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PHYSIOLOGICAL PARTICULARITIES OF THE SPECIES *VISCUM ALBUM L. ssp.album* AND *LORANTHUS EUROPAEUS Jack*, HEMI-PARASITES ON LIGNUOUS SPECIES FROM THE COMANESTI FOREST, ROMANIA

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ABSTRACT

In the study which had been carried out in the Comanesti forest from the Mehedinti County, Romania, there have been taken into consideration two hemi-parasitic species frequently found in this area: *Viscum album,* a parasite found on *Acer campestre*, and *Loranthus eurpaeus,* a parasite found on *Quercus cerris.*

During the summer time, the two hemi-parasitic species which have been takes under study show moderate values for the photosynthesis and transpiration, but increased values in the case of leaf respiration.

The leaf suction force presents high values, these ensuring both the absorption and the conduction of brute sap.

The chlorophyll pigment contents indicate higher values in the case of *Loranthus,* when compared with the other species, *Viscum,* in the same lighting conditions.

In *Viscum album*, the water content shows a seasonal variation, with a maximum during the spring time, after the appearance of leaves on the host plants.

In the case of *Loranthus,* the maximum water content is recorded in early June.

INTRODUCTION

Hemi-parasitic plants display a unique strategy of resource acquisition combining parasitism of other species and own photosynthetic activity. Despite the active photo-assimilation and green habit, they acquire substantial amount of carbon from their hosts. The organic carbon transfer has a crucial influence on the nature of the interaction between hemi-parasites and their hosts which can oscillate between parasitism and competition for light (Těšitel, J. et al, 2010).

Mistletoe can have a major impact on the health of the host plant. If there are several mistletoe species on the same host tree, the overall impact could be amplified. Different mistletoe species may be compatible with the same host species.

Therefore, compatibility (structural and physiological) could be an important factor for the appearance of mistletoe. Recent studies have shown that if the vase does not "recognize" the host species, the deposited seeds will germinate, but the haustorium will not enter the host branch. This is probably the main mechanism in establishing several mistletoe species on the same host, which can trigger a cascade of harmful effects on the host species (Arruda R.et al, 2013).

MATERIALS AND METHODS

The research aimed to establish the physiological peculiarities of the hemi-parasitic species *Viscum album L. ssp.album* and *Loranthus europaeus Jack*

Viscum album is a hemi-parasitic dioecious shrub growing on the branches of deciduous trees as well as conifers. It is an evergreen plant with leathery and opposite leaves. The inflorescences are axiliary or terminal. Male flowers consist of four perigones. Anthers are implanted on perigones and dehisce by numerous pores. The female flowers are smaller than the male ones. The ovary of *Viscum* generally demonstrates striking reduction phenomena. No ovules exist; instead, a central ovarian papilla, or mamelon, protrudes into the ovarian cavity and produces two or more embryo sacs (Zaki and Kuijt, 1995, cited by Mehrvarz S. et al, 2012)

The epidermis of leaves in front view is composed from cells with polygonal contour, with right walls. On transversal sections, in both epidermises, the cells have external wall thick and strongly cutinized. The palisade parenchyma is bi- or try-layered with short and large cells, sometime with waved lateral walls. The spongy parenchyma is multilayered (10-12 layers), the hypodermic tissue having taller cells. The veins are not prominent; in all of that a vascular bundle of different dimensions could be observed (Andronache A et al, 2006).

Primary haustoria of *Viscum album* have the tendency to dichotomic branch out, but, most of the time only a ramification grows more. The tissues of the host plant are affected by the contact with the parasite: they are hypertrophied, many of its cells being destroyed.

The penetration and development of the haustoria in host plant tissues is revealed in macroscopic longitudinally section of the parasite and host plant (Andronache A.et al, 2006).

In fruit, epicarp consists of one layer of epidermal cells. Mesocarp is parenchymatous with elliptic cells, intercellular spaces and fiber bundles. There are cubic al and stellate calcium oxalate crystals in mesocarp of *V. album subsp. album.* Endocarp in both taxa consists of viscin layer (Mehrvarz S. et al, 2012).

