

**ASSIMILATES AND NUTRIENTS PARTITIONING
AND EFFICIENCY OF NITROGEN FIXATION
OF BLACK LOCUST (*ROBINIA PSEUDOACACIA* L.)
GROWN ON THE EXCESS OF Cu IN SOIL**

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Summary. The effect was studied of Cu excess (200 and 400 ppm Cu) in soil on the growth, Cu accumulation, nitrogen fixation, nutrients and assimilates partitioning of nodulated black locust plants (*Robinia pseudoacacia* L.). The protective role of extracellular polysaccharide slime (EPS) produced by respective *Rhizobium* sp. (*Robinia*) strain was also studied in conditions of laboratory cultivation. The main quantity of accumulated Cu ions in the symbiotic plants was found in the roots < 1 mm and nodules. This led to strong imbalance of nitrogen exchange between the stressed roots and nodules. The higher entrance of Cu ions in the roots < 1 mm converted them in strong sink for C and N assimilates. This was connected with the change of patterns of total amino acids composition of roots and nodules. The cells structure of nodules was effected by Cu stress as well. All these alterations could result in the change of oxygen diffusion control of the stressed nodules and would have as consequence the decrease of efficiency of black locust nitrogen fixation.

Key words: amino acids, black locust (*Robinia pseudoacacia* L.), cell ultrastructure, copper stress, nitrogen fixation, soluble sugars

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Abbreviations: AA – amino acids, roots < 1 mm – roots with diameter less than 1 mm, EPS – extracellular polysaccharide slime, C and N – carbon and nitrogen, NR – nitrate reductase activity, NG – nitrogenase activity, ARA – acetylene reduction assay, PBU – perybacteroid unit, B – bacteroids, PBM – perybacteroid membrane, Pn – net leaf photosynthetic rate, Chl *a/b*, *a+b* – ratio of leaf chlorophyll content *a/b* and sum of leaf chlorophyll *a+b*, POA – nodule peroxidase activity, DW – plant dry matter accumulation

Introduction

A large quantity of data collected show that the process of nitrogen fixation is very sensitive to the changes of environment (Bergersen, 1984; Shows et al., 1985; Atkins, 1986). The main reason is the dependence of symbiotic relationships from the processes of assimilate partitioning in the plant (Streeter, 1993). Nevertheless, the investigations on the mechanism of stress tolerance of symbiotic nitrogen fixing legume plants are quite a few. Most of data are collected from annual legume crops (Andrew and Johnson, 1976; Lin and Lin-Lin, 1990). There is a little information about the stress tolerance of perennial symbiotic vegetation although its importance for the forest science (Foy et al., 1978). Black locust is perennial woody plant from Leguminosae family which is capable to fix atmospheric N₂ in its root nodules formed with the representatives of soil bacteria *Rhizobium* sp. (Reinsvold and Pope, 1987). The existing data show low dependence of black locust growth from the level of soil fertility and moisture supply (Keresteshi, 1990). However most of the investigations are concentrated mainly on the behaviour of plant macrosymbiont. Data about the growth of free-living bacteria *Rhizobium* sp. under stress conditions are rather scare. Despite the well documented negative effects which contaminated with heavy metals soils exert on plant biochemistry and physiology, almost no data about the effect of heavy metals on the root nodules functions of black locust plants exist (Reinsvold and Pope, 1987). A considerable ability of black locust trees to grow on polluted with heavy metals soil is observed as well (Foy et al., 1978; Georgiev and Kalmuckov, 1996).

The aim of this study was to investigate the effects of Cu ions excess in soil on the growth of micro- and macrosymbiont separately and symbiotically, on the tissue Cu accumulation, on the plant nitrogen fixing and nitrate assimilating abilities, on the nodulation and leaf CO₂ exchange rates and connected with them processes of assimilates and nutrients partitioning.

Materials and Methods

Plant material

The experiments were performed as soil pot culture in green house conditions. The planting medium was 1:1:1 mixture by volume of soil, sand and vermiculite. The soil was alluvial meadow. Pots contained 12 kg of planting medium and were watered daily up to the level of 70% of full moisture capacity of the mixture. In every pot was planted one root cutting of clone 6 (Svishtov) of black locust (*Robinia pseudo-acacia* L.). The plants were inoculated with the bacterial suspension (10^8 cells/ml) of an effective symbiotically isolate (DC-25-1) of *Rhizobium* sp. (Robinia) (Brown and Dilworth, 1975; Georgiev et al., 1996). Part of the pots were supplied with different quantities of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ to obtain the final concentration of Cu in pots – 200 and 400 ppm Cu.

