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FIRST REPORT OF LEAF SPOT ON SWEET POTATO (*IPOMOEA BATATAS*) CAUSED BY *ALTERNARIA ALTERNATA* IN BULGARIA

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Abstract

For the first time in Bulgaria, leaf spotting on sweet potatoes was investigated, and the causative agent, *Alternaria alternata*, was reported. The collected morphological data aimed to determine the identity of the fungus. According to Koch, the primary isolates from the damaged parts are identical to those produced from pathogenicity tests. In a laboratory we tested sixty-two leaves which had similar symptoms, with slight to medium-sized lesions with concentric circles and a yellowish halo. In general, the attack from *Alt. alternate* affected 7% of all plants and 32% of the examined leaves. The pathogen tests proved that the pathogen could also cause damage to tubers during storage. Knowing the etiology of diseases is essential for taking preventive measures and thus preserving the quality and quantity of agricultural production.

Keywords: *Alternaria alternata*, sweet potato, leaf spots, pathogen, pathogenicity

INTRODUCTION

The sweet potato (*Ipomoea batatas*) is a dicotyledonous plant belonging to the Boundweed or *Convolvulaceae* family. Potatoes (*Solanum tuberosum*) belong to the same taxonomic genus.

The tubers and leaves of the sweet potato, like other vegetables, contain many bioactive components such as coumarins, triterpenes, polyphenolic compounds, coroutines, polysaccharides callisthenics, and others, all of which have been studied many times with many proven benefits. In tropical and subtropical countries, the tubers and tender shoots of the plant are consumed for nutrition.

The sweet potato is the world's sixth most important food crop (CIP, 2020). Several diseases can play a crucial role in plant development, suppressing growth and significantly reducing yield and quality (see Clark et al., 2013).

Sweet potatoes are not among the traditionally viewed crops in our country. They have been growing gloriously recently. During the growing season 2020, a soft leaf spot was observed on the sweet potato plants. The lesions were initially small dots, which towards the end of the growing season, grew, united and occupied at most one-third of the leaf picture. For this reason, our study is about discovering and studying the aetiology of diseases in our country's soil and climatic conditions; this is the first report on *Alternaria alternata* causing necrotic leaf spots in the country.

MATERIALS AND METHODS

A sampling of sweet potato leaves and tubes

The survey was carried out in June-October 2020, covering the entire vegetation period up to and including harvest. In the region of Malo Konare, the sweet potato Orleans variety was grown in an area of 2.5 ha.

The plants with disease symptoms were observed in the field conditions on naturally infected plants.

The final diagnosis was made after identification in the laboratory.

Koch's postulates were applied to determine sweet potatoes' aetiology of leaf spots.

Disease prevalence in (%)

The study was done on randomly collected plants taken from the diagonals of the sweet potato field.

The percentage of diseased leaves was calculated according to the formula of Chumakov (1974).

P=a/A*100

P-Incidence of disease (%)

a-Number of diseased plants

A-Total number of reported plants

Isolation

Collected leaves with necrotic spots were placed in 15 cm Petri dishes with a filter paper soaked in distilled water to provoke mycelium formation and sporulation. From the site of damage (healthy-infected tissue), small pieces were cut, dipped briefly in ethanol 70%, then immersed in distilled water and transferred to a plate with PDA. They were incubated at room temperature 25ºC to develop fungal growth. Their effect was observed daily, and at the initial expansion, a piece of the fungus was taken and transferred independently to PDA slants.

Pathogenicity test

All isolates were tested for pathogenicity on a sweet potato leaf and a tuber slice (Moreira et al., 2013) for organotrophic specification. Visibly healthy leaves were taken, disinfected with ethanol 70% and washed with sterile distilled water. The leaves ready for inoculation were placed in plastic disinfected trays. A filter paper soaked in distilled water was placed on its bottom. Three disks with a diameter of 5 mm of the excised mycelium and one with only a nutrient medium for control at a temperature of 25 were placed on the leaf. The experiment was recorded on the seventh day. A mycelium disk with a diameter of 5 mm was placed on a sweet potato slice and reported under the same conditions. The test was considered positive when there was an indication of necrosis and mycelium on the inoculated part.

Cultural and morphological characterization of isolates

The cultural characteristics of Alternaria spp. were grown for seven days on potato-dextrose agar (PDA), oatmeal agar (OA), corn meal agar (CMA), water agar (WA) at a temperature of 25°C in the dark. After incubation, the cultures were examined for colony color, colony greyness and development of pigment or crystals in the agar medium. The morphological characteristics (spore shape, color, size) were determined using the microscopic method. The average spore size was calculated by measuring 100 conidia from 10-day-old cultures. Fungal isolates were identified according to Elis (1971, 1976) and Simmons (1967, 1997).