***Loranthus europaeus* Jack.**

The only European species of this genus, *Loranthus europaeus* Jacq. (synonyms: L. *dioicus* Stokes, *Hyphear europaeum* (Jacq.) Danser; vernacular name: yellow or yellow-berried mistletoe) is a widespread deciduous hemiparasitic plant (Uotila, 2011−onward). Primary hosts of L. europaeus are species of *Quercus* L. *(Fagaceae*), such as *Q. pubescens* Willd., *Q. cerris* L., *Q. robur* L., *Q. petraea* (Matt.) Liebl., though it may also occur on *Castanea sativa* Mill. *(Fagaceae)(*Krasilenko Yuliya *et al, 2019)* *Loranthus europaeus* is widely distributed in Central and Southeastern Europe, the Eastern Mediterranean region, and also has several isolated populations in Asia Minor (Glatzel et al., 2016).

The epidermis of leaf in front view has polygonal shaped cells, with right lateral walls. In the external wall cuticular parallel rows could be observed. . The stomata, from paracytic type, are present in both epidermises. On cross sections the mesophyll appears homogenous, spongy type, with large, isodiametric or slowly elongated cells. Here and there the mesophyll has all cells elongated al disposed perpendicularly on the epidermis, with an aspect of palisade parenchyma (Andronache A et al, 2006).

To grow and reproduce, mistletoe must successfully compete for a share of the host's water content, avoid mineral deficiencies, tolerate differences in host xylem sap chemistry and flower and form seeds within the host canopy (Glatzel, 2016).

Mistletoe does not produce functional roots but obtains water and solutes by diverting xylem sap from the host tree through direct xylem connections by haustoria (Kuijt and Toth 1976, cited by Gebaurer et al, 2012). There are very few phloem elements in the haustorium, and they terminate well before the host–mistletoe interface (Buchleitner 1982 in Glatzel 1916).

The vascular connections through the haustoria can provide a route for the molecular trafficking of sugars, water, amino acid, organic acids and ions between the host and parasitic plants (Okonkwo 1966; Hibberd and Jeschke 2001; Jiang et al. 2008, cited by Gebaurer et al, 2012).

In addition to nutritional molecules, informational macromolecules, including RNAs (Roney et al. 2007, cited by Gebaurer et al, 2012), proteins (Haupt et al. 2001; Birschwilks et al. 2006, Gebaurer et al, 2012) and DNA (Davis and Wurdack 2004, cited by Gebaurer et al, 2012), can also be translocated.

In a first stage, in winter (December 2019), on *Viscum album* plants were determined the water and assimilatory pigments contents, after which, the experiments took place in the spring and summer of 2020 in the Comanesti Hills.

The hills of Comăneşti are located in Mehedinţi county, west of the Motru river and they belong to the Bala commune, Comăneşti village. The altitude varies between 150-402 m, the coordinates being lat. 44 ^{0}58', long. 22 ^{0}54'.

Being located in the south-west of the country and of the Getic Piedmont, the researched territory is in the regime of the Central European climate with sub-Mediterranean influences. According to Köppen's classification, the researched territory falls in the area of cold humid climate, with conditions specific to oak (according to Buia et al. 1961, from Costache I., 2011).

The multiannual average air temperature which is characteristic for this area is in the range of 10.1 - 11.5 degrees Celsius.

The average isotherm for winter is between 0 and -10 ^{0}C, and the average temperature of the coldest month (Jan.) is -10 ^{0}C (www.mehedinti.insse.ro)

During the warm July-August period, the maximum temperature often exceeds 32^{0} C. Atmospheric precipitations amount to between 500–793 mm annually.

Regarding the relative humidity of the air, the maximum values are registered in autumn, and the minimum ones in summer (www.mehedinti.insse.ro)

The atmospheric circulation is marked by the advection of the maritime air masses from the west, with a high degree of humidity and of the subtropical or continental ones from the east. (www.mehedinti.insse.ro)

In the analyzed area, *Quercus* species are predominant. As a result, the semi-parasite species *Loranthus europaeus* is common. Young trees are the most affected by it (fig. 1).

Fig. 1. *Quercus cerris* strongly affected by *Loranthus*

Viscum has been found on species of *Tilia*, *Populus*, *Pinus*, but more frequently on *Acer campestre*. Therefore, the studies have been carried out on individuals which were semi-parasite on *Acer campestre*.

