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Physiological and Biochemical Changes in Host Leaf Tissues Associated with the Growth of Two Biotrophic Fungi Growing in Egypt

By

H. S. ALDESUQUY and Z. A. M. BAKA*)

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Summary

ALDESUQUY H. S. & BAKA Z. A. M. 1992. Physiological and biochemical changes in host leaf tissues associated with the growth of two biotrophic fungi growing in Egypt. – Phyton (Horn, Austria) 32 (1): 129–142. English with German summary.

Two biotrophic fungi, from different groups, growing in Egypt were selected for this study, *Puccinia lagenophorae,* infecting *Senecio glaucus* and *Albugo candida* infecting *Cakile maritima* during late infection. The intensity of growth of the two pathogens on their hosts was studied in relation to some physiological and biochemical aspects of infected leaves. The infection of *Senecio* caused a significant decrease of relative water content, while the infection of *Cakile* caused a non-significant decrease of the same parameter. The ionic content (Na^+, K^+, Ca^{2+}) of infected leaves of both *Senecio* and *Cakile* was greatly reduced. Also, there was a decrease in pigments (chl a, chl b and carotenoids) in infected leaves of both *Senecio* and *Cakile.* In addition, carbohydrate content (sucrose, reducing sugars and polysaccharides) was significantly decreased in infected leaves of *Senecio* but increased in case of *Cakile.* Seventeen amino acids were detected in both healthy and infected leaves of *Senecio* and *Cakile.* It was found that, most of these amino acids decreased after the infection by the two pathogens except the increase of some amino acids such as phenylalanine and histidine in case of *Senecio* and glycine, alanine, methionine and histidine in case of *Cakile.* The variation of these results were discussed in relation to the growth and behaviour of the two pathogens on their hosts.

*) Dr. H. S. ALDESUQUY, Dr. Z. A. M. BAKA, Department of Botany, Faculty of Science, University of Mansoura, Mansoura, Egypt.

Zusammenfassung

ALDESUQUY H. S. & BAKA Z. A. M. 1992. Physiologische und biochemische Veränderungen in Blattgeweben von Wirtspflanzen im Zusammenhang mit dem Wachstum zweier biotropher Pilze aus Ägypten. – Phyton (Horn, Austria) 32 (1): 129–142. Englisch mit deutscher Zusammenfassung.

Zwei biotrophe Pilze aus Egypten, verschiedenen Gruppen zugehörig, werden für diese Arbeit herangezogen: *Puccinia lagenophorae* auf *Senecio glaucus* and *Albugo candida* auf *Cakile maritima*. Untersucht werden jeweils die letzten Infektionsstadien. Die Wachstumsintensität dieser zwei Pathogene auf den Wirtspflanzen wird in Verbindung mit einigen physiologischen und biochemischen Aspekten der infizierten Blätter untersucht. Die Infektion von *Senecio* bewirkt eine signifikante Verringerung des relativen Wassergehaltes, wohingegen diejenige von *Cakile* nicht signifikant ausfällt. In befallenen Blättern sowohl von *Senecio* und *Cakile* ist der Gehalt an Ionen (Na^+, K^+, Ca^{2+}) deutlich reduziert. Weiters ist bei *Senecio* und *Cakile* eine Pigmentabnahme (Chl. a, Chl. b und Karotinoide) festzustellen. Zusätzlich ist bei befallenen Blättern von *Senecio* der Kohlehydratgehalt (Saccharose, reduzierende Zucker und Polysaccharide) signifikant vermindert, jedoch bei *Cakile* deutlich höher. Siebzehn Aminosäuren konnten in gesunden und infizierten Blättern von *Senecio* und *Cakile* bestimmt werden. Es zeigte sich, daß die meisten dieser Aminosäuren nach der Infektion durch die zwei Pathogene abnahmen, mit Ausnahme eines Anstieges von Phenylalanin und Histidin bei *Senecio* und Glycin, Alanin, Methionin und Histidin bei von *Cakile*. Die Unterschiede in diesen Ergebnissen werden in bezug auf das Wachstum und Infektionsverhalten dieser zwei Pathogene diskutiert.