Microbiological studies

The growth of free-living form of *Rhizobium* sp. strain DC 25-1, treated with different Cu concentrations in the culture medium (YEM broth) was studied (Brown and Dilworth, 1975). Extracellular polysaccharide slime and bacterial cells were analysed for Cu accumulation by atomic absorption spectrometry (Mort and Bauer, 1980; Georgiev et al., 1996).

Plant analysis

Plant material were harvested 30, 60 and 90 days after the planting. Dried and milled material was analysed for total nitrogen (N) by Keldahl digestion, for P_2O_5 – spectrometrically, for K_2O – by flame photometry, and for Ca and Mg – by atomic absorption spectrometry (Tchernavina, 1978). The ureides content (allantoin and allantonic acid) was analysed colorimetrically (Trijbels and Vogels, 1966). Soluble sugars were assayed after recovering 80% ethanol plant extracts colorimetrically (Turkina and Sokolova, 1971). After the acid hydrolysis (6N HCL, 120°C, 12 h) and recovering, total amino acids composition of nodules and roots < 1mm was analysed on an automatic aminoanalyzer T 339 (Check Republik) (Rjiadchikov, 1978). The leaf photosynthetic rate of black locust plants was studied by the IR-gas analyzer “Infralit-4” (Germany) supplied with leaf chamber (Gulyaev, 1983). The chlorophyll *a* and *b* of leaves were studied colorimetrically as well (Turkina, 1971).

Enzyme assay

Nitrate reductase activity (EC.1.7.13.1) was assayed by the method *in vivo* (Javorski, 1971). Peroxidase activity (EC.1.11.1.7) of plant extracts was analysed by the guacole-

hydrogen peroxide method (Pupo and Dimitrievic, 1982; Zhiznevskaya et al., 1992). Nitrogenase activity (EC.1.18.2.1) of fresh detached nodules was determined as acetylene reduction assay (Hardy et al., 1968).

Electron-microscopy and light microscopy studies

Samples of root nodules were fixed with 4% glutaraldehyde in 0.1 M phosphate buffer pH 7.4 (4 h, 20°C), rinsed and postfixed with 1.5% OsO₄. After dehydration procedure the specimens were embedded in Durcupan resin, sectioned on ultramicrotome LKB 7800, stained with uranyl acetate and lead citrate and were examined in a Hitachi M2 (60 kV) TEM as described O'Brien and McCully (1981). Part of these resin specimens were prepared as semithin sections (1 µm) and were stained with methylene blue-azur 2-basic fuccin as described Humphrey and Pittman (1974) with the modifications of Petkov and Dobrolubov (1988). Double stained specimens allowed to determine the metabolic changes of infected cells (Lilly, 1969). Data were processed statistically (Dospheov, 1985).

Results and Discussion

Black locust saplings showed considerable growth rate in soil containing excess of Cu ions (Georgiev and Kalmuckov, 1996). The dry matter accumulation of plants was not negatively effected by the stress of 400 ppm Cu in soil, although such kind of stress increased shoot/root ratio (Table 1). The changes of patterns of biomass partitioning under stress were strongly connected with the alterations in plant nitrogen metabolism and photosynthesis (Table 1, 2). Cu treated plants had higher CO₂ exchange rate from the beginning of the growth period. This photosynthetic activity of leaves was connected with higher chlorophyll *a* and *b* content (Table 2).

The increased photosynthetic activity of Cu treated plants was related to the higher nitrate assimilation rate in leaves (Table 2). This growth tolerance of Cu stressed plants was connected with the ability of black locust to nodulate and to fix atmospheric nitrogen. One of the reasons for the unchanged nodulation rate of plants could be related to the ability of free-living bacteria *Rhizobium* sp. (*Robinia*) to grow successfully in culture medium containing high concentrations of Cu ions (Table 3). Cu ions in the medium up to the minimal inhibitory concentration increased extracellular polysaccharide slime production (EPS) and played a protective role against the spreading Cu ions into the *Rhizobium* cells (Mort and Bauer, 1980; Georgiev et al., 1996).

Despite the considerable Cu stress growth tolerance expressed by the symbiotic organisms their nitrogen metabolism was deteriorated (Table 1, 4). The total nitrogen of plants was decreased following the uptake of Cu ions by the plant organs. Plant roots, stem and nodules N contents were negatively effected by the stress, but the nitrogen content of stressed leaves was not altered. These changes undoubtedly were

Table 1. Dry matter, total N and Cu accumulations of nitrogen fixing black locust plants (*Robinia pseudoacacia* L.) grown on the excess of Cu in soil