The analyzed physiological indices have been the photosynthesis intensity, respiration intensity, transpiration intensity, total water content, the water types (bound and unbound water), the content of pigments and the content of phosphorus and potassium.

Photosynthesis, respiration and transpiration were determined with the portable Lci apparatus.

The total water content was determined gravimetrically by drying the plant material at the oven at 105 ^{0}C.

The water forms (bound and unbound) were determined by the Artihovski method (Boldor O., 1983).

The suction strength of the parenchyma was determined by immersing equal portions of haustoria in solutions with different concentrations of sucrose and determining the isotonic solution (Boldor O., 1983).

The quantity of pigments from the leaves has been determined spectrophotometrically from the extract obtained from a gram of milled leaves and acetone 80% in a final volume of 50 ml of substance.

The extinction has been determined for a wavelength of 646, 663, 470 nm, and the quantity of pigments has been calculated with the following formulas:

Chlorophyll a (mg/100g) = (12,21 • D663) – (2,81 • D646) • 5

Chlorophyll b (mg/100g) = (20,13 • D646) – (5,03 • D663) • 5

Carotene + xanthophyll (mg/100g) = (1000 • D470) – (3,27 • cl a–1,04 cl b)

The ash has been obtained by incinerating the vegetal material at 550 degrees Celsius.

The phosphorus content (P_2O_5) has been determined spectrophotometrically. The process is based on the following property of the phosphoric acid: it gives a yellow color in contact with the ammonium molybdate.

The vegetal material is mineralized with the wet procedure, with the help of a mixture of perchloric acid, sulfuric acid and hydrogen peroxide. A mixture of KH_2PO_4, ammonium molybdate and acetone is used as the standard solution (Buliga,E., Unc, R., 1996).

The potassium quantity has been determined through a volumetric procedure

This method consists on the precipitation of potassium from the hydrochloric ash solution in the form of sodium cobalt nitrite and potassium.

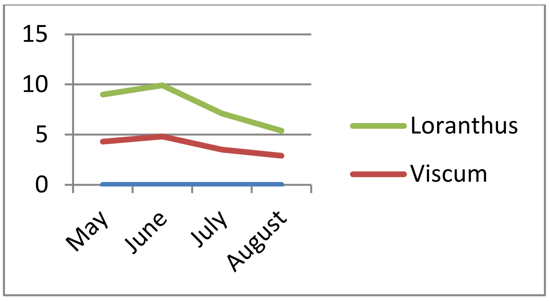
Subsequently, the cobalt nitrite is decomposed in an acidic environment with the excess of 0.1 n potassium permanganate. The excess of permanganate is titrated with oxalic acid (Buliga,E., Unc, R., 1996).

RESEARCH RESULTS

Transpiration intensity

During the summer time, the two hemi-parasitic species which have been takes under study show moderate values for the transpiration, average value 4 mmol / m ^{2} / s (graph 1)

The intensity of transpiration has maximum values during the June, being dependent on the air temperature, air currents, and also on the atmospheric humidity.

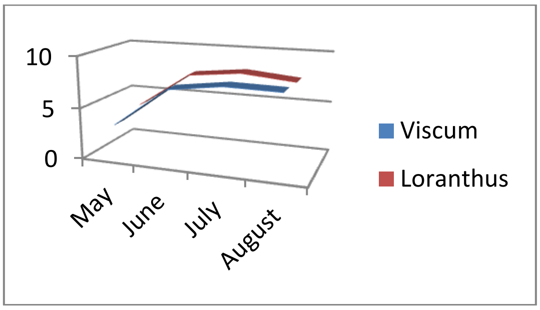


Graph. 1. The seasonal variation of leaves transpiration (mmol / m ^{2} / s)

Respiration intensity

According to the graph 2, the values of the respiration intensity in hemi-parasitic species are very high, close to those recorded for photosynthesis. This shows that, although these plants produce enough organic matter, much of it is oxidized by respiration in order to produce energy.

As in the case of the suction force, there is an intensification of this process when the temperature increases; the values recorded at 25 ^{0}C are almost double when compared with those measured at 10 ^{0}C during the spring .