1. Introduction

A biotrophic fungus may be defined as one which, as a parasite, must derive the nutrients it requires for growth and full development from the living tissues of a compatible host (INGRAM & al. 1976). For example, infection by biotrophic fungi may lead to substantial changes in the carbohydrate content of infected plants which may reflect the alterations in different metabolic processes favourable or unfavourable for fungal development (HWANG & HEITEFUSS 1986). In addition, the infected leaf by biotrophic fungi shows accelerated senescence and loss of chlorophyll (BRIAN 1967, BUSHNELL 1984, FARRAR & LEWIS 1987, SCHOLES & FARRAR, 1987).

The growth habit of biotrophic fungi on their hosts may differ from fungus to another and also depends on the stage of infection of the same fungus. AL-KHESRAJI & LOSEL 1980 reported morphological and quantitative comparison of the heteroecious rust fungus, *Puccinia poarum* in tissues of its alternative hosts, but without any physiological aspects. So far, the quantitative distribution and frequency of biotrophic fungal structures in relation to physiological or biochemical changes in their hosts still attracted a little attention.

Changes in structure, function and metabolism which occur during pathogenesis are obviously interrelated and inseparable in diseased plants.

Any change in one may caused or be caused by a change in one or both of the others. Abnormal patterns of translocation of organic and inorganic materials are commonly found in plant infected with virus or biotrophic parasites (MIROCHA & ZAKI 1966).

BAKA (unpublished data) recorded for the first time in Egypt that *Senecio glaucus (Compositae)* and *Cakile maritima (Cruciferae)* are new susceptible hosts to the infection of *Puccinia lagenophorae* and *Albugo candida* respectively.

The main objective of the present investigation is to correlate the changes in some physiological and biochemical aspects in the leaves of these hosts infected by their pathogens, with the quantitative distribution and frequency of such pathogens in their hosts.

2. Materials and Methods

Two biotrophic fungi belonging to two different groups were selected for the present study: *Puccinia lagenophorae* Cooke *(Uredinales)* with late infeciton (opened aecia) which infects *Senecio glaucus* L. and *Albugo candida* (Pers.) Kuntz. *(Oomycetes)* with late infection (opened conidial sori) infecting *Cakile maritima* Scop. The healthy and infected leaves by both pathogens were collected for different analyses from the field at Baltim area, Egypt.

2.1 Quantitative growth of both pathogens on their hosts:

The quantitative growth of the two pathogens on their hosts was carried out using Araldite semi-thin sections (0.5 μm thick) according to the procedure of AL-KHESRAJI 1981.

Growth measurements were made of the number of haustoria per host cell and the number of infected and uninfected cells of both hosts, each sample consisting of cells lying along 300 μm transect line in a section parallel to leaf surface.

2.2 Determination of relative water content (R. W. C.).

The relative water content of both healthy and infected leaves was determined according to the technique of WEATHERLY 1950.

2.3 Determination of ionic content:

Samples were dried in an oven at 80° C until constant weight. The dried matter was digested in concentrated HNO_3 and made up to volume with deionized distilled water. K^+ and Na^+ concentrations were measured by flame-emission spectrophotometer. Ca^{2+} concentration was measured by atomic absorption spectrophotometry (PHF 80, 3 biologie Spectrophotometer) (ABBAS 1981).

2.4 Estimation of pigments:

The plant photosynthetic pigments (chlorophyll a, b and carotenoids) in both healthy and infected leaves were determined according to spectrophotometric methods recommended by METZENER & al. 1965.

2.5 Estimation of carbohydrates:

The methods of extraction, clarification and measurements of carbohydrates were essentially those described by YOUNIS & al. 1969.

2.6 Quantitative determination of amino acids:

All amino acids were extracted with ethanol and then hydrolysed with 6N HCl for 24 h according to the procedure of SEMPIO & RAGGI 1966. The extracted amino acids were then measured using a LKB alpha high performance amino acid analyser (LKB Biochrom. LTD England). Retention time and area were determined using Hewlett Packard 3390 recording integrator. Concentration of each amino acid GM/16 GM nitrogen was calculated by special designed program.

Three samples from three different healthy and infected plants by the two pathogens were taken for fungal growth measurements, biochemical and physiological analyses. The samples of infected leaves which were subjected to these analyses were always taken from the centre of fungal pustules.

The results of R. W. C., pigments, ions and carbohydrates were subjected to an analysis of variance according to SENEDECOR & COCHRAN 1967.