Treatments	Plant organ	Dry matter (g/plant)	Total N		Cu content	
			%	% of control	mg/100 g DW	% of control
Control	nodule	1.68 ± 0.3	3.58	100	4.58	100
	root < 1 mm	1.86 ± 0.2	2.22	100	14.90	100
	root > 1 mm	6.98 ± 0.5	1.50	100	3.05	100
	stem	13.97 ± 1.0	1.75	100	3.95	100
	leaf	12.94 ± 0.4	2.17	100	4.70	100
	leaf veins	1.20 ± 0.1	1.10	100	5.00	100
	S/R ratio	2.67				
	total	38.65 ± 2.5				
200 ppm Cu	nodule	1.81 ± 0.3	3.20	89.3	6.00	123.7
	root < 1 mm	1.96 ± 0.1	1.57	70.7	45.30	304.0
	root > 1 mm	6.80 ± 0.3	1.07	71.3	8.27	226.8
	stem	15.50 ± 1.2	0.90	51.4	4.10	103.7
	leaf	14.58 ± 0.6	1.67	76.9	3.75	79.7
	leaf veins	2.10 ± 0.2	0.62	56.3	5.20	104.0
	S/R ratio	3.44				
	total	42.45 ± 3.1				
400 ppm Cu	nodule	2.30 ± 0.1	2.37	66.2	9.55	196.9
	root < 1 mm	2.74 ± 0.2	1.47	66.2	20.20	135.5
	root > 1 mm	9.90 ± 0.3	1.10	73.3	6.80	222.9
	stem	20.35 ± 2.2	0.67	38.2	5.60	141.7
	leaf	14.90 ± 0.4	2.45	112.9	5.10	108.5
	leaf veins	2.80 ± 0.2	1.15	104.5	20.40	408.0
	S/R ratio	2.54				
	total	53.32 ± 2.6*				

* Data are means of 4 replicates ± SE; S/R – shoot to root ratio of dry mass of plants

related to the patterns of dry matter accumulation between roots and shoot. The total N of black locust was a function of assimilating of soil nitrogen and fixing of atmospheric nitrogen. The Cu ions excess in soil exerted more negative effect on the nitrogen fixation than on the processes of nitrate assimilation.

These results correlated with the uptake, transport and accumulation of adverse Cu ions in plants. The main quantities of Cu under 400 ppm Cu stress was found to be in the roots < 1 mm and nodules after 90 days of treatment (Table 1). Surprisingly, the leaves of Cu treated plants did not accumulate the excess of Cu ions in their tissues. This result positively correlated with higher photosynthetic and nitrate assimilatory rates of leaves under stress. Despite the fact that nodules dry matter accumulation was not effected by the stress conditions their nitrogen metabolism and functional activity were heavily altered (Table 2). Obviously, the contradiction between

Table 2. Photosynthetic activity, nitrate assimilation and nitrogen fixation of nodulated black locust plants (*Robinia pseudoacacia* L.) grown on the excess of Cu in soil

Treatments	Days of treatments with Cu excess												
	30 days				60 days				90 days				
	Pn	Chll <i>a+b</i> (<i>a/b</i>)	NRa	POA	Pn	Chll <i>a+b</i> (<i>a/b</i>)	NRa	POA	Pn	Chll <i>a+b</i> (<i>a/b</i>)	NRa	POA	ARA, $\mu\text{mol/g FW/h}$
Control													
leaves	9.21±1.2	10.5 (2.2)	1.19±0.03	0.56±0.04	3.47±0.3	4.9 (2.5)	1.39±0.01	1.33±0.06	6.45±0.7	6.7 (2.5)	0.37±0.02	0.51±0.1	–
nodules	–	–	2.20±0.3	38.20±2.3	–	–	–	42.12±4.5	–	–	0.96±0.02	61.68±3.7	0.491±0.052
200ppm Cu													
leaves	15.37±1.6	7.2 (2.4)	1.29±0.05	–	13.00±0.8	4.9 (3.4)	0.46±0.01	–	7.32±0.8	8.5 (2.8)	0.47±0.03	0.65±0.02	–
nodules	–	–	1.79±0.04	–	–	–	–	–	–	–	0.59±0.02	69.30±5.7	0.216±0.034
400ppm Cu													
leaves	18.40±2.0	6.9 (2.6)	1.59±0.06	3.34±0.02	19.30±0.04	5.1 (3.9)	0.77±0.05	1.88±0.07	7.14±0.04	9.9 (3.1)	0.48±0.02	0.62±0.01	–
nodules	–	–	2.10±0.03	44.60±3.1	–	–	–	56.30±4.8	–	–	1.43±0.05	75.00±5.4	0.119±0.031

Pn – diurnal leaf photosynthetic rate, $\text{mgCO}_2/\text{gFW/h}$, Chll – leaf chlorophyll *a* and *b* content (mg/g DW); sum – (*a+b*) and ratio – *a/b*, NRa – nitrate reductase activity ($\text{mg NO}_2\text{-N/gFW/h}$), POA – peroxidase activity ($\text{OD}_{490}/\text{gFW/h}$), ARA – acetylene reduction rate of nodules ($\text{mmol C}_2\text{H}_4/\text{gFW/h}$), data are means of 3–5 replicates±SE