Graph. 2. The seasonal variation of respiration (μmol /m^2 /s)

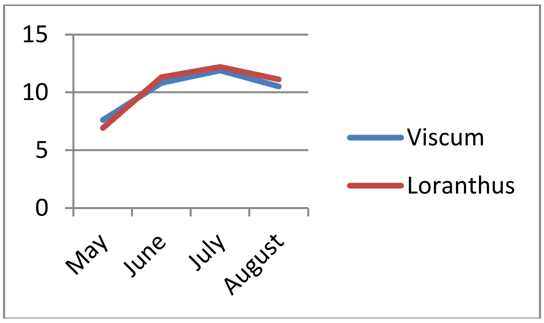
The intensity of photosynthesis

In *Loranthus* and *Viscum*, the values of photosynthesis (gr.3) are close to those recorded in autotrophic species, which demonstrates that they are able to obtain the necessary organic substances without consuming the elaborated sap from the host plant. The maximum values are registered for both species in July: 11.9 µmol / m / s for *Viscum* and 12.2 µmol / m / s for *Loranthus*.

However, some authors consider, based on biochemical determinations, that *Viscum* also uses organic compounds from the phloem vessels of the host trees. An evidence is that plants taken from trees belonging to different species have different chemical compositions.

Carbon budget is the key parameter of biology of individual hemiparasitic species. Hitherto published studies demonstrated that many hemiparasitic species are capable of efficient organic carbon abstraction from their hosts. It is however necessary to validate how the outcomes of the growth-chamber experiments translate into the natural conditions (Jacub T et al, 2010).

Hemi-parasitic plants have an ambiguous relationship with their hosts which, on the one hand, represent exclusive sources of inorganic nutrients but on the other hand, the co-occurrence of these host plants in the hemi-parasite vicinity imposes competition for light. The nature and intensity of this competitive relationship varies across different groups and species of hemi-parasites. The ability of hemi-parasites to acquire organic carbon (largely in the form of xylem-mobile organic and amino acids) is certainly the key factor affecting this interaction -since hemiparasites that are capable of efficient organic carbon abstraction should be minimally affected by shading from their host. The fact that hemi-parasites can exhibit substantial carbon heterotrophy is now supported by a large number of studies, although a traditional point of view on hemi-parasites that highlights the importance of inorganic resources (mainly nitrogen) acquisition is still prevailing (Jacub T. et al, 2010).

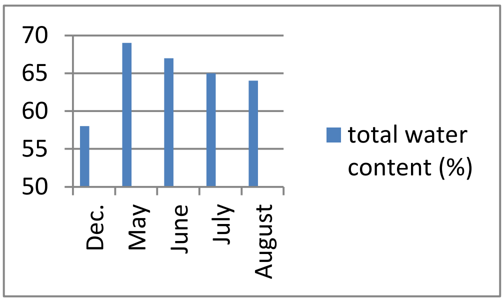


Graph. 3. The seasonal variation of photosynthesis (μmol /m^2 /s)

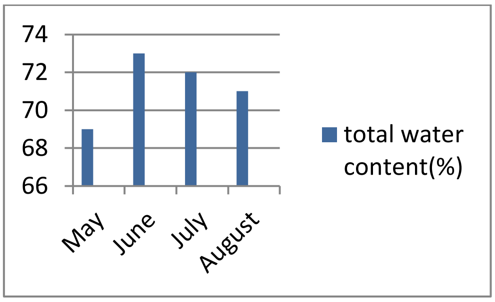
The total water content

In the case of *Viscum album*, the water content shows a seasonal variation, with a maximum during the spring (69%), after the appearance of leaves on the host plants (graph 4).

In the case of *Loranthus,* the maximum water content is recorded in early June (73%) (graph 5).



Graph. 4. The total water content of *Viscum album* leaves (%)

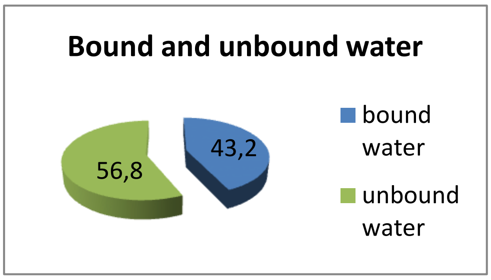


Graph. 5. The total water content of *Loranthus* leaves (%)

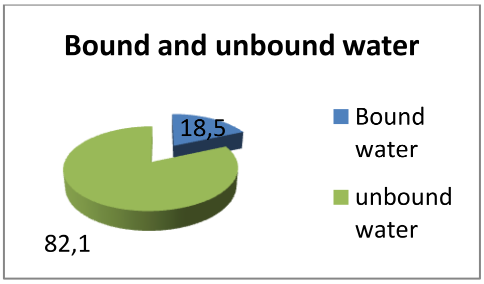
The bound and unbound water

At *Viscum album*, during the winter, the percentage of bound water increases a lot, representing 43.2% of the total water. This high percentage ensures the resistance of the species to the negative temperatures recorded at this time of year(Graph 6). During the warm period of the year, the percentage of bound water decreases significantly for this species (graph 7).

