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IV° SEMINAIRE SUR L'ENERGIE SOLAIRE

(10 - 21 septembre 1984)

L'ENERGIE SOLAIRE ET L'ASSIMILATION DE L'AZOTE PAR LES VEGETAUX

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(150x → A. MOYSE) ①

Energie Solaire et l'assimilation de l'azote par les végétaux

A. MOYSE

NO_3^-

$NO_3^- + 2\ NAD(P)H_2 \xrightarrow{(2e^-)} NO_2^- + NAD + H_2O$

nitrate réductase

$NO_2^- + 6$ Ferrédoxine réduite $+ 6H^+ \xrightarrow{(6e^-)} NH_4^+ + 6$ Fd oxydée $+ 2H_2O$

nitrite réductase

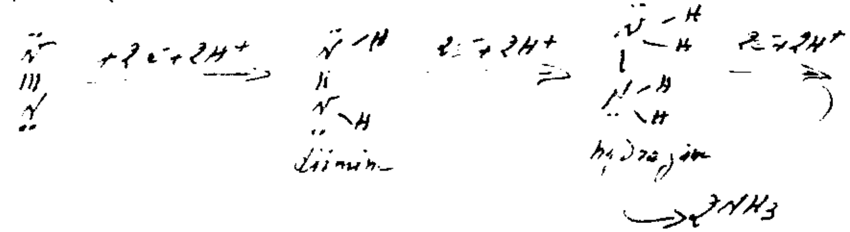
glutamate $+ NH_4^+ \xrightarrow{+ATP}$ glutamine $+ ADP + Pi$

glutamine synthétase

glutamine + α-cétoglutarate $\xrightarrow{+2Fd\ réduite}$ 2 glutamate

glutamate synthase (GOGAT).

N_2 Bactéries libres ou symbiotiques



Nitrogénase

Inorganic Plant Nutrition. Encyclopedia of Plant Physiology New Series. Vol 15A. A. Läuchli & R.L. Bieleski ed. Springer Verlag 1983

Table 1. List of selected genera of nitrogen-fixing organisms

	Genus or type	Species
A. Free-living nitrogen-fixers		
1 Obligate aerobes		
	Azotobacter	*vinelandii, chroococcum, paspali, beijerinckii*
	Beijerinckia	*indica*
	Derxia	*gummosa*
	Azotococcus	*agilis*
2 Obligate aerobes that fix nitrogen only at low oxygen tensions		
	Azospirillum	*brasilense, lipoferum*
	Xanthobacter	*autotrophicus, flavus*
	Thiobacillus	*ferro-oxidans*
	Rhizobium	cowpea group
	Methylosinus	*sporium*
	Methylococcus	*capsulatus*
3. Facultative anaerobic bacteria that fix nitrogen only under anaerobic conditions		
	Klebsiella	*pneumoniae*
	Bacillus	*polymyxa, macerans*
	Propionibacterium	*shermanii, petersonii*
	Escherichia	*intermedia*
	Citrobacter	*freundii*
	Enterobacter	*cloacae, agglomerans*
	Erwinia	*herbicola*

Inorganic Nutrition - Encyclopedia of Plant Physiology, New Series (Plant) Vol 15A, A. Läuchli & R.L. Bieleski ed. Springer Verlag 1983

Table 1 (continued)

	Genus or type	Species
4 Obligate anaerobes		
	Clostridium	*pasteurianum, butyricum*
	Desulfovibrio	*gigas, desulfuricans, vulgaris*
	Desulfotomaculum	*ruminis*
5. Phototrophic bacteria		
	a) Rhodospirillaceae	
	Rhodospirillum	*rubrum, tenue, fulvum, molischianum, photometricum*
	Rhodopseudomonas	*palustris, viridis, capsulata, spheroides*
	b) Chromatiaceae	
	Chromatium	*vinosum, gracile, minus, violacea*
	Thiocystis	*violacea*
	Thiocapsa	*roseopersicina, pfennigii*
	Amoebobacter	*roseus*
	Ectothiorhodospira	*spaposhnikovii*
	c) Chlorobiaceae	
	Chlorobium	*limicola, vibrioforme*
	Pelodictyon	*luteolum*
6 Blue-green algae or cyanobacteria		
	a) Unicellular, aerobically fixing strains	
	Gloeothece (formerly *Gloeocapsa*)	*alpicola* (5 strains)
	Aphanothece	
	b) Filamentous, heterocystous forms which fix aerobically and anaerobically	
	Nostoc	*muscorum, commune*
	Anabaena	*cylindrica, variabilis*
	Aphanizomenon	*flos - aquae*
	Cylindrospermum	Various strains
	Calothrix	Various strains
	c) Filamentous, non-heterocystous forms which fix microaerobically	
	Plectonema	*boryanum*
	Oscillatoria	Various strains
	Pseudanabaena	Various strains
	Lyngbya	Various strains
	Phormidium	Various strains