Table 3. Growth and copper accumulation of *Rhizobium* sp. DC 25-1 cells and EPS under excess of Cu in the medium

Concentration of Cu in medium ($\mu\text{mol/l}$)	Growth of <i>Rhizobium</i> sp. (cells/ml $\times 10^7$)	Cu content of <i>Rhizobium</i> sp. cells (mg/g DW)	Cu content of extracellular polysaccharide slime (EPS) (mg/g DW)	Ratio of EPS/cells (g/g)
5 (control)	180.3	0.05	0.158	1.95
50	185.4	1.127	0.577	0.53
100	180.3	1.640	1.541	2.95
250	91.4	4.512	1.810	1.70
500	4.4	6.750	1.750	–

not changed leaves metabolism and sharply decreased one of roots and nodules concerned the sink-source relationships between shoot and root under Cu stress conditions. Analysis of data showed that the pool of carbon entering the root and nodules was not so changed. At the same time export of nitrogen compounds mainly from the nodules was significantly decreased. This could be result of some shift from symbiotic to parasitic relationships in stressed nodules. All this imbalanced carbon (C) and nitrogen (N) nutrition of underground plant parts under Cu stress conditions. Stressed roots < 1 mm possessed more competitive sink for shoot C and N assimilates in comparison with the stressed nodules. This fact was directly related to the nodule function disorders. The state of C and N deficiency of stressed nodules was connected with altered patterns of major assimilates partitioning. Despite the fact that the roots < 1mm were more stronger acceptor of harmful Cu ions in their tissues, their amino acids (AA) composition was less influenced than that of the nodules (Fig.1). Under the effect of 400 ppm Cu in soil roots < 1 mm contained 70% of total amino acid pool of the control. At the same time stressed nodules decreased drastically their total amino acid content under 400 ppm Cu in soil (30% of control). Consequently,

Table 4. Nitrogen compounds and soluble sugars of nitrogen fixing black locust plants (*Robinia pseudoacacia* L.) grown on the excess of Cu in soil

Treatments	Plant organs	Total N content ($\mu\text{mol N/g FW}$)	Content of ureides ($\mu\text{mol allantoin/g FW}$)	Content of amino acids ($\mu\text{mol/g FW}$)	Content of soluble sugars ($\mu\text{mol sucrose/g FW}$)
Control	nodules	1278.4	0.730	14.20	26.34
	root < 1mm	792.6	0.467	9.06	11.60
	leaves	775.0	2.312	15.30	29.80
400 ppm Cu	nodules	846.0	0.571	4.38	15.40
	roots < 1mm	525.3	0.628	6.79	17.50
	leaves	876.4	1.241	13.60	32.70

