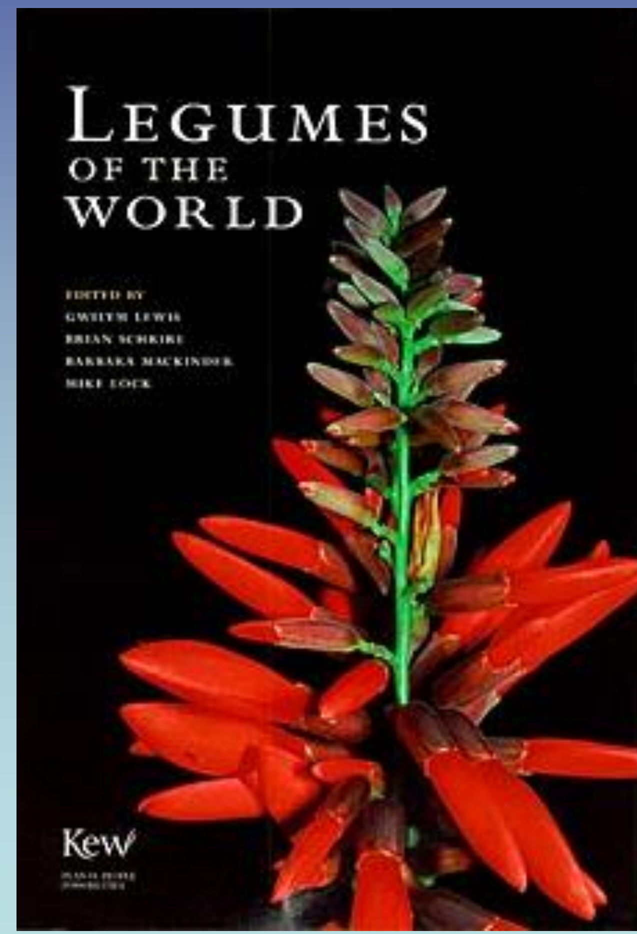
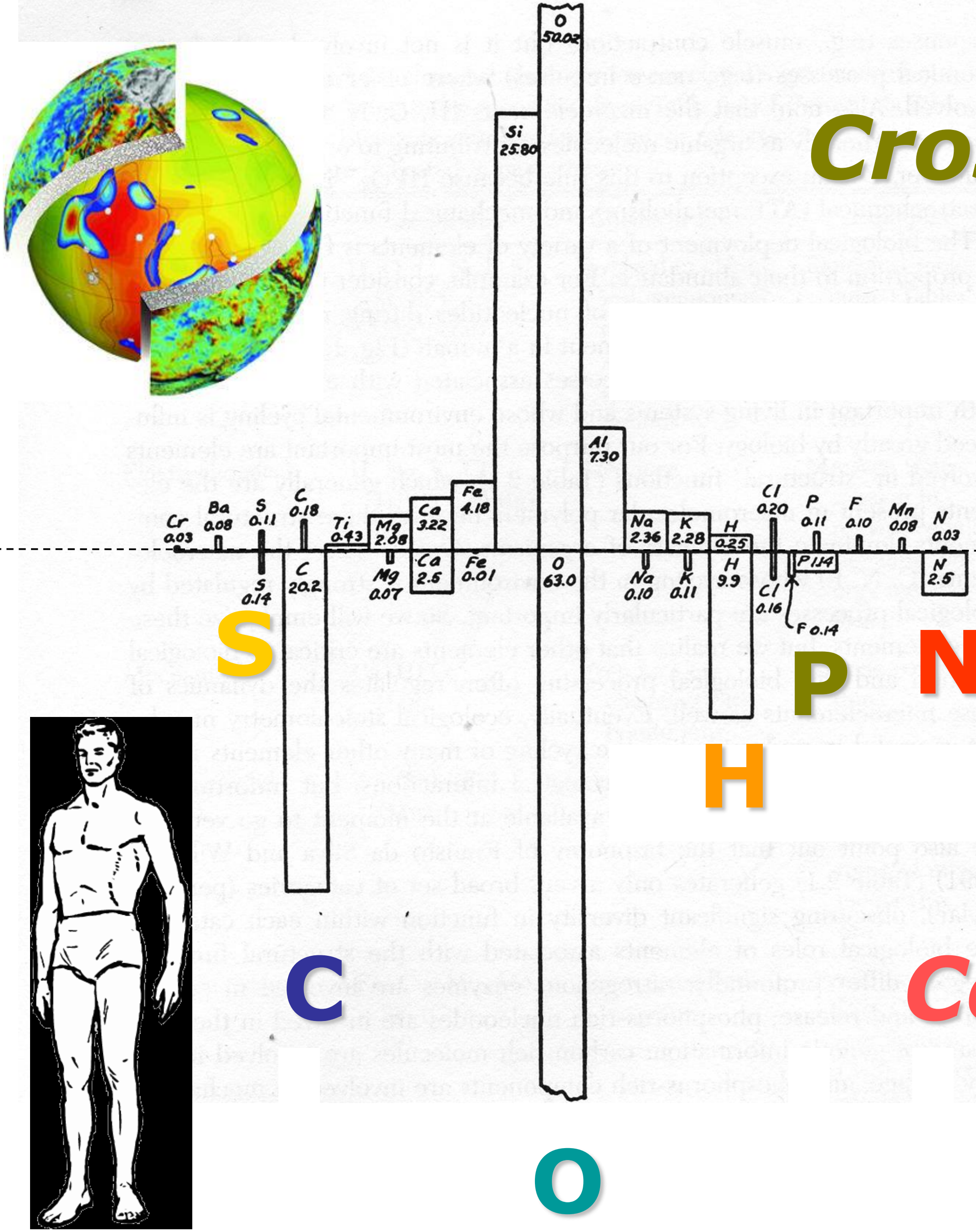
Alle radici della nutrizione, l'azotofissazione simbiotica delle leguminose come paradigma di sostenibilità del nostro ecosistema