B. Symbiotic nitrogen-fixing systems and associations

Host family	Host genus	N_2-fixing microorganism
a) *Rhizobium* symbioses		
Leguminosae	Most species	*Rhizobium* species
Ulmaceae	*Parasponia*	*Rhizobium*

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Inorganic Plant Nutrition. Encyclopaedia of Plant Physiology, New Series. Vol. 15A. A. Läuchli & R.L. Bieleski ed. Springer Verlag 1983

Table 1 (continued)

Host family	Host genus	N_2-fixing microorganism
b) Non-*Rhizobium* symbioses		
Betulaceae	*Alnus*	
Myricaceae	*Myrica, Comptonia*	
Eleagnaceae	*Eleagnus, Hippophae, Shepherdia*	In all cases
Rhamnaceae	*Ceanothus, Trevoa, Discaria*	*Actinomycetes* "*Frankia*"
Rosaceae	*Dryas, Cercocarpus, Purshia*	
Coriariaceae	*Coriaria, Colleta*	
Casuarinaceae	*Casuarina*	
c) Lichens	*Collema, Peltigera, Dendriscocaulon*	*Nostoc, Scytonema*
d) Liverworts	*Anthoceros, Blasia, Cavicularia*	*Nostoc sphaericum*
e) Waterfern	*Azolla*	*Anabaena azollae*
f) Cycads	*Cycas, Ceratozamia, Encephalartos, Macrozamia, Dioon* etc.	*Nostoc, Anabaena*
g) Higher plants		
Haloragaceae	*Gunnera*	*Nostoc punctiforme*
h) Associative symbioses and casual associations		
Phyllosphere	Leaves	*Azotobacter* spp
Rhizosphere	Roots of grasses	
	Paspalum notatum	*Azotobacter paspali*
	Zea mays	*Azospirillum*

Compiled mainly from data by Burns and Hardy 1975, Sprent 1979, Postgate 1978, Siefert 1976, Rippka et al. 1979

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Inorganic Plant Nutrition – Encyclopaedia of Plant Physiology, New Series. Vol. 15A. A. Läuchli & R.L. Bieleski eds. Springer Verlag, 1983

Table 2. Cross-inoculation groups in the *Rhizobium* symbioses, in which mutual infections by the corresponding *Rhizobium* are possible

Group	*Rhizobium*	Plants which are regularly infected	Occasional infections
Fast growing			
Pea group	*R. leguminosarum*	*Pisum, Vicia, Lens, Lathyrus, Cicer*	*Trifolium*
Clover group	*R. trifolii*	*Trifolium*	
Bean group	*R. phaseoli*	*Phaseolus*	
Medicago group	*R. meliloti*	*Medicago, Melilotus, Trigonella*	
Slow growing			
Soybean group	*R. japonicum*	*Glycine*	
Lupinus-Lotus group	*R. lupini*	*Lupinus, Ornithopus, Lotus, Anthyllis, Astragalus, Caragana, Ononis, Dorycnium*	
Cow-pea miscellany specialized	*R.* strains	*Stylosanthes, Centrosema, Desmodium, Lotononis, Leucaena*	
promiscuous	*R.* strains	*Vigna sinensis, Phaseolus lathyroides, Ph. atropurpurea, Arachis hypogaea, Parasponia*	Other *Phaseolus* species *Medicago, Glycine*

