

INFLUENCE OF TWO CAUSAL AGENTS OF RUST IN LEGUMES TO CHLOROPHYLL CONTENT IN LEAVES

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ABSTRACT

Rust diseases are of economic importance for many crops including legumes. The aim of the present study is to investigate the influence of *Uromyces appendiculatus* (Pers.:Pers.) Unger and *Uromyces striatus* Schroet. to chlorophyll content in leaves of common bean and alfalfa, respectively. The investigation was carried out during 2024 in Technical University of Varna, Bulgaria. Leaves from common bean and alfalfa with different disease intensity (DI) (from 5 to 100%), and leaves without symptoms were collected. Plant pigments are extracted according to the generally accepted protocol. The content of chlorophyll „a“ and chlorophyll „b“ were determined by spectrophotometer. The results show that in both crops there is an inversely proportional relationship between the content of chlorophyll “a” and “b” and the DI. In common bean, chlorophyll “a” /chlorophyll “b” is between 247.16 / 125.6 mg/m³ in symptomless leaves and 98.63 mg/ 37.33 mg/m³ at DI=100%. The differences are significant (p<0.05) except for the values at DI=5%. In alfalfa, in leaves without rust symptoms, the content of the plant pigment is respectively 226.87 / 53.30 mg/m³ and 134.6 / 41.07 mg/m³ at DI=75%. For chlorophyll “a”, the differences are significant only at DI=50% and DI=75%, and for chlorophyll “b” at DI=75%. The results show that these two rust pathogens in common bean and alfalfa affect leaf chlorophyll content differently in the two crops. This is likely a result of different host-pathogen interactions, leading to differences in basic physiological processes.

Keywords: *rust, common bean, alfalfa, chlorophyll content.*

INTRODUCTION

Legume crops (*Fabaceae* family) are influenced by numerous abiotic and biotic stress factors among which diseases are of economic importance. Plant fungi dominate over viruses and bacteria in these crops (Aglave et al., 2012). The most extensive taxonomic order of plant pathogenic fungi is *Pucciniales*, the causal agents of rust diseases (Toome-Heller, 2016).

Rust species are macrocyclic, heteroecious or homoecious fungi, producing five types of spores: teliospores; basidiospores; pycnidiospores; aeciospores and urediniospore, of which urediniospores can inoculate many times during the growing

season. Their life cycle combines sexual and asexual stages, which make them high-risk pathogens, capable of overcoming plant resistance (Mapuranga et al., 2022; Osuna-Caballero et al., 2024).

Rust symptoms are very specific regardless of the host plant. They comprise yellow brown to reddish brown pustules occurring on stems, pods, leaves (abaxial and adaxial leaf surface) resulting from epidermis rupture by forming spores. Rust pathogens extract nutrients from infected plant cells through specialized structures called haustoria (Voegelé and Mendgen, 2003).

Uromyces appendiculatus (Pers.:Pers.) Unger, the causal agent of common bean rust, is distributed worldwide (Stavely et al., 1983). The pathogen is homoeocious. Hosts are species of the genus *Phaseolus* – *P. vulgaris* L., *P. coccineus* L., *P. acutifolius* L., *P. lunatus* L., species of the genus *Vigna*. (Stavely and Pastor-Corrales, 1989). The disease is favored by high humidity, leaf wetness, and moderate temperatures (16–22°C) (Araya and Steadman, 1994). Yield losses can reach 25 to 100% in susceptible varieties under severe epidemics, especially in early growing stages (Stavely and Pastor-Corrales, 1989; Steadman et al., 1996).

Uromyces striatus J. Schröt. is dispersed worldwide and has broad host range which comprises at least 141 species and subspecies from the tribes *Trifolieae*, *Cicereae*, and *Vicieae*, including alfalfa (*M. sativa* L.) (Skinner, 1995; Kemen et al., 2005). The pathogen is heteroecious forming pycnidia and aecia on *Euphorbia cyparissias* L. (Maneval, 1924; Koepfer, 1942). Optimal conditions for disease development are temperatures between 20–25°C and high humidity (>90% relative humidity) (Koepfer, 1942).