3. Results

3.1 Quantitative assessment of growth of *P. lagenophorae* and *A. candida* on their hosts:

The intensity of infection of host cells by haustoria of *P. lagenophorae* on *Senecio* was compared with that by haustoria of *A. candida* on *Cakile* in Table 1. This study indicated that 81 % of the upper mesophyll cells in infected regions of *Senecio* leaves were penetrated by one, two, three or four haustoria, while 38 % of the upper mesophyll cells of *Cakile* were invaded by one or two haustoria but not by three or four. This was manifested from semi-thin sections which showed a heavy infection of leaves of *Senecio* by *Puccinia* and a light infection of leaves of *Cakile* by *Albugo*.

Table 1

Quantitative comparison of intensity of infection of *Puccinia lagenophorae* on leaves of *Senecio glaucus* (S) and *Albugo candida* on leaves of *Cakile maritima* (C)
Values of means of 25 counts ± standard errors of means, each sample consisting of cells lying along a 300 µm transect line in a section through the upper mesophyll, parallel to epidermis.

Mean number of cells containing each number of haustoria					Mean no. of cells per sample	Percentage of cells infected
Number of haustoria in each host cell						
	1	2	3	4		
S.	5.60±0.19	1.80±0.16	0.25±0.10	0.15±0.10	10.40±0.32	75.00
C.	2.25±0.10	0.40±0.14	0	0	6.80±0.18	38.97

3.2 Changes in relative water content (R. W. C.) and saturation water deficit (S. W. D.)

It is clear from Table 2 that the infection by *P. lagenophorae* caused a significant decrease ($P<0.05$) in the R. W. C. of *Senecio* leaves while in *Cakile* leaves infected by *A. candida*, there was a non-significant decrease in the same parameter. Therefore, S. W. D. was significantly ($P<0.01$) or non-significantly increased in infected leaves of *Senecio* or *Cakile* respectively.

3.3 Changes in ionic content:

The ionic content (Na^+, K^+, Ca^{2+}) of infected leaves of *Senecio* and *Cakile* was greatly reduced ($P<0.01$) if they compared with the results obtained from healthy leaves (Table 3).

Table 2

Relative water content (R. W. C.) and saturation water deficit (S. W. D.) in healthy and infected leaves of *Senecio glaucus* and *Cakile maritima*.

Leaf tissues		% R. W. C.	% S. W. D.
• *Senecio glaucus*			
• Healthy		76.89	23.11
• Infected		63.20	36.80
• *Cakile maritima*			
• Healthy		78.14	21.86
• Infected		72.16	27.84
L.S.D.	5%	10.56	10.63
	1%	19.40	19.53

Table 3

Content of Na^+, K^+ and Ca^{2+} in healthy and infected leaves of *Senecio glaucus* and *Cakile maritima*. Values are expressed as $mg.g^{-1}$ dry weight.

Leaf tissues		Na^+	K^+	Ca^{2+}
• *Senecio glaucus*				
• Healthy		0.140	0.205	0.08
• Infected		0.012	0.098	0.03
• *Cakile maritima*				
• Healthy		0.134	0.151	0.063
• Infected		0.028	0.175	0.038
L.S.D.	5%	0.003	0.010	0.015
	1%	0.05	0.018	0.029

3.4 Changes in pigment content:

Regarding the effect of the two provided biotrophic fungi on pigment content of leaves of *Senecio* or *Cakile,* it was clear that chl a, chl b and total pigments were clearly lower in the infected *Senecio* leaves than healthy ones. Also, Chl a/b ratio was not significantly changed due to *Puccinia* infection. Carotenoids in infected *Senecio* leaves by *Puccinia* were nearly as high as in healthy ones. On the other hand, carotenoids were drastically decreased ($P < 0.01$) in infected *Cakile* leaves, while chl a, chl b and chl a/b were non-significantly affected (Table 4).

3.5 Changes in carbohydrate content:

Data presented in Table 5 show a significant decrease ($P < 0.01$) in sucrose, polysaccharides and total carbohydrates content of infected leaves of *Senecio*. On the other hand, an obvious increase was detected in these carbohydrate fractions in addition to reducing sugars in infected leaves of *Cakile*.

3.6 Changes in amino acids:

Mainly, 17 amino acids were detected in infected and healthy leaves of *Senecio* and *Cakile* when amino acid analyser was used. These amino acids in addition to ammonia are presented in Table 6. These data indicated that cystine was absent in infected and healthy leaves of *Senecio* and *Cakile*. In addition, the infected leaves of *Senecio* contained low amount of glycine, alanine, valine, leucine and isoleucine. On the other hand, infected leaves of *Cakile* showed a pronounced increase in such types of amino acids.

