HELIA, **16**, Nr. 19, p.p. 85-92 (1993)

NUTRIENT METABOLISM IN SUNFLOWER LEAVES INFECTED WITH *Alternaria helianthi*

D.T.Prasad

AICRP on Sunflower, Department of Biochemistry, University of Agricultural Sciences, GKVK, Bangalore-560065, India.

SUMMARY

Nutrient assimilation and metabolism in sunflower leaves were examined in both healthy and *Alternaria helianthi* infected plants. An increase in the total nitrogen and accumulation of ammonia was observed in the *Alternaria* infected leaves. The nitrate accumulation following infection was found to be non-significant. A significant increase in total phosphorus was observed in infected leaves whereas the available phosphorus contents was reduced drastically. The deamination of aspartic acid, glutamic acid, tyrosine and arginine *in vivo* was found to be significantly higher in infected leaves than healthy ones. The nitrite and nitrate reductase activities were found to be higher in infected leaves. Whereas the ratio of chlorophyll *a/b* was higher in healthy leaves. The soluble sugar content was observed to be more in infected than the healthy leaves of sunflower. The possible explanations for these alterations in nutrient metabolism of sunflower leaves infected with *Alternaria* have been discussed.

Key words: Sunflower (*Helianthus annuus* L.), *Althernaria*, nitrogen, phosphorus, carbohydrates, metabolism.

INTRODUCTION

Considerations of the physiological and biochemical aspects of host/pathogen interactions are crucial for a better understanding of infection and yield loss relationships. Foliar diseases cause substantial alterations in the assimilation and metabolism of nutrients in the host (Bassham et al., 1981; Roberts and Walters, 1988; Sadler and Scott, 1974). Further, the translocation of photosynthates and nutrients from unifected tissues or leaves to the infected ones under the influence of obligate biotrophic phytopathogens was observed (Goodman et al., 1986; Pozsar and Kirlay, 1966; Walters, 1985). Alterations in the levels of nitrate/ammonia was reported for barley infected with powdery mildew (Sadler and Scott, 1974; Walters and Ayers, 1980), followed by increase in the activities of enzymes involved in the biosynthesis of polyamines (Walters et al., 1985). Further, it has been shown that Pi markedly affect both photosynthesis and apportioning of newly fixed carbons between storage carbohydrates like starch and cytoplasmic metabolites (Bassham et al., 1981; Herald and Walker, 1979).

Sunflower, one of the important oilseed crops in the world, suffers yield loss due to the infection by foliar diseases caused by pathogens like *Alternaria helianthi, Puccinia helianthi, Plasmopara halstedii*, etc. Data on the alterations in the nutrient metabolism as a result of host/pathogen interaction in sunflower are scant. In this paper, the results of the studies carried out in our laboratory on some aspects of nitrogen, carbon and phosphorus metabolism in sunflower leaves infected with *Alternaria helianthi* is discussed.

MATERIALS AND METHODS

Sunflower (*Helianthus annuus* L.), EC.68415, seedlings were raised in earthen pots for four weeks. The plants were inoculated by spraying a spore suspension of *Alternaria helianthi*. After the inoculation the plants were kept under plastic covering for 48 hr to maintain high humidity necessary for spore germination. Analyses were performed 12 days after the inoculation. All the results reported are means of four replicates.

The total nitrate and ammonia contents in the water extract of fresh tissues, total nitrogen, total and available phosphorus content in leaves, were determined by standard assay methods (A.O.A.C., 1980). The soluble and reducing sugar content, total phenolic and chlorophyll content were determined by the A.O.A.C. method (1980).

The rate of ammonia metabolism *in vitro* was measured by the method of Kumar et al. (1988) and Roberts and Walters (1988). About 1g. of fresh leaf discs (1cm dia) were tranferred to a beaker containing 20 ml of distilled water, leaf discs were placed with their lower part immersed in water and transferred to a dessicator. Twenty ml of 5% boric acid in a petri dish were also placed in the dessicator to absorb the evolved ammonia. Incubated at room temparature for 24hr in illuminated chamber. The ammonia in samples (both in water and boric acid) was estimated by nesslerisation.