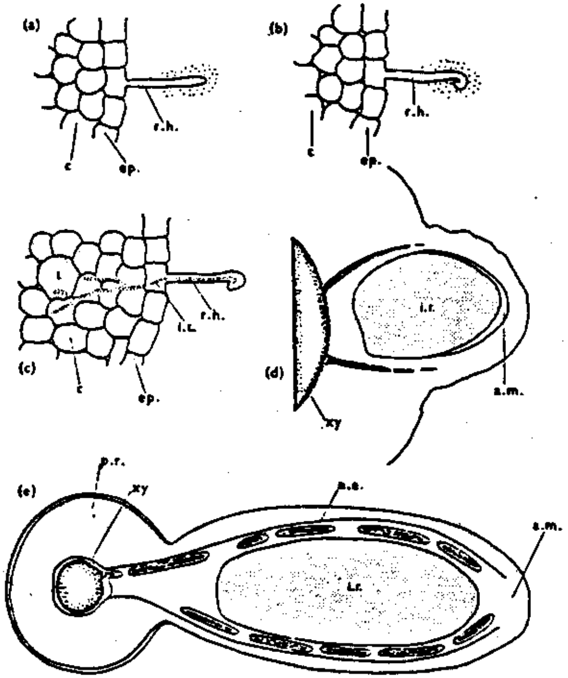
Fixation de N_2

(3)

type de fixation	N_2 fixé : T × 10^6 par an
Fixation biologique directe	
Agriculture	
Légumineuses	35
non :	
Riz	4
Autres	5
prairies permanentes	45
Forêts, bois	40
Terrains incultes	10
Total des zones terrestres	139
Océans, mers	36
Total de la fixation biologique	**175**
Fixation non biologique	
Atmosphère (orages)	10
Combustions	20
Industrie	
engrais	40 } énergie consommée 3×10^8 barils de pétrole 1b = 159 l
autres activités	10
(≃ 100 en 1983)	
Total de la fixation non biologique	**80** en 130
Total Général	**255** en 305

Les processus biologiques représentent 69 % (en 57 %) de la fixation totale.

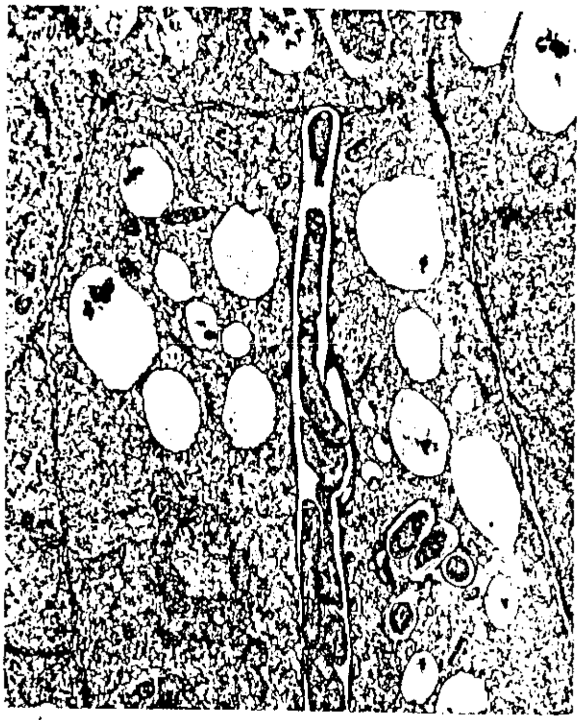
D'après SKINNER K J. Chemical and Engineering NEWS, 4 Oct. 1976, 22-35



Initiation et structure des nodules de Pois.
a - Rhizobium autour d'un poil absorbant
b - poil absorbant enroulé
c - infection
d - région centrale infectée
e - section longitudinale au travers du nodule
am: méristème apical, c: cortex, ep: épiderme, i.r.: région infectée, it: fil d'infection, n.e.: endoderme du nodule, p.r.: racine primaire, r.h.: poil absorbant, t: cellule tétraploïde, xy: xylème.
BEEVERS L., Nitrogen metabolism in Plants. E. Arnold, éd., Londres, 1976.

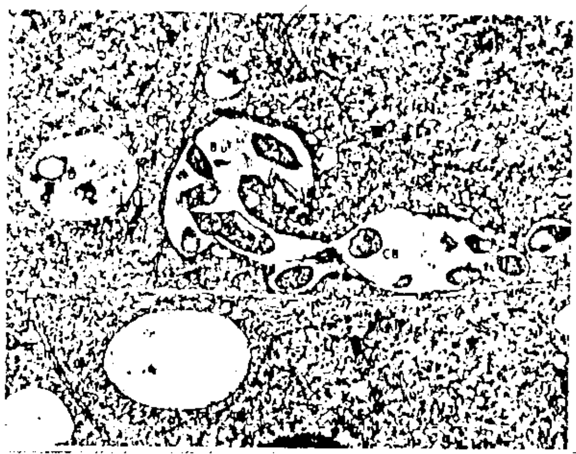
N5

N7



Filament d'infection d'une racine de Soja (x 10 000)
Le filament est enclos dans une paroi de cellulose et une membrane .
BEEVERS L., Nitrogen metabolism in Plants. E. Arnold éd., Londres, 1976.