Table 4

Content of pigments in healthy and infected leaves of *Senecio glaucus* and *Cakile maritima*. Values expressed as $mg.g^{-1}$ fresh weight.

Leaf tissues		chl. a	chl. b	chl. a/b	Caro-tenoids	Total pigments
• *Senecio glaucus*						
• Healthy		0.426	0.217	1.980	0.149	0.792
• Infected		0.231	0.137	1.704	0.143	0.511
• *Cakile maritima*						
• Healthy		0.304	0.246	1.239	0.111	0.661
• Infected		0.286	0.222	0.892	0.059	0.567
L.S.D.	5%	0.071	0.037	0.389	0.044	0.031
	1%	0.130	0.068	0.715	0.080	0.058

Table 5

Content of different carbohydrates in healthy and infected leaves of *Senecio glaucus* and *Cakile maritima*. Values are expressed as mg glucose g^{-1} dry weight.

Leaf tissues	Reducing sugars	Sucrose	Polysaccharides	Total carbohydrates
• *Senecio glaucus*				
• Healthy	9.32	7.07	115.00	131.39
• Infected	8.04	1.66	18.04	27.77
• *Cakile maritima*				
• Healthy	9.27	1.99	19.26	30.52
• Infected	12.98	2.91	29.12	45.01
L.S.D. 5%	1.790	2.038	7.001	8.666
L.S.D. 1%	3.302	3.744	12.859	15.918

Table 6

Content of amino acids in healthy and infected leaves of *Senecio glaucus* and *Cakile maritima*. Values are expressed as mg/100 mg dry weight.

Leaf tissues	*Senecio glaucus*		*Cakile maritima*	
Amino acid	Healthy	Infected	Healthy	Infected
–Aspartic acid	5.207	4.914	3.398	2.633
–Threonine	1.733	1.365	1.328	1.040
–Serine	2.394	1.906	1.691	1.234
–Glutamic acid	7.569	6.117	4.687	2.999
–Proline	4.781	2.845	2.304	0.092
–Glycine	2.648	2.078	0.660	1.598
–Alanine	2.359	2.065	0.881	1.593
–Cystine	0.000	0.000	0.000	0.000
–Valine	2.479	1.354	1.612	1.858
–Methionine	1.795	1.453	0.410	2.056
–Isoleucine	1.673	0.905	1.161	1.253
–Leucine	5.832	4.559	4.428	3.805
–Tyrosine	0.154	0.353	0.217	0.906
–Phenyl alanine	2.057	2.470	1.264	0.578
–Histidine	1.032	1.327	1.156	3.678
–Lysine	3.078	2.522	1.842	1.623
–Arginine	3.101	2.247	1.280	1.150
–Ammonia	5.677	4.929	4.702	3.305
Total determined amino acids	47.889	38.480	28.320	27.280
Total determined amino acids and Ammonia	53.466	43.409	33.022	30.585

With regard to the effect of the two provided biotrophic fungi on serine and threonine, it was noticed that the infection caused an obvious decrease in serine and threonine in the leaves of both *Senecio* and *Cakile*.

Also, a slight decrease in sulphur containing amino acid (methionine) was manifested in infected leaves of *Senecio* while, a drastic increase was observed in infected leaves of *Cakile*. On the other hand, a slight increase in aromatic amino acids (phenyl alanine, tyrosine) in infected leaves of *Senecio* was detected, while in infected leaves of *Cakile* the level of such types of amino acids were either sharply decreased or increased respectively. In addition, there was an obvious decrease and a slight increase in heterocyclic amino acids (i. e. proline and histidine) in both infected leaves of *Senecio* and *Cakile* respectively.

A noticeable reduction in acidic amino acids was observed in infected leaves of *Senecio* and *Cakile,* but the degree of this reduction was more pronounced in *Cakile* than *Senecio* leaves. Also, there was a remarkable reduction in the amount of basic amino acids (i. e. ariginine and lysine) and ammonia. This reduction was higher in *Senecio* leaves than in *Cakile* leaves.

It was clear from the present data that, the total determined amino acids and ammonia were reduced in infected *Senecio* leaves by *Puccinia* and *Cakile* by *Albugo*.

4. Discussion

The rust fungus, *Puccinia lagenophorae* caused remarkable changes in the morphology of its host, *Senecio glaucus*. The infected whole plant appeared shorter than healthy one. The infected leaves became rolled up and the size of root was reduced. In contrast, *Albugo candida* caused no much changes in the morphology of *Cakile maritima*.