The method of Roberts and Walters (1988) was followed to study the amino acid metabolism *in vivo* in infected and healthy leaves of sunflower. Leaf discs of 1 cm diameter (500 mg fresh weight) were floated in 10 ml of 10 mM amino acid solution in petri dishes. Incubated for 18 hr under constantly illuminated flourescent light. After incubation, the ammonia content in water (in which leaves have been suspended) as well as in boric acid, were determined by nesslerization.

Nitrite and nitrate reductase activities in the healthy and infected leaves of sunflower *in vivo*, were determined by the methods described elsewhere (Sadler and Scott, 1974; Deane-Drommond et al., 1979).

RESULTS

Nitrogen content – A significant increase in the total nitrogen as well as the ammoniacal nitrogen in the infected leaves was recorded. The moisture content in healthy and infected leaves was determined to be 81.7% and 82.52%, respectively. Although a small increase in nitrate content was observed in infected leaves, the variation was found to be significant (Table 1). The evolution of ammonia (collected in boric acid) as well as ammonia exuded into the water by *Althernaria helianthi* infected leaves was higher than in healthy leaves (Table 2).

Phosphate content – The total phosphorus content in the infected leaves of sunflower was significantly higher than in healthy leaves. Conversely, the available phosphate content was found to be higher in healthy leaves than in the infected ones (Table 1).

Phenolic and soluble sugar content – The total and reducing sugar contents (80% aqueous ethanol soluble) in the infected leaves were found to be considerably higher than in healthy leaves. Whereas the total phenolic content was observed to be reduced drastically. The infected leaves were found to exude significantly higher amounts of phenolics and sugars than healthy leaves (Table 3).

Table 1. Total nitrogen, nitrate, ammonia and phosphorous in healthy and *Alternaria* infected leaves of sunflower

	Healthy	Infected
	(mg.per g. fresh weight)	
1. Total phosphorus*	0.126±0.010	0.218±0.008
2. Available phosphorus*	0.112±0.008	0.021±0.006
3. Total nitrate@	0.080±0.020	0.120±0.030
4. Total ammonia*	0.330±0.040	0.080±0.051
5. Total nitrogen*#	26.100±1.612	32.624±2.213

* Significant at 1% level (t=3.707)
@ Non significant
# mg. per g. dry weight

Table 2. Ammonia evolved/exuded by healthy and *Alternaria helianthi* infected sunflower leaves

	μg $NH_3.g^{-1}$ fresh weight/hr.	
	Collected in boric acid*	Exuded into water**
1. Healthy	0.022±0.006	0.062±0.041
2. Infected	0.033±0.004	0.906±0.142

* Significant at 1% level (t=3.707)
** Significant at 0.1% level (t=5.959)

Table 3. Phenolic* and sugar* content in healthy and *Alternaria* infected sunflower leaves

	Healthy	Infected
	(mg. per g. fresh weight)	
A. EXTRACT		
1. Sugar content		
(a) Total	2.190±0.080	2.790±0.110
(b) Reducing	2.040±0.020	2.620±0.040
2. Phenolic content	9.990±0.531	6.712±0.532
B. EXUDED INTO WATER		
1. Sugar content		
(a) Total	0.130±0.020	0.660±0.020
(b) Reducing	0.100±0.020	0.278±0.024
2. Phenolic content	0.044±0.015	0.210±0.020

* Significant at 0.1% level (t=5.959)

Chlorophyll content – Although there was a significant increase in the chlorophyll *b* content, a reduction in the chlorophyll *a* content due to *Alternaria helianthi* infection was observed. The reduction in the chlorophyll *a* to *b* ratio due to infection was highly significant (Table 4).

Nitrite and nitrate reductase activity – The nitrite reductase activity was higher in both healthy and infected leaves as compared with nitrate reductase (Table 5). Both nitrite and nitrate reductase activities were significantly higher in infected leaves than in healthy leaves.

Degradation of amino acids – The results in Table 6 show that all amino acids tested seem to serve as substrates for deamination *in vitro* in both healthy and infected leaves.