Biological interactions between plant and pathogen result in negative impact of several physiological processes of the host like photosynthetic light reaction, carbon assimilation, respiration, translocation of metabolites and nutrients (Mandal et al., 2009). Being biotrophic fungi rust pathogens as long as downy mildew pathogens reduce chlorophyll substantially, resulting in severe damage to photosynthesis in different crops (Lindenthal et al., 2005). The reduction in photosynthesis can be result of impairment of photosynthetic apparatus, light harvesting photosystems and the enzymatic processes of carbon assimilation (Mandal et al., 2009). Mishra et al. (2015) noticed that abnormalities in form and functions of photosynthesis resulted in reduction of chlorophyll content in wheat infected by rust. According to Mandal et al. (2009) reduction in photo assimilation lowers biomass accumulation and thereby reduces the grain yield.

The chlorophyll is a green pigment found in almost all plants, which allows plants obtain energy from light. The loss of chlorophyll content of leaves occurs due to abiotic (nutrient imbalance, excessive use of pesticide, environmental changes, aging) and biotic stress, especially due to diseases (Aglave et al., 2012). According to Mahlein et al. (2012) chlorophyll content can be used to quantify disease severity. The aim of the present study is to investigate the influence of *U. appendiculatus* and *U. striatus* on chlorophyll content in leaves of common bean and alfalfa, respectively.

MATERIALS AND METHODS

The investigation was carried out during 2024 in Technical University of Varna, Bulgaria. Leaves with rust pustules and without symptoms were collected from naturally infected common bean and alfalfa under field conditions. Leaves were then separated into six classes according to disease intensity (DI) (percentage leaf area covered by pustules) (DI=5; 10; 25; 50; 75; 100) using modified Cobb scale (Stavely, 1985; Beleva, 2010), put in paper bags, labeled and stored in fridge until use.

For pigment extraction 0.100 g of leaf tissue selected randomly from each DI variant, in three replications, is cut into small pieces with scissors. The weighed sample is ground in a mortar to a homogeneous mass with a little quartz sand, calcium carbonate and 1-2 ml of 80% acetone (from an initial volume of 10 ml). The sample is quantitatively transferred to a beaker with several portions of acetone until the upper volume is exhausted. The resulting green extract is filtered using a funnel and filter paper, then diluted 5-fold, by pouring 0.5 ml of filtrate into a test tube with a pipette and the volume is brought to 2.5 ml with acetone (Tzvetkova and Anev, 2017).

The extract prepared in this way is placed in a spectrophotometer Pharo to determine the content of chlorophyll “a” (chl “a”) and chlorophyll “b” (chl “b”).

Data was analyzed by Duncan’s multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Symptomless leaves from common bean have chl “a” 247.16 mg/m³ and chl “b” 125.6 mg/m³ (Table 1). Chlorophyll “a” in infected by *U. appendiculatus* leaves is from 240.56 (DI=5%) to 98.63 (DI=100%) and chl “b” is between 124.82 (DI=5%) and 37.33 (DI=100%) respectively.

The differences between symptomless leaves and those with symptoms of common bean rust are significant (p<0.05) except for the leaves with DI=5% for both types of chlorophylls (Table 1).

Table 1. Chlorophyll content in common bean and alfalfa leaves infected by rust pathogens *Uromyces appendiculatus* and *Uromyces striatus* with different disease intensity (DI).

Pathosystem	Chlorophyll, mg/m ³	No symptoms	Disease intensity (DI), %					
			5	10	25	50	75	100
Common bean – <i>U. appendiculatus</i>	Chl "a"*	247.16 a	240.56 a	204.87 b	195.83 b	192.63 b	115.53 c	98.63 c
	Chl "b"	125.6 a	124.82 a	66.17 b	72.77 b	67.60 b	51.37 b	37.33 c
Alfalfa - <i>U. striatus</i>	Chl "a"	226.87 a	209.60 a	208.13 a	195.20 a	178.50 b	134.60 c	No data
	Chl "b"	53.30 a	53.10 a	52.87 a	51.73 a	51.37 a	41.07 b	

* Chl “a” – chlorophyll “a”; Chl “b” – chlorophyll “b”

** a, b, c – Duncan’s multiple range test ($p < 0.05$)

In alfalfa, in leaves without rust symptoms, the content of the plant pigment is 226.87 mg/m³ for chl “a” and 53.30 mg/m³ for chl “b” (Table 1). In leaves infected by *U. striatus* chl “a” range from 209.60 mg/m³ (DI=5%) to 134.60 mg/m³ (DI=75%) and chl “b” is between 53.10 mg/m³ (DI=5%) and 41.07 mg/m³ (DI=75%).

For chlorophyll “a”, the differences are significant only at DI=50% and DI=75%, and for chlorophyll “b” at DI=75% (Table 1). It has to be noticed that leaves with DI=100% were not observed.