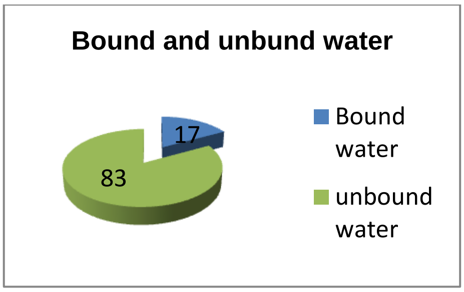
In *Loranthus* leaves, determinations made in June indicate a percentage of bound water of 17% (graph 8).



Graph. 6. The content in bound and unbound water in *Viscum album* during the winter (%)



Graph. 7. The content in bound and unbound water in *Viscum album* during the summer (%)



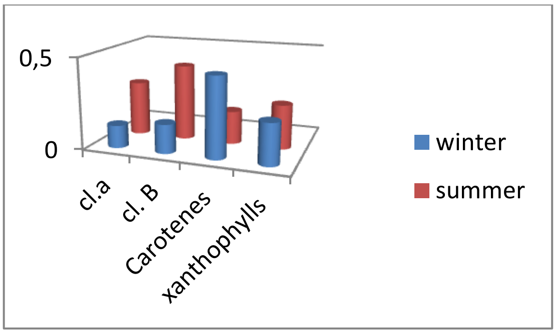
Graph. 8. The content in bound and unbound water in *Loranthus* during the summer (%)

The pigments content

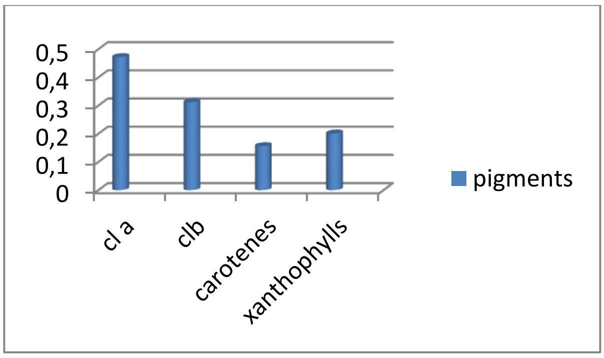
The spectrophotometric determination of the pigment content in the two studied species indicated significant differences.

In the case of *Viscum*, chlorophyll **b** predominates. During the winter, the chlorophyll content decreases in *Viscum album* and the carotenoid content increases (graph 9).

In the case of *Loranthus* (graph 10), the chlorophyll **a** content is predominant.



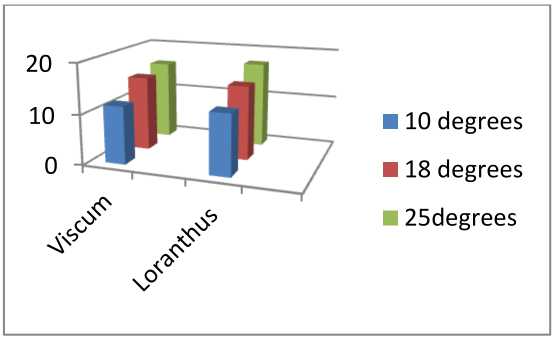
Graph. 9. The content in pigments in *Viscum album (g/100g veg. mat.)*



Graph. 10. The content in pigments in *Loranthus* (g/100g veg. mat.)

The suction force of tissues

The suction force of the leaves in the hemi-parasitic species *Viscum* and *Loranthus* has very high values, which explains the ability of these plants to extract large amounts of raw sap from the body of the host plant. The value of the suction force is dependent on the temperature of the environment, having at 25 ^{0}C values higher by about 5 atm compared to the values recorded at 10 ^{0}C (graph 11).