N8



Libération des Bactéries à partir d'un filament d'infection (x 8 000) à partir d'un "ballon" cellulosique (Bu) continué par un "ballon" sans cellulose (CB). Les Bactéries sont absorbées par les cellules, par endocytose (Bi: bactérie dans une vacuole, HN: noyau de l'hôte, CE: paroi cellulosique du filament).
BEEVERS L., Nitrogen metabolism in Plants. E. Arnold éd., Londres, 1976.

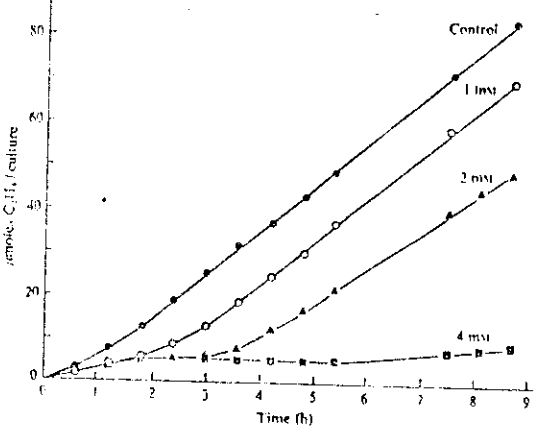


Fig. 1.3. Effect of different amounts of ammonium acetate (final concentrations 1, 2 and 4 mM) on the nitrogenase activity of a growing culture of *A. chroococcum* (Brotonegoro, 1974).

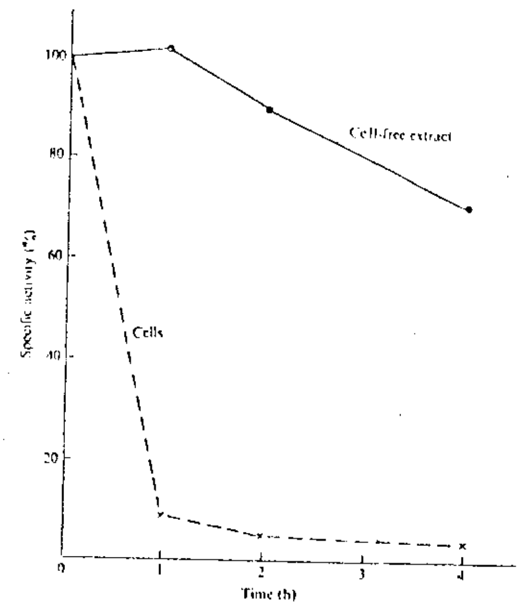


Fig. 1.4. Nitrogenase activity of living cells of *A. chroococcum*, measured at different intervals after the addition of ammonium acetate (final concentration 5 mM) to the culture, as compared to the activity of cell-free extracts prepared from the same culture at the same time (Brotonegoro, 1974).

STEWART W.D.P. Ed., Nitrogen fixation by free-living micro-organisms. IBP Cambridge University Press, 1975

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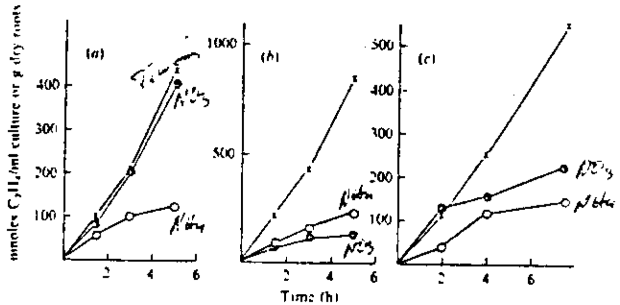

Fig. 3.6. Effects of ammonia and nitrate on nitrogenase activity of *A. paspali* in pure culture (*a*), on roots of *P. notatum* removed from soil (*b*) and intact systems with *P. notatum* (*c*). For pure culture assays, 1 ml of shallow layer batch culture (56.3×10^7 cells/ml) was injected into 60 ml bottles containing 1 ml of 20 mM NH_4^+ or NO_3^- in medium at the time of acetylene injection (time zero). To root samples, 5 ml of 10 mM NH_4^+ or NO_3^- in 0.025 M phosphate buffer (pH 6.8) and acetylene were applied at time zero. Intact soil–plant cores received 11.2 ppm N (soil basis) in solution at time zero. All nitrogen sources were $(NH_4)_2SO_4$ or KNO_3. All points are means of four replicates. ○, NH_4^+; ●, NO_3^-; ×, control.

