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RESEARCH ON THE INFLUENCE OF COMMON LEAF SPOT ATTACK ON THE MINERAL CONTENT IN LUCERNE

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Abstract

Common leaf spot of alfalfa is one of the most important diseases of alfalfa that primarily affects the foliage of plants. Our research followed the influence of the attack of this disease on the mineral content of the plants affected by the attack of the micromycete Pseudopeziza medicaginis f.sp. medicaginis-sativae to the causative agent of common leaf spot of lucerne. Samples were made with plants free from the pathogen and with characteristic attack and the concentration was determined (g per 100g dry plant). The attack of the fungus Pseudopeziza medicaginis f. sp. medicaginis-sativae (Schmiedeknecht) Schmiedeknecht influenced the concentration of micro and macronutrients in alfalfa. For some elements (Cd, Co, Ga, In, Pb) the concentration values were below the detection limit of the method.

Key words*: alfalfa, fungus, mineral.*

INTRODUCTION

Alfalfa is one of the most important leguminous plants grown for fodder (Askar et al., 2012) due to its fodder quality (Sun et al., 2012), but also as a plant with a role in nitrogen fixation and honeydew (Yang et al., 2008, Stutewille and Erwin, 1990). In our country, alfalfa has an important role for agriculture (Schitea et al., 2020). Alfalfa is attacked by a large number of pathogens that can cause significant losses both in the green table crop and in the seed crop (Cîrstea et al, 2022; Al-Askar et al., 2012). *Pseudopeziza medicaginis* f. sp. *medicaginis-sativae* (Schmiedeknecht) Schmiedeknecht is a micromycete that attacks the aerial organs of the plant, and the attack on the leaf mass is characteristic. Micromyceta produces the disease called brown leaf spot or common leaf spot (Yuan et al., 2011). The disease is manifested by the presence of yellow, then brown spots on the leaves, which causes their qualitative deterioration and leads to defoliation of the plants (Frate and Devis, 2008; Cristea, 2005). In autumn, the fungus forms non-pedunculated apothecia in the center of the spot, with asci with ascospores (Gheorghieş and Cristea, 2001; Meyer and Lutrell, 1986). The presence of spots on leaves reduces the photosynthetic activity of plants and causes harvest losses (Vincelli and Smith, 2014). Attack of the micromycete *Pseudopeziza medicaginis* f. sp. *medicaginis-sativae* (Schmiedeknecht) Schmiedeknecht reduces the assimilation rate in leaves and delays flowering. Under phytotron conditions, production drops up to 40% (Morgan and Parbery, 1977; Morgan and Parbery, 1980). The attack of the pathogen reduces forage production, but its quality is also affected by decreasing the carbohydrate and protein content (Mainer and Leath, 1978; Hwang et al., 2006). The healthy development of plants requires micronutrients such as iron, zinc, copper, manganese, cobalt, nickel, boron and molybdenum. Molybdenum is present in soils as anions and require active transport across the plasmalemma of plant root cells for uptake. Boron can be found in most soils as an anion or as a neutral molecule. When boron is taken up by plants in the form of a neutral molecule it will pass across biological membranes (Stangoulis et al., 2001). Most debates on boron uptake by plants is in about boron being actively transported into plants. It has been reported that boron may be taken up as a

neutral molecule and its transport is facilitated when soil solution concentrations are low which is the case in acid soils (Stangoulis et al., 2001). Marschner (1995) reported that "B has been shown to reduce diseases caused by *Plasmodiophora brassicae* (Woron.) in crucifers, *Fusarium solani* (Mart.) (Sacc.) in bean, *Verticillium albo-atrum* (Reinke & Berth) in tomato and cotton, tobacco mosaic virus in bean, tomato yellow leaf curl virus in tomato, *G. graminis* (Sacc.) (Graham and Webb, 1991) and *Blumeria graminis* (D.C.) (Speer) in wheat".