The lower frequency of penetration of host cells by haustoria in *Cakile* leaves and much denser in case of *Senecio* leaves may suggest that there was a difference in pathological behaviour between the members of *Oomycetes* and *Uredinales*. These differences may also lead to the variation of physiological characteristics of the host.

Reaction of host plants to the different diseases caused by biotrophic fungi may be related to the constituents of the host. Thus, in infected leaves of *Cakile* by *Albugo* a great variation in their physiological and biochemical aspects were manifested if compared with the infection of *Senecio* by *Puccinia*.

In infected leaves of *Senecio* and *Cakile* a slight and noticeable decline in the R. W. C. was manifested respectively, which was accompanied by a simultaneous increase in their S.W.D. The changes in these parameters may be used here as reliable indices for the biochemical changes within the two different types of leaves of *Cakile* and *Senecio*.

It was clear from the results that the two pathogens induced water

stress in the infected leaves of the hosts. These observations are in agreement with the results of WHEELER & HANCHEY 1968. They concluded that, imbalance in water relations will be one result of alterations in cell permeability which appeared as characteristic of diseased plants.

Abnormal patterns of translocation of organic and inorganic materials are commonly found in plants infected with viruses and biotrophic parasites (WHEELER 1975). The observed reduction in the amount of Na^+, K^+ and Ca^+ in the infected leaves of *Senecio* and Na^+ and Ca^{2+} in infected leaves of *Cakile* was in agreement with the observation of AHMED & al. (1982). They reported that the infection by biotrophic fungi may modify root activity. For example, ion uptake by intact barley plants was inhibited by the infection of *Puccinia recondita* which was generally attributable to the reduced size of root system of infected plants. Also, water stress appearently inhibits the mechanism of uptake of nutrients (HSIAO, 1973). Our observation on *Senecio* and *Cakile* may fit these conclusions. The high significant decrease of Na^+ in infected leaves of the two hosts is in contrary to the observation of ROBERTS 1987 as cited in PAUL & AYRES 1988. He found an increase in the concentration of Na^+ in case of leek infected by *Puccinia alli*. Our interpretation for this disagreement is, that *Senecio* and *Cakile* are growing in soils rich in Na^+ but leek may be not. ABU EL-SOUD 1991 confirmed that *Senecio glaucus* and *Cakile maritima* are growing in the transition zone between salt marshes and sand dunes close to the sea and this zone is rich in Na^+. Furthermore PAUL & AYRES 1988 investigated that the uptake of different ions in case of *Senecio vulgaris* infected by *Puccinia lagenophorae* depends on the concentration of these ions in nutrient media. It is also possible that Na^+ is absorbed by the root system of infected plants and circulates within them and is not transported towards the shoot.

Chlorosis resulting from loss of chlorophyll is a common symptom in plants infected with foliar pathogens (SEMPIO 1959). Thus, the obvious decrease in chl a, chl b and total pigments of infected leaves of *Senecio* and the slight decrease in pigment content of *Cakile* were in agreement with many studies which report that biotrophic pathogens cause a reduction in green leaf area and in chlorophyll concentration (DOODSON & al. 1965; CALONGE 1967, OWERA & al. 1981, SCHOLES & FARRAR 1985). In addition, the loss of chlorophyll in infected leaves of the present studied hosts coincides with the ultrastructural changes of chloroplasts in infected leaves of *Senecio vulgaris* by *Puccinia lagenophorae* and *Albugo candida* (BAKA 1987, 1989) and infected leaves of *Tussilago farfara* and *Poa pratensis* by *Puccinia poarum* (ALKHESRAJI 1981). On the other hand, the elevation of carotenoids in case of infected *Senecio* was in conformity with the results obtained by SZIRAKI & al. 1984 in case of *Phaseolus vulgaris* infected by *Uromyces phaseoli*. This increase may come from the carotens which are present in the spores of *Puccinia*. It is well known that rust spores contain large quantity of carotens (GOODWIN 1980, BAKA 1987).