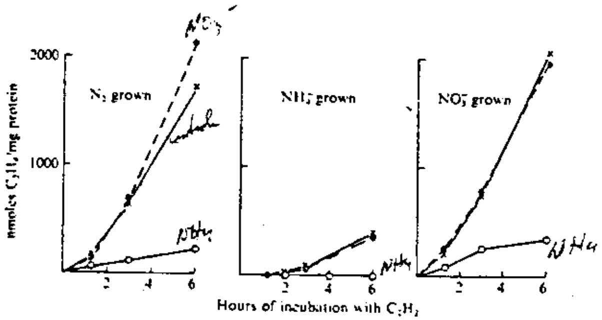

Fig. 3.7. Effects of ammonia and nitrate on nitrogenase activity of *A. paspali* grown in pure culture on N_2, ammonia or nitrate. One ml of washed cell suspensions, obtained from shake cultures grown with 10 mM $(NH_4)_2SO_4$ or KNO_3, or on N_2 (1.32, 0.44, 0.51 mg protein/ml resp.), was injected into 60 ml bottles with air, containing 1 ml of 20 mM NH_4^+ or NO_3^- in medium, at time zero. All points are means of three replicates. ○, NH_4^+; ●, NO_3^-; ×, control.

STEWART W.D.P. éd. Nitrogen fixation by free-living micro-organisms. IBP Cambridge University Press, 1975.

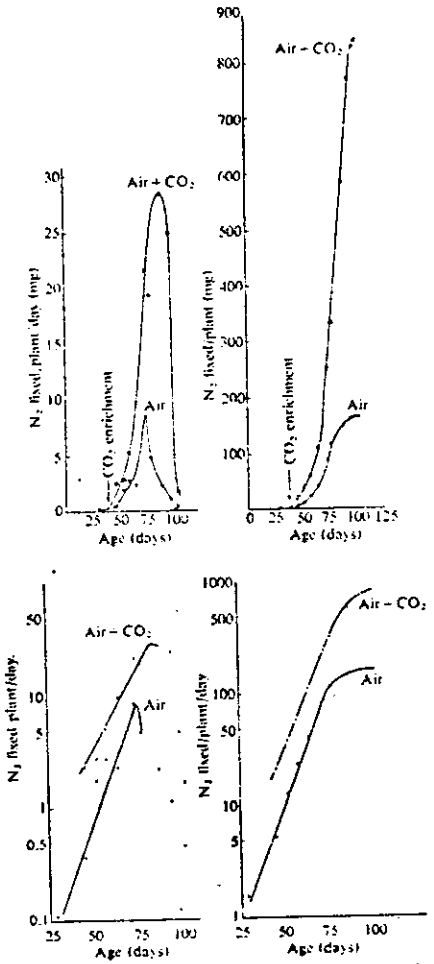

Fig. 31.7. Profiles as in Fig. 31.1 of nitrogen fixation by control and carbon dioxide enriched soybeans.

NUTMAN P.S. – Symbiotic nitrogen fixation in plants. IBP, Cambridge University Press, 1976.

TABLEAU I

Influence de la concentration en NO_3^- du milieu de culture sur les quantités de matière sèche, les teneurs en chlorophylle et en azote protidique de l'ensemble des feuilles de Soja non pourvu de nodosités (–) ou pourvu de nodosités à *Rhizobium japonicum* (+).

Effect of nitrate concentration in the nutrient solution on dry weight, chlorophyll content, organic N content of the shoots of soybean not inoculated (–) or inoculated (+) with Rhizobium japonicum.

Plantes de 51 j. Moyennes de 6 plantes. Comparaison des moyennes au risque 5 % des plantes inoculées et non inoculées : *ts*, différence très significative; *s*, différence significative; *ns*, différence non significative.