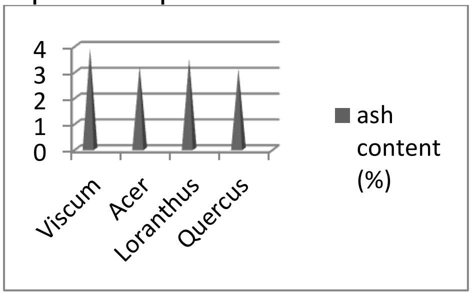


Graph. 11. The suction force of the tissues (atm)

The ash content

Since it is evident that xylem-tapping mistletoes depend completely on their hosts for their mineral nutrition, comparisons of ash content of the two partners have been performed already in the last century (Tubeuf 1923, cited by Pop Marianne and Richter A., 1998). However, even at this early stage of investigations controversy arose which organs of the two members of an association should be compared. Tubeuf (1923) pointed out that it would be more meaningful to draw comparisons between the leaves (‚krautartige Teile autotropher Pflanzen') of both partners than between leaves of *Viscum album* and host branches as done in earlier studies (Popp Marianne and Richter A. ,1998).

The ash percentage was determined in the host plant branches and in the hemi-parasitic plant branches. The data presented in the graph 12 clearly indicates the higher values in the case of hemi-parasitic plants.



Graph 11. The ash content of hemi- parasitic plants and their host plants (%)

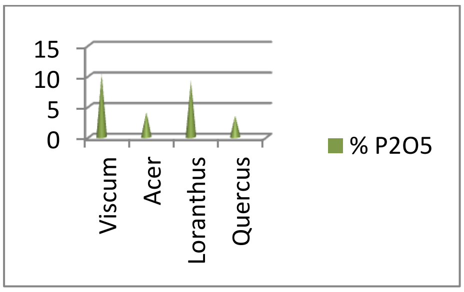
The phosphorus and potassium content (P_2O_5)

In terms of the phosphorus and potassium content, the differences between host and semi-parasitic plants are clearly visible. Thus, if in the case of *Quercus cerris*, P_20_5 is found in a percentage of 3.5%, in *Loranthus*, the maximum value registered is of 9.2%.

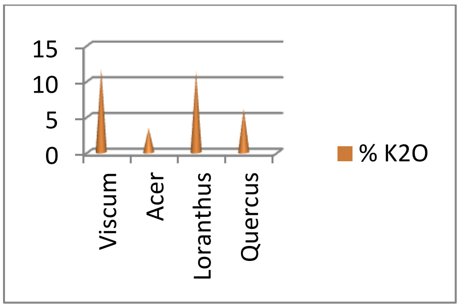
In the case of *Acer campestre*, P_20_5 represents 4.1%, and at *Viscum* it reaches 10.3% (graph 12).

K_2O is found in a percentage of 6.1% in the ash of *Quercus* and reaches 11.3% in the ash of *Loranthus*.

At *Viscum album*, the percentage of K_2O reaches 11.7%, while its host plant, *Acer campestre*, has a value of only 3.4% (graph 13). The large influx of potassium in this plants is probably determined by its participation in the circulation of sugars.



Graph 12.The percentage composition of phosphorus (P_2O_5) in host and parasite plants (% of ash)



Graph. 13.The percentage composition of potassium (K_2O) in host and parasitic plants (% of ash)

CONCLUSIONS

- *Viscum* and *Loranthus* are hemi-parasitic plants with chlorophyll, capable of photosynthesis, which take from the host plants the raw sap (water and mineral salts) which is then processed into organic substances using their own pigments
- Out of the total assimilating pigments, chlorophyll **a** predominates in *Loranthus*, but in Viscum a higher amount of chlorophyll **b** has been found.
- The pigment content changes during the year in *Viscum*; during the winter, carotenoids are predominant
- Research has shown that hemi-parasitic plants have a very intense respiration process
- In the case of hemi-parasitic plants, the values of the suction force are higher than those registered in autotrophic plants. This force contributes to the absorption of an optimal amount of raw sap from the host plant.
- The amount of ash is higher in hemi-parasitic plants than in the case of their host plants
- The percentages of P_2O_5 and K_2O in hemi-parasitic plants are also clearly higher when compared with the values recorded for their hosts

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