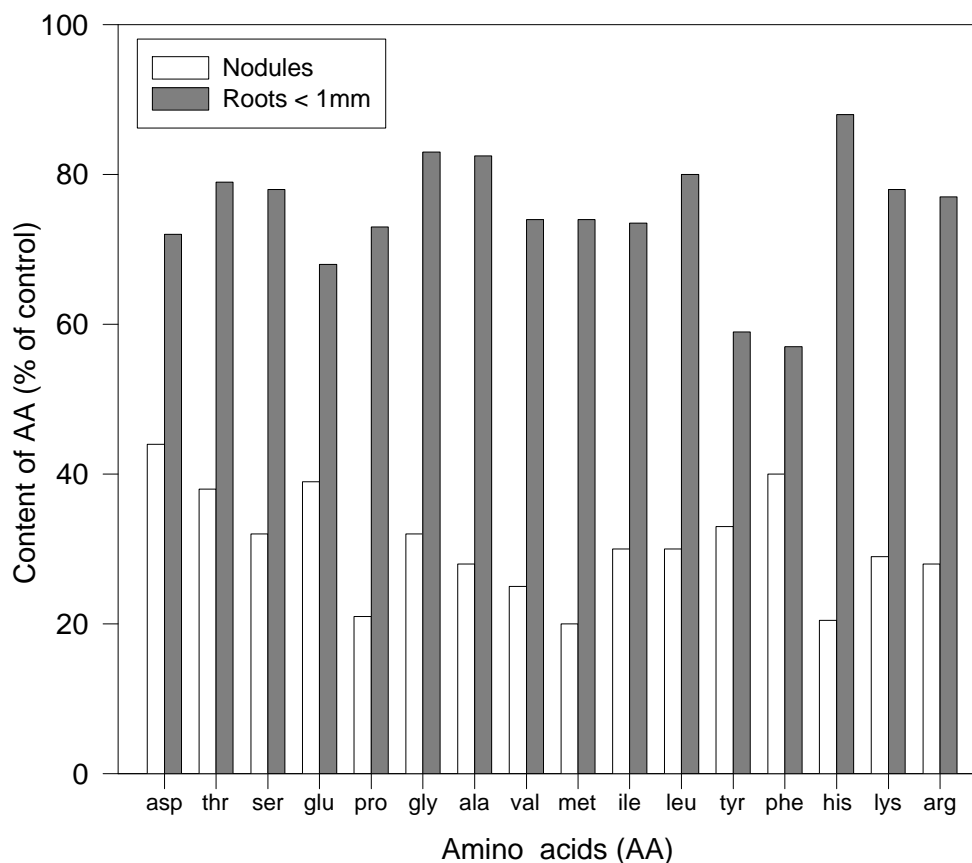


Fig. 1. Total amino acids content of nodules and roots <1 mm of black locust plants (*Robinia pseudoacacia* L.) after 90 days of treatment with 400 ppm Cu in soil

the contents of ureides and soluble sugars in the stressed roots <1 mm were increased (Table 4). This could be accepted as an evidence for the role of the roots <1 mm as a major place for Cu detoxification in plants (Foy et al., 1978). The altered amino acid composition of underground plant parts showed some changes in the ratio of individual amino acids in the total AA pool. Cu stressed nodules contained more aspartate, glutamate and phenylalanine. At the same time roots <1 mm under 400 ppm Cu showed some increase of the relative values of histidine, glycine and alanine. The reason for these changes could be the disturbed C/N balance of nodules and roots of stressed plants. A considerable flow of C belonging to the AA pool of control nodules was found to come from the aspartate, lysine, glutamate, leucine, phenylalanine and valine (Table 5). The contribution of this C in the nodules was accounted for 51.3% of the total C of amino acids. Under 400 ppm Cu stress the quantity of C delivered by amino acids in the nodules was 30% of that of control plants. The main

Table 5. Budget of C and N of amino acids composition of nodules and roots of black locust plants subjected to 90 days 400 ppm Cu stress

Composition of amino acids	Gain of C and N in plant organs	Nodules (mg/gDW/plant)		Roots< 1mm (mg/gDW/plant)	
		control	Cu stress	control	Cu stress
Asp	C	54.1	23.4	47.3	33.7
	N	15.7	6.8	13.8	9.8
Threo	C	29.3	10.3	21.5	16.8
	N	8.5	3.1	6.2	4.9
Ser	C	27.6	9.0	24.1	18.4
	N	10.7	3.5	9.3	7.1
Glu	C	83.4	31.8	60.8	40.5
	N	19.4	7.4	14.1	9.4
Pro	C	49.5	10.4	28.2	20.3
	N	11.5	2.4	6.5	4.7
Gly	C	31.2	10.0	19.2	16.1
	N	18.2	5.8	11.2	9.4
Ala	C	54.7	15.6	23.9	20.0
	N	21.2	6.0	9.3	7.7
Val	C	74.1	18.2	40.8	30.2
	N	17.2	4.2	9.5	7.0
Met	C	20.1	4.1	5.7	4.2
	N	4.7	0.9	1.3	0.9
Ileu	C	48.0	14.2	33.7	24.7
	N	9.3	2.7	6.5	4.8
Leu	C	74.7	22.0	49.1	39.5
	N	14.5	4.3	9.5	7.7
Tyr	C	32.6	10.9	27.9	16.4
	N	4.2	1.4	3.6	2.1
Phen	C	54.0	21.6	44.7	25.4
	N	7.0	2.8	5.8	3.2
His	C	40.9	8.4	22.4	19.9
	N	23.8	4.9	13.1	11.6
Lys	C	74.0	21.4	44.8	34.6
	N	28.8	8.3	17.4	13.4
Arg	C	68.9	19.2	33.9	26.0
	N	53.6	14.9	26.3	20.2
Total	C	835.8	250.9	527.8	386.7
	N	268.3	79.4	163.4	123.9

part of this C was coming from the compounds of aspartate, leucine and lysine. Its value was accounted for 39.2% of the total amino acid C pool of stressed nodules. At the same time, the C delivered by the amino acids in the roots < 1 mm was calculated as 63.1% of the total C amino acids pool. The N was, consequently, calculated as 29.5% of that quantity found in the control nodules. When the Cu stress was applied, the flow of amino acids C entering the roots < 1 mm was increased up to 73.2% compared with nodules. And the N content of AA of roots < 1 mm was increased up to 156% of that quantity contained in the stressed nodules. All these calculations showed that under Cu stress conditions nodules experiencing strong nitrogen deficiency produced more amino acids which possessed C/N molecular ratio ranging from 3.4 to 7.7. Correspondingly, the stressed roots < 1 mm which under stress were supplied with more carbon assimilates produced relatively higher amount of amino acids with C/N ratio equal to 1.7–2.8.