	Nodosités	NO_3^- dans le milieu de culture (méq.l^{-1}) 0		0,5		1,5		2,5	
Matière sèche (mg MS.plante^{-1})	–	310	s	728	ns	1 003	ns	1 052	ns
	+	520		763		992		1 163	
Chlorophylle (mg.g^{-1} MS)	–	2,7	ts	7,4	ts	8,4	s	7,6	ts
	+	8,8		10,1		9,2		9,8	
N protidique (mg.g^{-1} MS)	–	16,3	ts	30,7	ts	35,8	ns	37,0	ns
	+	33,1		37,2		35,2		36,2	
N protidique (mg.plante^{-1})	–	5,1	ts	22,3	ts	35,9	ns	38,9	ns
	+	17,2		28,4		34,9		42,2	

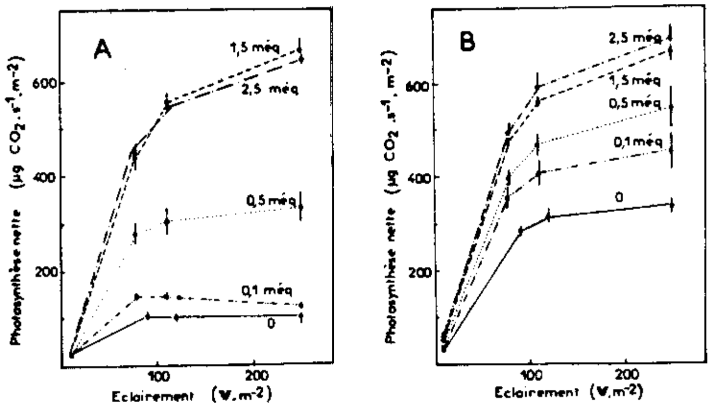

FIG. 3. – Influence de la concentration en NO_3^- du milieu de culture sur la variation de la photosynthèse nette par unité de surface foliaire du Soja en fonction de l'éclairement. A, plantes sans nodosités. B, plantes pourvues de nodosités.

– *Effect of nitrate concentration in the nutrient solution on net photosynthesis per unit leaf area of soybean versus light intensity. A, not inoculated. B, inoculated.*

Plantes de 33 j. Moyennes de 3 plantes (± écart-type). 0, absence de NO_3^-.

JATIMLIANSKY J.R., CHAMPIGNY M.L., PRIOUL J.L., BISMUTH E. et MOYSE A. Physiologie Végétale, 1982, 20, 407-422.

TABLEAU III

Influence de la concentration en NO_3^- du milieu de culture sur la masse de matière fraîche et sur les capacités nitrate réductase *in vitro* et nitrogenase des nodosités.

Effect of nitrate concentration in the nutrient solution on fresh weight in vitro *nitrate reductase and nitrogenase activities of the nodules.*

Plantes de 51 j.

	NO_3^- (méq.l^{-1}) 0	0,5	1	1,5	2	2,5	5
Matière fraîche (mg M.F. plante^{-1})	1 310	630	400	280	150	200	100
Nitrate réductase (µéq.h^{-1}.g^{-1} MF)	–	1,53	1,67	1,46	1,16	–	0,66
Nitrogenase (nmol.h^{-1}.g^{-1} MF)	10,0	15,5	–	11,2	–	5,0	4,0

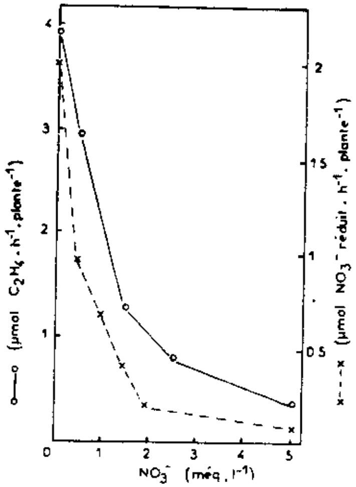

FIG. 7. – Influence de la concentration en NO_3^- du milieu de culture sur les capacités nitrate réductase (– – –) et nitrogénase (———) des nodosités de Soja.

– *Effect of nitrate concentration in the nutrient solution on* in vitro *nitrate reductase* (– – –) *and nitrogenase* (———) *of the nodules of soybean inoculated.*

Plantes de 51 j.

JATIMLIANSKY J.R., CHAMPIGNY M.L., PRIOUL J.L., BISMUTH E. et MOYSE A. – Physiologie Végétale, 1982, 20, 407-422.