RESULTS AND DISCUSSION

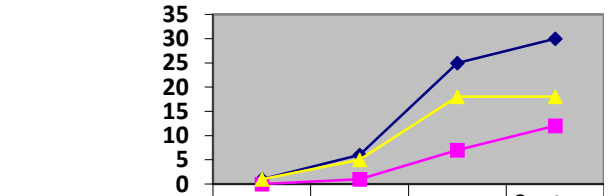
The symptoms observed on sweet potato leaves were irregularly shaped necrotic spots. The heels were dark brown to black with dark concentric circles in them and a yellow halo around the spot. Over time, the spots grew, coalesced and formed large lesions (Fig. 1). Usually, the attack started from the periphery of the leaf, but it could also be found all over the leaf petiole. The isolates obtained from the damaged parts of the leaf and those with a positive reaction had similar morphological characteristics.

Of the shed leaves, 32% were diseased, and only 7% of all plants examined were infected.

Sweet potatoes were planted in the field in July. Fig. 2 shows that during the first month, almost no spots were observed on the leaves. The plants were young and had yet to accumulate leaf mass.

Figure 1. Necrotic spots on a sweet potato leaf

In the following months, an increase in spotted leaves was also observed when they fell out and covered the field with their biomass. At the end of the growing season and with the senescence of the aerial parts, the reported attacks were the most significant. The above-ground mass was cut and removed from the field at the end of September.



	June	July	August	September
Pathogenicity test	1	6	25	30
Positive	0	1	7	12
Negative	1	5	18	18

Figure 2. Dynamics of disease spreading during the growing season

The fastest growth fungi were in OA, followed by PDA, and the slowest growth was in CMA and WA (Fig. 3).

The mycelium grown on PDA was fluffy; a white border encircled the periphery of the colonies, followed by greyish-green brown. The underside was greenish brown.

The OA colonies were radial, mycelium raised, and cottony, with white color on the top and concentric circles of grey-brown greenish, on the underside green-brown.

As in the first environment, CMA, the mycelium was ethereal, light, soft white, aerial hyphae surrounded in an almost perfect circle. Towards the middle of the concentric circles, it went from a soft brownish-green to a lighter color below.

On water agar, colonies were radial. The colour was a translucent rim saturated with CMA followed by olive green to brownish green.

A mycelium was attached to the surface of the agar. From the lower part, the color was greenish-brown green.

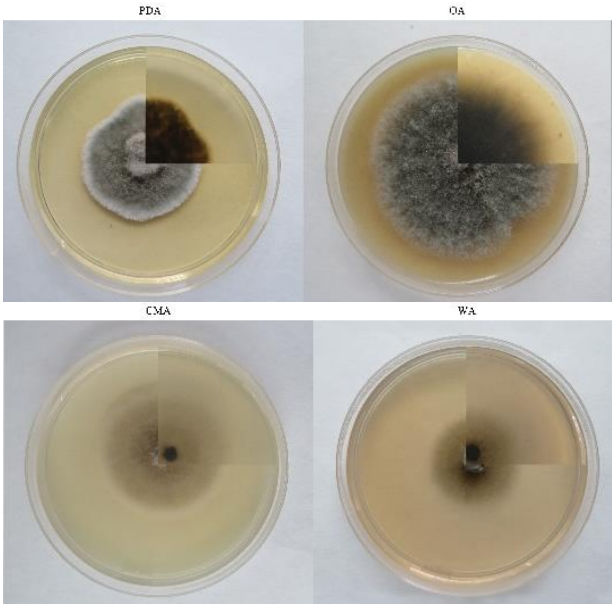


Figure 3. View of the surface and the bottom of PDA, OA, CMA and WA plates of the representative isolate of a sweet potato leaf (a monospore culture of *Alt. alternata*) at 25ºC for 7 days.

The conidiophores of *Alt. alternata* were septate, olive brown. They may be branched or unbranched with one or more apical conidiogenous loci. Conidia were roughly similar in shape and size, club-shaped, with a short beak, transverse septa of 1-5, and vertical septa of 0-1 (fig. 4). Simple or branched chains that range in size from 10.2-43.4 µm x 5.1-11.6 µm (average is 25.8 x 74 µm). Morphologically and culturally,

the results were compatible with those of *A. alternata* described in previous studies (Yin et al., 2022).