Table 4. Chlorophyll content of healthy and *Alternaria helianthi* infected sunflower leaves

	Healthy	Infected
I. Chlorophyll content (mg g^{-1} fresh weight)	0.81±0.04	0.80±0.02
a) Total*	0.81±0.04	0.80±0.02
b) Chlorophyll *a**	0.55±0.20	0.52±0.01
c) Chlorophyll *b***	0.60±0.01	0.69±0.03
II. Chlorophyll *a/b* ratio **	0.90±0.03	0.75±0.04

* Non-significant
** Significant at 1% level (t=3.707)

Table 5. Nitrite and nitrate reductase activity in healthy and *Alternaria helianthi* infected sunflower leaves

	μn moles of $NO_2.g^{-1}$ fresh weight	
	Healthy	Infected
1. Nitrite reductase*	4.47±0.38	5.43±0.48
2. Nitrate reductase**	1.21±0.31	2.78±0.42

* Significant at 5% level (t=2.447)
** Significant at 0.1% level (t=5.959)

Table 6. Effect of *Alternaria* infection on the rate of deamination of aminoacids* by sunflower leaves

	Healthy	Infected
	(μg. of ammonia per g. fresh weight)	
1. Aspartic acid	17.10±1.04	26.00±1.03
2. Glutamic acid	10.29±1.05	31.38±1.18
3. Tyrosine	7.66±1.02	12.20±0.61
4. Phenyl alanine@	13.18±0.87	11.16±1.02
5. Arginine	15.52±1.22	23.43±1.21

* Significant at 0.1% level (t=5.959)
@ Significant at 5% level.

The degradation of glutamic acid, aspartic acid, tyrosine and arginine was significant in *Alternaria* infected leaves. Whereas, the degradation of phenylalanine was found to be higher in healthy than in infected leaves of sunflower.

DISCUSSION

Nitrogen plays a vital role in plant growth and metabolism. The propitious effect of nitrogen on plant growth is often indeminified by the increased severity of pathogen infection. The powdery mildew infection in barley has been shown to result in a reduction in nitrate and total nitrogen content of all the tissues of infected plants than the controls (Goodman et al., 1986; Walters, 1985), whereas an increase in nitrate and total nitrogen content has been reported in the rusted leek leaves (Roberts and Walters, 1988). Further, in barley leaves infected with *Erysiphe graminis*, accumulation as well as evolution of ammonia in the early stages of development has been observed (Sadler and Scott, 1974; Goodman et al., 1986; Walters and Ayers, 1980). The present results, on sunflower leaves infected with *Alternaria helianthi*, a notable increase in the accumulation of ammonium ions followed by a significant increase in the exuded and evolved ammonia was observed (Tables 1 and 2). The observed increase in the ammonium ions in the leaves of infected plants may be attributed to (a) increase in nitrite reductase activity, (b) reduction in ammonia uttilization, and (c) breakdown of amino acids. The results also indicate that except for phenylalanine, the deamination rate of all other amino acids tested are highly significant in *Alternaria* infected sunflower leaves (Table 6). Such reduction in free amino acid content of leaves and roots of *Poa pratensis* infected with rust has been attributed to carbohydrate starvation (Hodges and Robinson, 1977).