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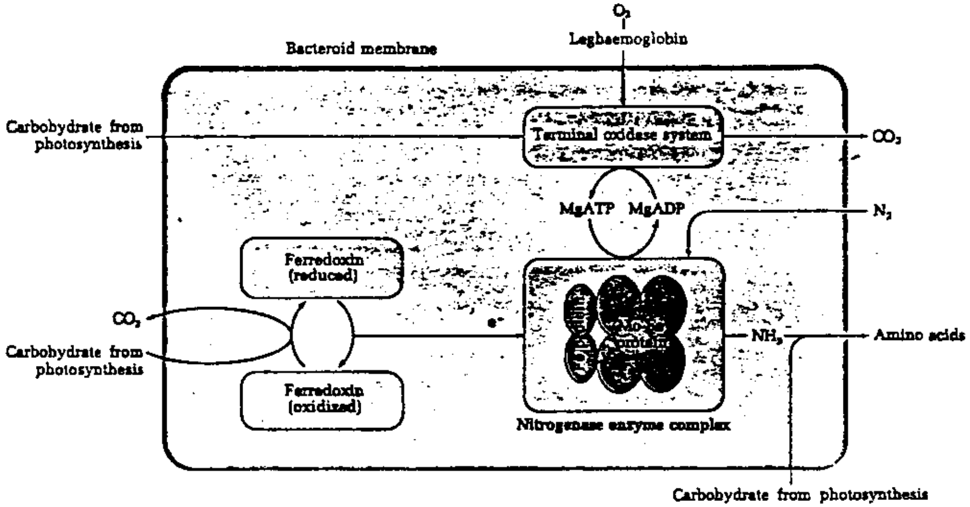
These are the major elements of the nitrogenase reaction



Two protein components—the Fe protein and the Mo-Fe protein—comprise the nitrogenase enzyme complex. The Fe protein is composed of two identical subunits. The Mo-Fe protein contains four subunits. Electrons flow from a reducing agent such as a reduced ferredoxin into the Fe protein, then into the Mo-Fe protein, and finally onto the substrate, usually nitrogen. MgATP, which binds to the Fe protein, becomes hydrolyzed as the substrate is reduced. The precise time at which MgATP binds and dissociates and the exact role it plays in the reduction process have not been fully elucidated. The leghaemoglobin provides for the diffusion of oxygen in such a way that ATP is produced most efficiently, but the oxygen-sensitive nitrogenase is not destroyed. In symbiotic relationships, the photosynthetic products of the plant serve as not only a carbon skeleton for the synthesis of molecules containing fixed nitrogen, but as an energy source for the production of reductant and ATP

Chemical and Engineering NEWS, 4 Oct. 1976

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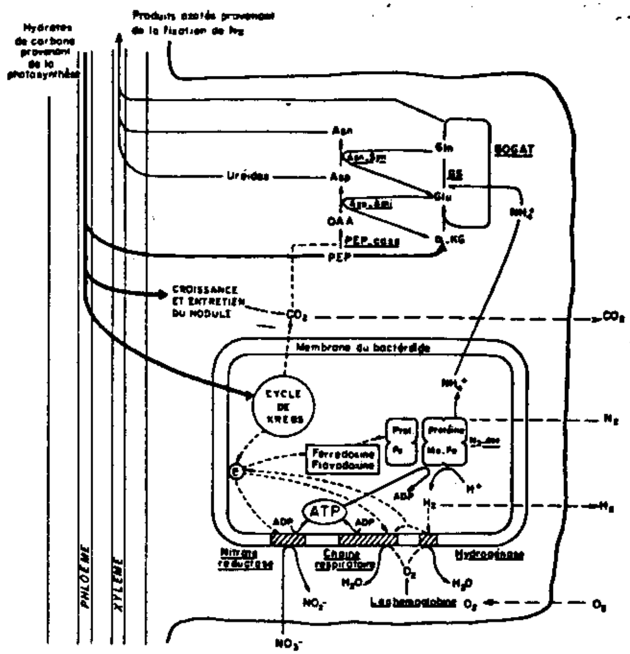
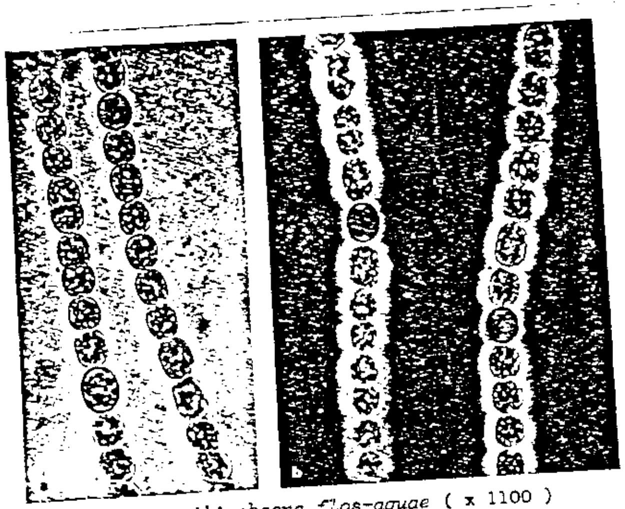