Iron, cobalt, copper, manganese, nickel and zinc are generally absorbed as divalent ions via divalent ion channels which have a high specificity for each element, and for which homeostasis is achieved by active-excretion mechanisms controlled by cytoplasmic concentrations (Welch, 1995).When a plant is infected by a pathogen its physiology is modified and nutrient uptake, assimilation, translocation from the root to the shoot and utilization is altered (Marschner, 1995). Diseased plants may show a reduction of the minerals concentration in roots and aerial organs. Several diseases such as rust in wheat leaves, smut in wheat and *Colletotrichum musae* in banana plants can be controlled thanks to Fe uptake by plants. Moreover for cabbage plants a high Fe concentration in the soil solution may overcame a possible fungus-induced Fe deficiency in the host but will not control the extent of infection. (Liu et al., 2021). The mechanism due to which Fe is taken up by pathogens like fungi is mediated by a group of low molecular weight (500-1500 Da) ferric-iron-specific chelators named siderophore produced by fungi as well as bacteria. Moreover the fungi can produce extracellular and intracellular siderophores, the main group of siderophore produced by fungi being the hydroxamates (Liu et al., 2021) which will decrease the Fe content in the host plant.

MATERIALS AND METHODS

The aim of our research was to carry out an analysis of the content of different minerals in alfalfa plants originating from plants attacked by the fungus *Pseudopeziza medicaginis* f. sp. *medicaginis-sativae* (Schmiedeknecht) Schmiedeknecht and healthy plants (free from the attack of the pathogen *Pseudopeziza medicaginis* f. sp. *medicaginis-sativae* (Schmiedeknecht) Schmiedeknecht). There were samples of plants attacked by the pathogen and samples made of plants free from the attack of the pathogen. The biological materials used was the Dobrogea variety. From the samples received, approximately 2 g of plants were chosen, which were dried (for 72 hours at 65C in the ventilated oven). Then they were mortared and homogenized. Cold samples digestion was done for 3 weeks 10 cm3 in spectral pure (Merck) nitric acid. A clear solution was obtained which was then brought to the mark with ultra-pure water in a 100ml volumetric flask. The method of analysis is the one communicated by Naizuka et al. (2011). RSD values for spectral lines selected for element analysis ranged from 0.5 to 2.7. The SRM material used was NIST® SRM® 1515 (apple leaves). For the preparation of calibration standards, Merck Certipur® Multi-Element Standard ICP was used – standard solution IV having 23 elements in dilute nitric acid, with a concentration for each element of 1000 mg/l: Ag, Al, B, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, In, K, Li, Mg, Mn, Na, Ni, Pb, Sr, Tl, Zn. The standards prepared to achieve the calibration curves of the chemical elements had concentrations of 0.001 mg/l, 0.01 mg/l, 0.1 mg/l, 1 mg/l and 5 mg/l. As a blank solution, a solution of ultrapure water with a concentration of 10% spectrally pure nitric acid was used. For statistical analysis of data, the MedCalc® Statistical Software version 22.021 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2024) was used. The minerals total concentrations in plants with attack of *Pseudopeziza medicaginis* f. sp. *medicaginis-sativae* (Schmiedeknecht) Schmiedeknecht and in plants free from the attack of *Pseudopeziza medicaginis* f. sp. *medicaginis-sativae* samples were compared using the bilateral t test..

RESULTS AND DISCUSSIONS

Common leaf spot attack caused by the micromyceta of *Pseudopeziza medicaginis* f. sp. *medicaginis-sativae* (Schmiedeknecht)

Schmiedeknecht was observed on leaves and stems. On the leaves, the attack was manifested by the appearance of circular or oval yellow spots that later evolved into brown ones (Figures 1, 2). The most serious attack was observed during the flourishing. In the center of the brown spots, on the upper side of the leaves, the fungus has fruited, forming apothecia in the form of point-shaped protrusions. The leaves with strong attack dried and fell off, the pathogen causing their defoliation. On the stems, the disease was manifested by elongated brownish-blackish spots. The disease appears in every year in alfalfa crop with different levels of attack (Cîrstea et al., 2023).