The changed C and N balance of stressed nodules related to the decrease of nitrogen fixing efficiency of nodules (Georgiev et al., 1996). The synthesis of compounds with higher C/N ratio could be result of the altered metabolism not only of the plant cytozol but the bacteroids in the infected cells of nodules as well (Streeter, 1993). This result could be connected with the ability of free-living forms of *Rhizobium* sp. to produce in the carbon rich media increased quantities of EPS (Georgiev et al., 1996). The increase of large quantity of wall of EPS surrounding the bacteroids in the peribacteroids unit (PBU) could result in a decrease of the processes of assimilates and nutrients exchange between plant cytozol and bacteroids, which would have as a consequence the cease of metabolite activity of PBU (Bergersen, 1984). This would lead to the bacteroids degradation and decreasing of nitrogen fixing efficiency (Atkins, 1986; Streeter, 1991). On the Fig. 2 and 3 could be seen that under 400 ppm Cu stress bacteroids degradation and PBU wall destroying took place.

The changed C and N metabolism of nodules under stress was connected with nutrients distribution alterations as well (Table 6). The increase of Cu in the nodules and roots < 1 mm led to decrease of the content of Ca, K and P, but Mg concentration increased. The leaves of stressed plants showed considerably higher content of N and K. The last alterations in leaves could be connected with their improved photosynthetic and nitrate assimilatory activities (Bershtain and Ockanencko, 1979; Stoyanov et al., 1980).

All these alterations, found in the stressed nodules, can relate to the changes of O₂ diffusion control in the nodules as well (Minchin et al., 1985; Minchin et al., 1994). There are two mechanisms of oxygen diffusion control described recently (Minchin et al., 1994). The one is connected with the function and structure of leghaemoglobine (Lb) in the nodules and the other is related to so called “variable oxygen diffusion barrier” in the nodules (Minchin et al., 1994). The importance of the existing mechanisms of control of oxygen in the nodules is determined by requirements of nitrogenase function for low pO₂ (Streeter, 1993). The increased entrance of Cu ions in the

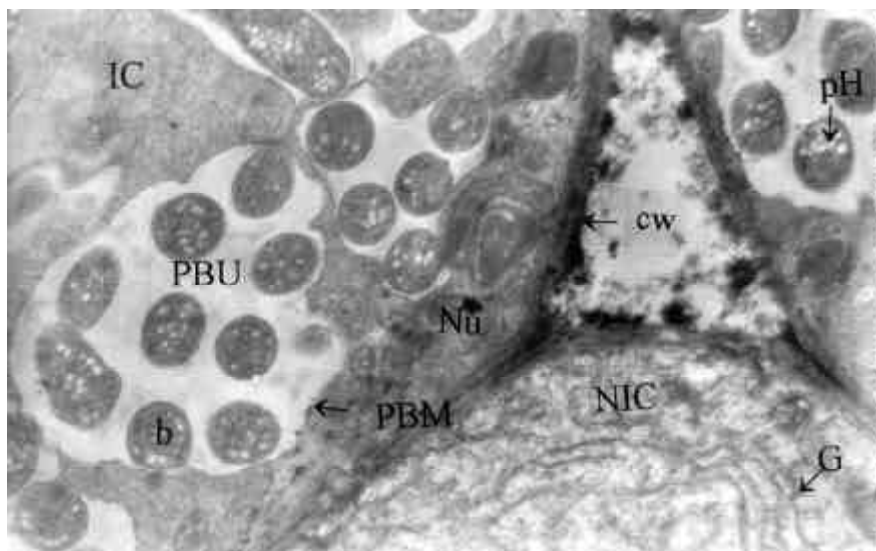


Fig. 2. TEMicroscopy photograph of the infected cells from the nodules of control black locust plants ($\times 12000$). Intact PBM (peribacteroids membranes) surrounding a number bacteroids found in the infected region of nodule. PBU – peribacteroid unit, b – bacteroid, Nu – nucleus, cw – cell wall, pH – poly- β -xydroxybutyrate granules, G – golgi bodies, IC – infected with bacteroids cells, NIC – non-infected cells.