FIG. 5. — Schéma représentant le fonctionnement d'une nodosité (d'après SCOTT *et al.*, 1976; EVANS *et al.*, 1977; CHRISTELLER *et al.*, 1977; ATKINS *et al.*, 1978; SHANMUGAM *et al.*, 1978)
— *Schematic diagram of a working nodule*

Gln : glutamine; Glu : glutamate; α-KG : α-cétoglutarate; PEP : phosphoénolpyruvate; OAA : oxaloacétate; Asp : aspartate; Asn : asparagine; PEP-case : phosphoénolpyruvate carboxylase; GS : glutamine synthétase; GOGAT : glutamate synthase; Asn-syn : asparagine synthétase; Asp-ami : aspartate-aminotransférase; N_2-ase : nitrogénase.

In : La symbiose Rhizobium Légumineuses : rôles respectifs des partenaires. J. DENARIÉ et G. TRUCHET.
Physiologie Végétale, 1979, 17 (3), 643-667.



Micrographies d'*Anabaena flos-aquae* (x 1100)

FOGG G.E. *et al.*, The Blue-green Algae. Academic Press, Londres et New-York, 1973

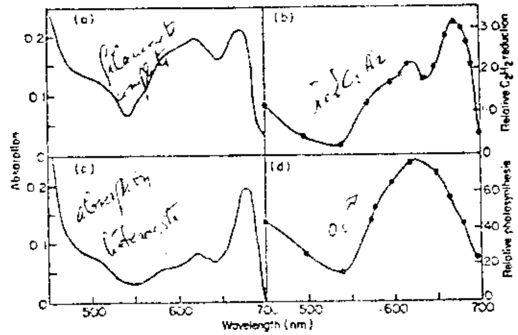


FIG. 10.7. Absorption spectra of (a) intact filaments and (c) isolated heterocysts of *Anabaena cylindrica*. (b) C_2H_2 reduction and (d) net oxygen evolution at different wavelengths by *Anabaena cylindrica*. After Fay (1970) *Biochim. biophys. Acta.* **216**, 353–356.

FOGG G.E. *et al.*, The Blue-green Algae. Academic Press, Londres et New-York, 1973

Table V. Estimated Costs of Nitrogen Fixation in Soybeans and Corn

	Soybeans	Corn
Total dry matter ($kg \cdot ha^{-1} \cdot yr^{-1} x 10^{-3}$)[a]	3.7	7.0
Total plant N ($kg \cdot ha^{-1} \cdot yr^{-1}$)[b]	110	40
Total plant C ($kg \cdot ha^{-1} \cdot yr^{-1} \times 10^{-3}$)[c]	1.5	2.8
Seed dry weight ($kg \cdot ha^{-1} \cdot yr^{-1} x 10^{-3}$)	1.5	2.8
Seed protein (% by weight)[d]	38	10
Seed N ($kg \cdot ha^{-1} \cdot yr^{-1}$)[d]	36	18
Estimated total photosynthesis ($KgC \cdot ha^{-1} \cdot yr^{-1} x 10^{-3}$)[e]	5	5
Estimated Costs N_2 fixation		
Carbon cost ($kgC \cdot ha^{-1} \cdot yr^{-1}$)[f]	660	240
% total photosynthesis	12	5
"Predicted" yield decrease ($bu \cdot acre^{-1}$)[g]	6.6	5.5
Dollar equivalent[h]	43	14

[a]Calculated from average seed yields for world production of corn and soybeans from FAO Handbook.

[b]Calculated from experimental measurements of protein or N content.

[c]Assumes plant C = 40% plant dry weight.

[d]Values taken or calculated from values given in (91).

[e]Estimated from values for dry matter and estimated respiratory losses.

[f]Assume value of 6 kg C used per kg N fixed.

[g]Assume % of photosynthate used to support N_2 fixation leads directly to reduction in yield by same %.

[h]Based on current prices for soybeans and corn and "predicted" yield decrease.

The Energetics of Biological Nitrogen fixation.
Workshop Summaries I .
American Society of Plant Physiologists, 1982.