In most of the host/pathogen systems reported previously, a decrease in net photosynthesis and increased respiratory rate have been observed. This has been related to (a) increase in stomatal resistance, (b) increase in mesophyll resistance due to loss of chlorophyll, and (c) reduced RuBPcase level or specific alterations in the contents of certain cytochromes (Goodman et al., 1986; Walters, 1985; Gordon and Donniway, 1982; Walters and Ayers, 1984). These changes have been correlated with the observed alterations in the concentrations of nitrate, ammonia, and inorganic phosphate of the infected plants. Because the changes in the nitrate/ammonia balance have been shown to affect RuBPcase activitity (Bassham et al., 1981; Walters and Ayers, 1980) and reduction in available Pi has been correlated with the observed reduction in the PePcase activity in the infected leaves (Walters and Ayers, 1984; Magyarosy et al., 1976; Waygod et al., 1974). Further, it is also shown that (a) reduction in Pi results in the decline of ATP/ADP levels, which in turn affect the activity of RuBPcase, and (b) low cytoplasmic Pi leads to reduction in PePcase activity, thus affecting the photosynthesis, and (c) regulates the translocation rate of triosphosphate for carbohydrate metabolism (Bassham et al., 1981; Herald and Walker, 1979). Further, the results in Table 3 indicate significant increase in the content of soluble sugar in the *Alternaria* infected leaves. This may possibly be an infection related process wherein the infected plants may have the need for redirecting more carbon skeletons to TCA cycle for balancing the energy requirements (Goodman et al., 1986). Although, it has been shown that a correlation may exist between the degree of resistance and the phenolic level in the healthy plants, the positive role of phenolics as resistance factors is not unanimous (Goodman et al., 1986). The observed reduction in the total phenolic content (Table 3) of the infected leaves of sunflower in the present study can be correlated with the observed reduction in the degradation phenylalanine which is one of the procesors in the phenolic biosynthesis (Goodman et al., 1986).

Most of the evidences for possible relationships between chlorophyll loss to fungal infection and subsequent reduction in photosynthesis are found to be contradictory. The net photosynthesis per mg of chlorophyll has been found to be higher in mildewed than in healthy leaves of wheat (Waygood et al., 1974). In barley, decline in the total chlorophyll before photosynthesis, with an increase in the chlorophyll a/b ratio upon powdery mildew infection has been observed (Goodman et al., 1986; Paulech and Haspelova-Harvatovicova, 1970). In sunflower, the total chlorophyll content remained almost the same, but the ratio of chlorophyll a/b ratio was reduced significantly upon infection (Table 4). In general, it is evident from the results of this study that *Alternaria helianthi* infection causes substantial alterations in the nitrogen metabolism, especially ammonium and aminoacid metabolism, and availability of inorganic phosphate, which probably regulates the carbon metabolism *in planta*.

REFERENCES

A.O.A.C., 1980: Official methods of analysis, Association of Official Agricultural Chemists, 13th Edition, Washington DC.

Bassham, J.A., P.O.Larsen, A.L. Lawyer, and Cornewell, K.L., 1981: Nitrogen and Carbon Metabolism (ed.J.D.Bewely) Martinus Nijhoff, the Hague, pp 135-163.

Bedbrook, J.R., and Metthews, R.E.F., 1973: Changes in the flow of early products of photosynthetic carbon fixation associated with the replication of TYMV. Virol, 53,84.

Deane-Drummond, C.E., Clarkson, D.T., and Johnson, C.B, 1979: Effect of shoot removal and malate on the activity of nitrate reductase assayed in vivo in barley leaves. Plant Physiol, 64.660-662.

Kumar, G.N.M., Mukunda, G.K., Prasad, D.T., Subbaiah, T.K., and Shashidhar, V.R., 1988: Biochemical study in relation to powdery mildew resistance in different cultivars of mango. Crop Res, 1, 163-168.

Goodman, R.N., Kirlay, Z., and Nood, K.R., 1986: Biochemistry and physiology of plant diseases, University of Missouri Press, Columbia.

Gordon,T.R., and Dunniway, J.M., 1982: Effect of powdery mildew infection on the efficiency of carbon dioxide fixation and light utilization by sugarbeets. Plant Physiol, 69.139-142.

Herald,A., and Walker, D.A., 1979: Hand Book on transport across membranes (eds.Geibrisch, G., Tosteson, D.C., and Using, H.H.) Springer-Verlag, Berlin, pp 411-439.

Hodges,C.F., and Robinson, P.W., 1977: Sugar and aminoacid content of *Poa pratensis* infected with *Ustilago striiformis* and *Urocystis agropyri*. Physiol. Plantarum. 41,25-28.

Magyarosy, A.C., Schurman, P., and Buchanan, B.B., 1976: Effect of powdery mildew infection on photosynthesis of leaves and chloroplasts of sugarbeets. Plant Physiol, 57.486-489.