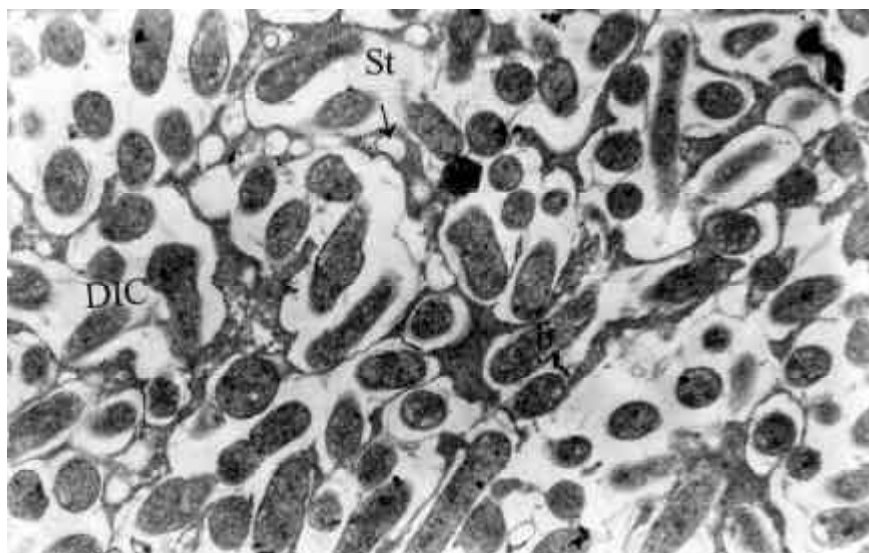


Fig. 3. TEMicroscopy photograph of the infected cells from 400 ppm Cu stressed nodules ($\times 7500$). Bacteroids are in the process of degradation, PBM wall is ruptured and bacteroids amalgamate. DIC – degradating infected cells, b – bacteroids, St – starch granule depositions around PBU.

Table 6. Mineral nutrients of nitrogen fixing black locust plants (*Robinia pseudoacacia* L.) grown on the excess of Cu in soil

Treatments	Content (in %) of						Dry matter (mg/plant)
	Ca	Mg	N	P ₂ O ₅	K ₂ O	Cu	
Control							
nodules	1.74	0.58	3.58	0.50	2.55	4.85	1.68
roots<1mm	2.16	0.65	1.93	0.32	1.26	14.90	1.86
roots>1mm	1.87	0.21	1.74	0.22	1.12	3.05	6.98
leaves	3.26	0.40	2.37	0.25	1.22	4.70	12.94
400ppm Cu							
nodules	1.56	1.00	2.34	0.21	1.22	9.55	2.30
roots<1mm	1.47	0.78	1.30	0.20	1.72	20.20	2.74
roots>1mm	0.87	0.20	1.35	0.37	1.50	6.80	9.90
leaves	1.87	0.27	2.43	0.15	1.12	5.10	14.90

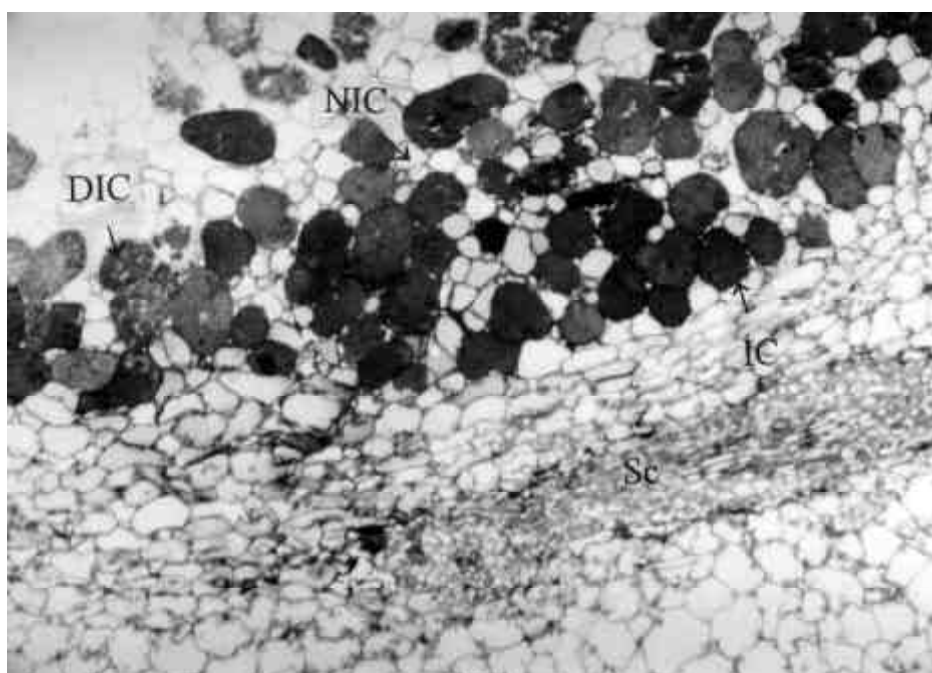


Fig. 4. Light microscopy photographs of semithin section of nodule from black locust (control). Double staining allow to discover the infected cells (IC) in physiological state from the infected cells in the process of degradation found in the black locust nodules ($\times 100$). NIC – non-infected cells, surrounding infected with bacteroids cells, DIC – infected cells in the process of decay, Sc – schlerenhim cell layer