Chlorophyll “a” is the primary photosynthetic pigment found in all oxygenic photosynthetic organisms, including plants, algae, and cyanobacteria. It is crucial for capturing light energy and converting it into chemical energy during photosynthesis (Lichtenthaler, 1999). It absorbs light energy, primarily in the blue (~430 nm) and red (~662 nm) regions of the spectrum and directly involved in photochemical reactions by transferring high-energy electrons to the electron transport chain. It has functions as the reaction center pigment in both Photosystem I and II (Taiz et al., 2015). All organisms that perform oxygen-producing photosynthesis contain chlorophyll “a” (Jeffrey et al., 2005).

Chlorophyll “b” is an accessory pigment found in green plants and green algae. It cannot drive photosynthesis alone but enhances the efficiency of light absorption by complementing chl “a” (Lichtenthaler, 1987). Acts as a light-harvesting pigment in the antenna complex of photosystems (especially Photosystem II), expands the absorption spectrum, especially in the blue-green region and transfers the excitation energy to chlorophyll “a”, which drives the photochemical reactions (Taiz et al., 2015). It increases the plant’s ability to harvest light in shaded environments or low-light conditions (Gross, 1991).

Diseased plants often show a decrease in total chlorophyll, especially chl “a” and “b”, due to disruption of chloroplast structure, increased activity of chlorophyll-degrading enzymes like chlorophyllase, oxidative stress and reactive oxygen species (ROS) produced during pathogen attack (Berger et al., 2004).

Arunyanart and Boonyakiat (2004) investigated the chlorophyll content in rye inoculated with *Xanthomonas oryzae* (Ishiyama) Swings and found significant chlorophyll loss. The authors announced that *Erysiphe* spp. decreased chlorophyll due to chloroplast damage and *Phytophthora infestans* (Mont.) de Bary broke down the photosynthetic pigments. Goh et al. (2016) determined that the chlorophyll content in Ganoderma-infected oil palm seedlings declined as the infection progressed or during the pathogenesis of Ganoderma.

Bilgin et al. (2010) determined that infected plants show reduced photosynthetic rate, stomatal conductance, and chlorophyll fluorescence resulted from toxins or effectors that pathogens may produce that damage the chloroplast or interfere with light-dependent reactions.

The results in present study show that these two rust pathogens *U. appendiculatus* and *U. striatus* affect leaf chlorophyll content in common bean and alfalfa,

respectively differently. In all cases, the relationship between chl „a“ and chl “b” content and DI is inversely proportional. In common bean leaves with DI=5% and symptomless do not have significant differences in terms of the two chlorophyll types. In alfalfa symptomless leaves and those with DI=5, 10 and 25% also do not have significant differences in chlorophyll content. This is likely a result of different host-pathogen interactions, leading to differences in basic physiological processes. According to Zhao et al. (2011) effects of rust diseases on leaf photosynthesis vary with the types of pathogens, disease severity, and host plant species and/or crop genotypes.

It has to be mentioned that symptomless leaves were also collected from field, and it is not excluded they were inoculated with pathogens but in incubation period.

Reduction in chlorophyll content directly reduces the leaf’s ability to absorb light, leading to lower rates of photosynthesis (Lichtenthaler and Wellburn, 1983). Roberts and Walters (1988) detected significant reduction in photosynthesis in pustule regions in rust infected leek (*Allium porrum*) leaves but found that photosynthesis did not differ from the uninfected controls in the region between pustules. In rust infected *Vicia faba* L. plants photosynthetic rate was substantially reduced in the lower, rusted leaves, but net photosynthesis was significantly increased in the upper, uninfected leaves (Murray and Walters, 1992). Leaves were randomly selected in the present study and such kinds of conclusions cannot be made but further investigations must be done to determine the influence of *U. appendiculatus* and ‘*U. striatus*’ on photosynthetic rate of common bean and alfalfa using conventional laboratory methods or digital tools.

CONCLUSION

The results from the present investigation about the influence of two causal agents of rust, *U. appendiculatus* and *U. striatus* in legumes to chlorophyll content in leaves give us the basis to draw the following conclusions:

U. appendiculatus, the causal agent of common bean rust, significantly reduce chlorophyll content (chlorophyll “a” and chlorophyll “b”) in leaves with disease intensity 10% and above.

U. striatus, which cause alfalfa rust, significantly reduce chlorophyll “a” content in leaves with disease intensity 50% and above, and chlorophyll “b” content in leaves with disease intensity at least 75%.

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