Paulech. C., and Haspelova-Horvatovicova, A., 1970: Photosynthesis, plant pigments and transpiration in healthy barley and barley infected with powdery mildew. Biologia (Bratislava), 25.477-484.

Pozsar, B.L., and Kirlay, Z., 1966: Phloem transport in rust infected plants and cytokinin directed long distance movements of nutrients. Z. Phytopathol. 56.297-309.

Roberts, A.M., and Walters, D.R., 1988: Nitrogen asimilation and metabolism in rusted leek leaves. Physiolo. MOL. Plant Pathol. 32,329-339.

Sadler, R., and Scott, K.J., 1974: Nitrogen assimilation and metabolism in barley leaves infected with powdery mildew fungus. Physiol. Plant Pathol. 4,235-247.

Walters, D.R., 1985: Shoots:roots relationships: The effects of obligately bitrophic fungal pathogens. Biol. Rev. 60.47-79.

Walters, D.R., and Ayres, P.G., 1980: Effects of powdery mildew disease on uptake and metabolism of nitrogen by roots of infected barley. Physiol. Plant Pathol. 17.369-379.

Walters, D.R., and Ayers, P.G., 1984: Ribulose biphosphate carboxylase and enzymes carbon dioxide assimilation in a compatible barley/powdery mildew combination. Z. Phytopathol, 109.208-218.

Walters, D.R., Wilson, P.W.F., and Shutteeton, M.A., 1985: Relative changes in the levels of polyamines and activities of their biosynthetic enzymes infected with powdery mildew fungus, *Erysiphe graminis*.D.C. ex Merat f.sp*hordei* Marchal. New Phytol, 101.695-705.

Waygood, E.R., Pao, L.Y., and Godavari, H.R., 1974: Stimulation of phosphoenol pyruvate carboxylase activity in rust infected wheat leaves. Experientia, 15.986-988.

METABOLISMO DE NUTRIENTE EN HOJAS DE GIRASOL INFECTADAS CON *Alternaria helianthi*

RESUMEN

La asimilación de nutrientes y metabolismo en hojas de girasol fueron examinadas en ambas plantas sanas e infectadas con *Alternaria helianthi*. Un incremento de nitrogeno total y acumulación de amomio fue observada en las hojas infectadas por *Alternaria*. La acumulación del nitrato despues de la infección no se encontró significativa. Un incremento significativo en el fosforo total fue observado en hojas infectadas mientras el contenido disponible de fosforo fue reducido drasticamente. La deanimación del ácido aspástico, ácido glutámico, tirosina y arginina *in vivo* fue encontrada significativamente más alta en las hojas infectadas que en las sanas. Las actividades de nitrato reductasa se encontraron más altas en las hojas infectadas. Mientras la relacción clorofila *a*/*b* fue más alta en las sanas.El contenido de azucar soluble fue más alto en las hojas infectades que en las hojas sanas de girasol. Las posibles explicaciones para estas alteraciones on el metabolismo de nutrientes de las hojas de girasol infectadas con *Alternaria* ha sido discutida.

MÉTABOLISMES DES FEUILLES DE TOURNESOL INFECTÉES PAR *Alternaria helianthi*

RÉSUMÉ

Nous avons comparé l'assimilation des nutriments et le métabolismes de feuilles de tournesol saines et de deuilles infectées par *Alternaria helianthi*. Une augmentation de l'azote total et une accumulation d'ammonium ont été observées an niveau des feuilles infectées. L'accumulation de nitrate aprés infection n'atteint pas un niveau significatif. Par contre une augmentation significative du phosphore total a été notée dans les tissus infectés alors que la teneur en phosphore disponible avait considérablement diminuée. Les dosages des acides aspartique et glutamique, de la tyrosine et de l'arginine *in vivo* se sont révélés significativement supérieurs dans les feuilles contaminées. L'activité nitrite et nitrase réductase était plus élévée dans les feuilles infectées. Le taux de chlorophylle *a* / chlorophylle *b* était supérieur dans les feuilles saines. La teneur en sucres solubles augmente dans les organes infectés par rapport aux organes sains. Des interprétations de l'altération du métabolisme des fuilles de tournesol infectées par *Alternaria* sont discutées.