tissues of nodules under stress is accompanied by strong changes of their ions distribution, C and N amino acid metabolism disturbances which would have as a consequence deterioration of oxygen diffusion control mechanism. The changed nutrients concentration of Ca, Mg, K and P of nodules could influence the control of “variable oxygen diffusion barrier” as well (Minchin et al., 1994). The tissue structure of nodules under Cu stress was found to be changed as well (Fig. 4 and 5). The decrease of ratio between infected and uninfected cells in the nodule central zone could be seen. This could be result of altered assimilation and ion supplies of nodules under Cu stress (Baird and Webster, 1982; Streeter, 1993). The changes of nodule tissue structure under long term influence of the increased Cu concentration could affect the quantities and properties of specific glucoproteins which fill the intercellular spaces of nodule cortex as well (Iannetta et al., 1993). It is believed that these compounds are primary responsible for the regulation of the “variable oxygen diffusion barrier” in nodules. All this alterations could contribute for the disturbance of oxygen diffusion pathways through the nodule tissue to the bacteroids zone and could have as a consequence fall of the efficiency of nitrogen fixation of black locust plants under Cu stress.

Cu ions entering the nodules under Cu stress conditions could have an adverse effect on the function of Lb in the nodules as well (Puppo and Dimitrievic, 1982).

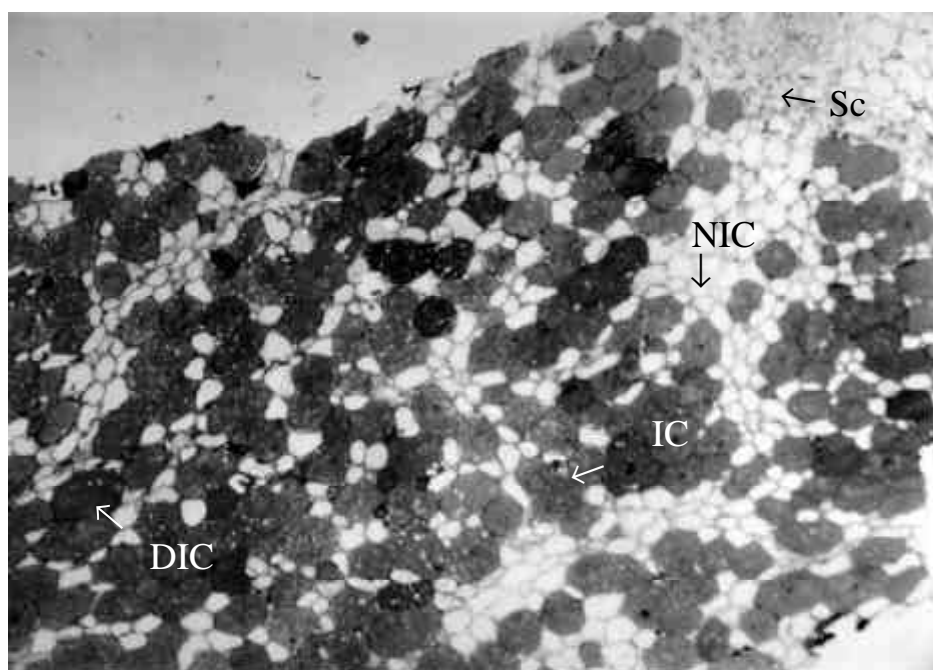


Fig. 5. Light microscopy photographs of semithin section of nodule from 90 days stressed with 400 ppm Cu black locust plants ($\times 100$). See the abbreviations on the Fig. 4.

Under unfavourable conditions of growth, Lb Fe^{2+} could be oxygenated to the state of LbFe^{4+} , which is not active to attract the oxygen. This action is accompanied by the production of active oxygen species and H_2O_2 , which leads to the activating POA function in the nodules (Table 2) (Zhiznevskya et al., 1992).

Conclusions

Under high Cu concentration in the soil, the carbon and nitrogen nutrition of nitrogen fixing black locust plants are changed substantially. The nitrogen exchange of roots and nodules is strongly imbalanced under stress conditions. It is mainly due to the accumulation of adverse concentration of Cu ions in the underground part of the plant. The major place for Cu accumulation are roots < 1 mm. This makes them strong sink for assimilates and ions and leads to imbalance of C and N nutrition of stressed nodules. The less influenced C nutrition of stressed nodules allow the plant to keep the nodulation rate non-changed. Significant protective role for that play the formed EPS around the bacteroids. This leads to the decrease of nitrogen fixing efficiency mainly because of disturbed assimilates and nutrients exchange and altered control on oxygen diffusion through the nodules.

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