wildpretii* was found in arid zones - Canary Islands and Chihuahuan Desert Mexico (Beltrán-Tejera et al., 2010).

A particular anatomical adaptation of *Stemonitis* which might have allowed them to colonize arid zones is the stalk of sporocarps

which elevates spores above the substrate, allowing them to dry out and become airborne (Rikkinen et al., 2019). The authors proposed this characteristic as evidence of strong environmental selection favouring the maintenance of adaptations that promote wind dispersal in this lineage of myxomycetes.

CONCLUSIONS

This first report of myxomycete - crop association in Romania reveals another example that naturally occurring species in forests may shift to man-made monoculture ecosystems and complete their life cycle without harming the associated plants. Further molecular identification is required as well as field observations during present year to determine if myxomycete has established in the soil where sweet potatoes are planted.

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