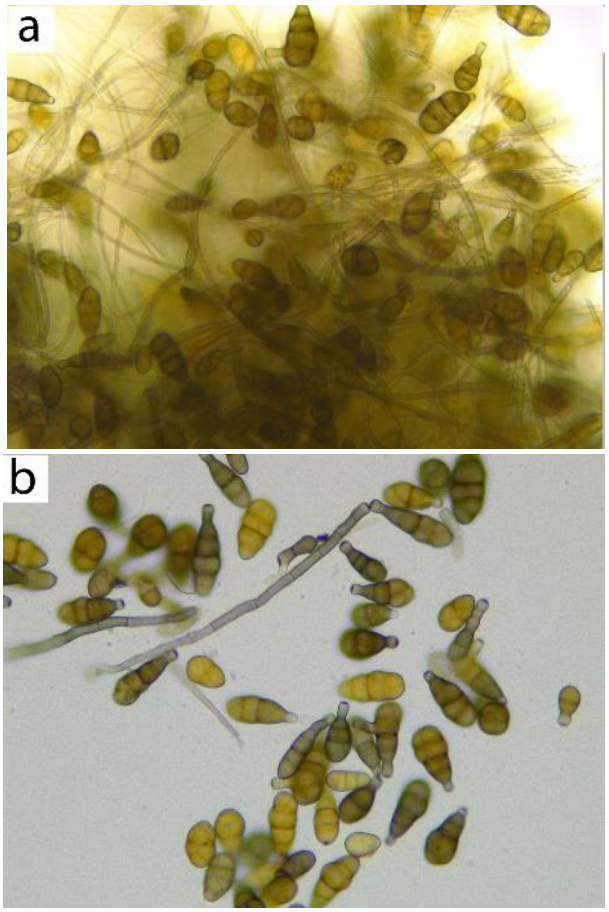


Figure 4. Mydelium,conidiophores and conidia of *Alt. alternata* a-PDA, b-OA

Pathogenicity tests proved that *Alt. alternata* could infect sweet potato leaves and tubers. Cut discs with a diameter of 5 mm were placed in three folds and one on a tuber slice (Fig. 5). On day 7, after inoculation, necrotic tissue was observed at the site of infection. The mycelium had also covered the tissue, and necrosis and the beginnings of mycelium formation were observed on the lower side. The necrotic damage was similar to the field damage. In a tuber mycelium slice, it developed slowly, causing wilting. The damaged parts darkened and became brownish. The sponge was extremely cumbersome, and it did not go deep.

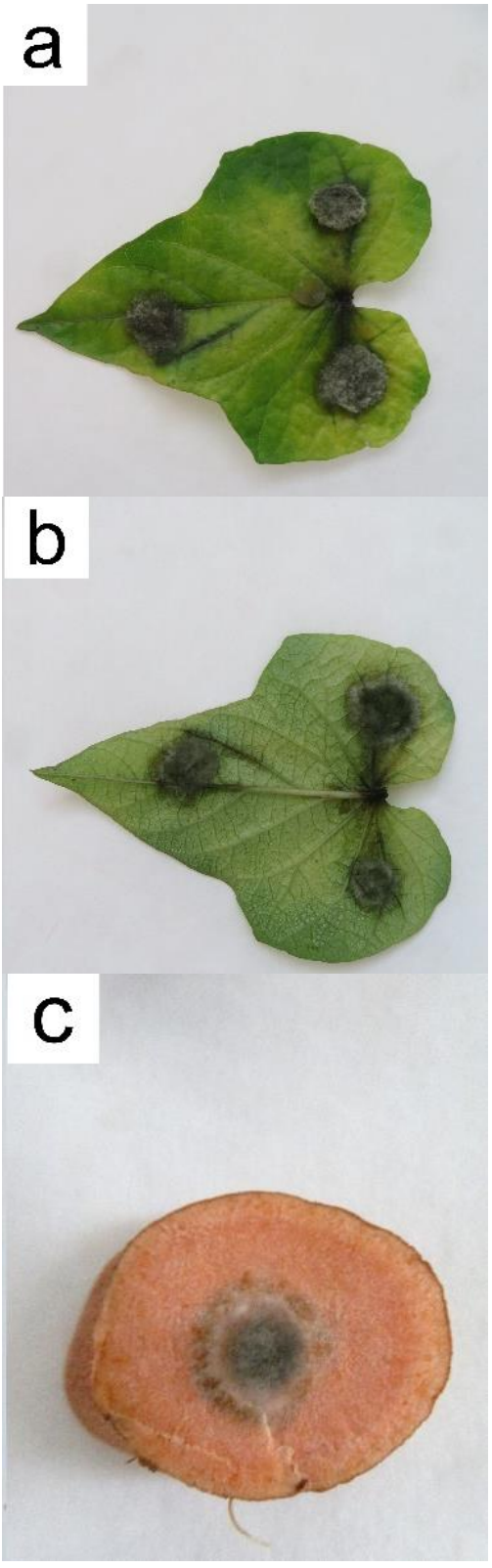


Figure 5. Pathogenicity test on a sweet potato leaf (a, b) and a tuber slice (c)

CONCLUSION

One of the isolates obtained turned out to be a sweet potato pathogen. It caused leaf spots, and later in storage, could also affect the tubers. Since it is not one of the most aggressive pathogens and grows relatively slowly, it is not of primary importance to growers of this crop. Overall, the foliage was mildly attacked, and it is not economically justifiable to take action against this pathogen. The study was done to determine the causative agent and the most suitable crop to plant after the sweet potato.

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