It was interesting to observe that infected leaves of *Senecio* contained lower amount of polysaccharides and total carbohydrates than healthy leaves. This reduction in sugar content was probably due to the increase in respiration of infected plant (SHAW & SAMBORSKI 1956; DALY & al. 1961) and the increase in dehydrogenase and pentose cycle enzyme activities (CUTTER 1951; LUNDERSTADT 1964). Another interpretation was coming from the fact that the diminished sugar content could also be derived for the higher supply of the fungus and also be due to reduced photosynthesis (OWERA & al. 1981, AHMED & al. 1983). The diminished chlorophyll content which detected in the present investigation due to the infection may lead to the reduction of photosynthesis rate. In contrast to healthy leaves, the amount of sucrose in leaves of *Senecio* infected by *Puccinia* was greatly decreased, while it was increased in leaves of *Cakile* infected by *Albugo*. In the first case, complete sporulation may demand an energy from host tissue and for biosynthetic intermediates for the growth and sporulation of the pathogen (KOSUGE 1978). The sucrose may be the carbon source used by rust fungi, which is hydrolysed before or during transfer to the fungus. Furthermore, sucrose is the form of carbohydrates taken up by some biotrophic fungi (HEWITT & AYRES 1978, MANNERS & GAY 1982). Some of these evidences came from an examination of invertase activity in rust-infected leaves. Increases in acid invertase activity have been found in several biotrophic infection (WHIPPS & LEWIS 1981). Moreover, sucrose, glucose and fructose often increased sevenfold in rusted leaves as part of the juvenile host response prior to fungus sporulation and then decline rapidly thereafter (LUNDERSTADT 1966, MITCHELL & al. 1978). Similar patterns occur with bean rust (INMAN 1962) and rust of *Poa pratensis* (LEWIS 1976). The sugars (sucrose and reducing sugars) are later depleted during the autolytic host response as the sporulating fungus uses increasing amounts of carbohydrate (BUSHNELL 1984). In our case, infected *Senecio* is considered as autolytic host due to the aggressive infection and much sporulation of *Puccinia* while infected *Cakile* is a juvenile host due to the weak infection and slight sporulation of *Albugo*. Changes in starch content following infection have been observed in many foliar diseases. Depletion of starch in the chloroplast during sporulation has been reported by several authors (SCHOLES & FARRAR 1987, SZIRAKI & al. 1984). These results suggested that the reduction in the amount of starch in rusted *Senecio* leaves, particularly when sporulation has started, was likely to be due to decreased photosynthetic efficiency and the diversion of host assimilates to the pathogen. On the other hand, the concentration of reducing sugars, sucrose, polysaccharides and total carbohydrates was greater in infected leaves of *Cakile* than those in healthy leaves. The observed accumulation of these compounds might have resulted from the increase of transport into infected area. The weak infection by *Albugo* might stimulate the metabolism in pustule area (LONG & al. 1975). This was manifested by the presence of green island around the

pustule of *Albugo*. Many workers investigated that the green islands are rich with metabolites including carbohydrates (HARDING & al. 1968, SZIRAKI & al. 1984, BAILEY 1987).

The detected seventeen amino acids in infected and healthy leaves of *Senecio* and *Cakile* showed great variations. These variations depend mainly on the type of the pathogen. It is striking to observe that glycine, alanine and methionine were increased after the infection of *Cakile* only, while histidine was increased in infected leaves of both *Senecio* and *Cakile*. We can speculate that these amino acids were preferable to take up by *Oomycetes* rather than *Uredinales*. The increase of histidine after the infection was in agreement with the results obtained by SEMPIO & RAGGI 1966 in case of bean plants infected with *Uromyces fabae*. They concluded that histidine seems to be necessary to the process of differentiation of the mycelium. KIM & ROHRINGER 1969 found that in rusted wheat leaves many free amino acids diminish except glutamine which increases considerably and seems to be necessary to the formation of the membrane of the uredospores.

The observed decline of proline in infected leaves of both *Senecio* and *Cakile* compared with healthy leaves may be attributed to the fact that the demand of proline by the two biotrophs was more than its accumulation in infected leaves to overcome the water stress. There was a positive correlation between the accumulation of proline in plants and water stress (SINGH & al. 1972).

We can conclude from the present study that there was a relationship between the growth intensity of the pathogen and physiological and biochemical changes of the host. Also, the pathological behaviour of biotrophic fungi and their effects on the physiology and biochemistry of the hosts varies from pathogen to another and the degree of infection.

In fact, this study is considered as a preliminary work between different healthy and infected hosts with different biotrophic pathogens, but the study of the development of these pathogens on their hosts may be necessary to obtain precise informations about physiological and biochemical changes and their relation with different stages of pathogen growth.

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