Figure 1. Common leaf spot on alfalfa leaves (original)

Figure 2. Alfalfa common leaf spot attack (original)

The concentration of different minerals in samples of alfalfa plants with micromycete attack and samples with plants free of attack was analyzed (Table 1).

Table 1. Concentrations of micro- and macronutrients

Chemical element	Plants with attack of *Pseudopeziza medicaginis* f. sp. *medicaginis-sativae* sample (Psm) concentration in g per 100 g dry plant	Plants free from the attack of *Pseudopeziza medicaginis* f. sp. *medicaginis-sativae* sample concentration in g per 100 g dry plant (control)	p value bilateral t test
Al	0.006438844	0.004854135	<0.05
B	0.008599419	0.007070324	<0.05
Ca	3.443726085	4.168781194	<0.05
Cd	SLDM	SLDM	-
Co	SLDM	SLDM	-
Cr	5.27774 x10 $^{-6}$	0.000165056	<0.001
Cu	0.000982979	0.001605727	<0.001
Fe	0.016390685	0.017835939	<0.05
Ga	SLDM	SLDM	-
In	SLDM	SLDM	-
K	1.554294762	2.967565197	<0.001
Mg	0.298588204	0.408452855	<0.001
Mn	0.006999604	0.008223255	<0.05
Na	0.165951973	0.181956139	<0.05
Ni	0.00018736	0.000315824	<0.05
Pb	SLDM	SLDM	-
Sr	0.00836522	0.011746099	<0.001
Tl	0.002979615	0.003969235	<0.05
Zn	0.002591041	0.006193308	<0.001

SLDM = BELOW METHOD DETECTION LIMIT

The concentration of B is significantly higher ($p<0.05$) in plants with attack of *Pseudopeziza medicaginis* f. sp. *medicaginis-sativae* (Schmiedeknecht) Schmiedeknecht sample compared to plants free from the attack of fungus sample. The main functions of boron relate to the strength and development of the cell wall, cell division, the development of fruits and seeds, sugar transport and the development of hormones. Some functions of boron interact with those of nitrogen, phosphorus, potassium, and calcium in plants. The effects of boron accumulated by plants in combating pathogens such as the development of *Fusarium oxysporum* have also been reported (Dong et al., 2016). Therefore, an increase in B uptake by the plants with attack may indicate a defense response mechanism to *Pseudopeziza medicaginis* (Schmiedeknecht) Schmiedeknecht. The low concentration of Sr and Tl in plants with attack compared to plants free from the attack of fungus may also indicate altered mechanisms of nutrient uptake at root level. Sr, Ca, and Mg are behaviorally similar in plants and may compete for the same receptors at the level of cell membranes and both Sr and Ca are accumulated in the cell walls. Obviously the Ca and Sr uptake of plants with attack of mcromycetes was affected as both minerals have a significantly lower concentration in plants with fungus attack compared to plants free from fungus attack. Plants with attacked by the pathogen accumulated significantly less calcium (3.44 g/100 g dry plant) than plants free from pathogen attack (4.16 g/100 g dried plant). Calcium total concentration in plants with patogen attack is 17,30% lower compared to Ca total concentration in plants free from the patogen attack and Sr total concentration in plants affected by pathogen attack is 29.05% lower compared to Sr total concentrations in plants free from fungus attack. These facts demonstrates that the Ca/Sr uptake mechanism

was significantly affected by pathogens. Although between healthy (free from fungus attack) and diseased plants there is a significant difference in iron concentration, (table1) the difference is at the limit of significance ($p = 0.041$). The fungus presence generated a high decrease in Cu total concentration in the attacked plants. Cu concentration in diseased plants was 59.9% lower compared to Cu total concentration in plants free from the disease. Moreover the K, Mg and Zn in the attacked plants was respectively 52.37%, 73.03% and 21.18% lower than in plants free from the attack of the fungus.

CONCLUSIONS

The attack of the fungus in alfalfa influenced the concentration of micro and macronutrients in alfalfa. For some elements (Cd, Co, Ga, In, Pb) the concentration values were below the detection limit of the method.

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