Andrea Squartini squart@unipd.it

Le Leguminose rappresentano una delle più vaste famiglie di piante vascolari, con oltre 18,000 specie. Pari a circa un dodicesimo del totale delle specie di piante terrestri.

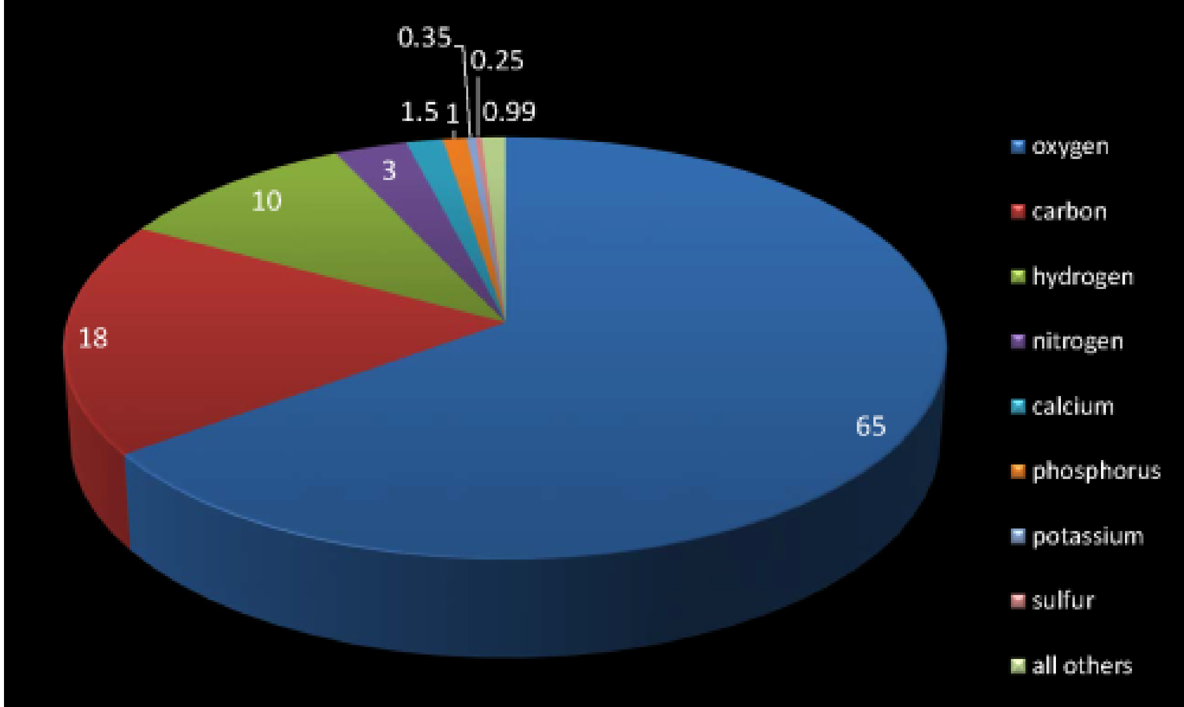


Crosta terrestre

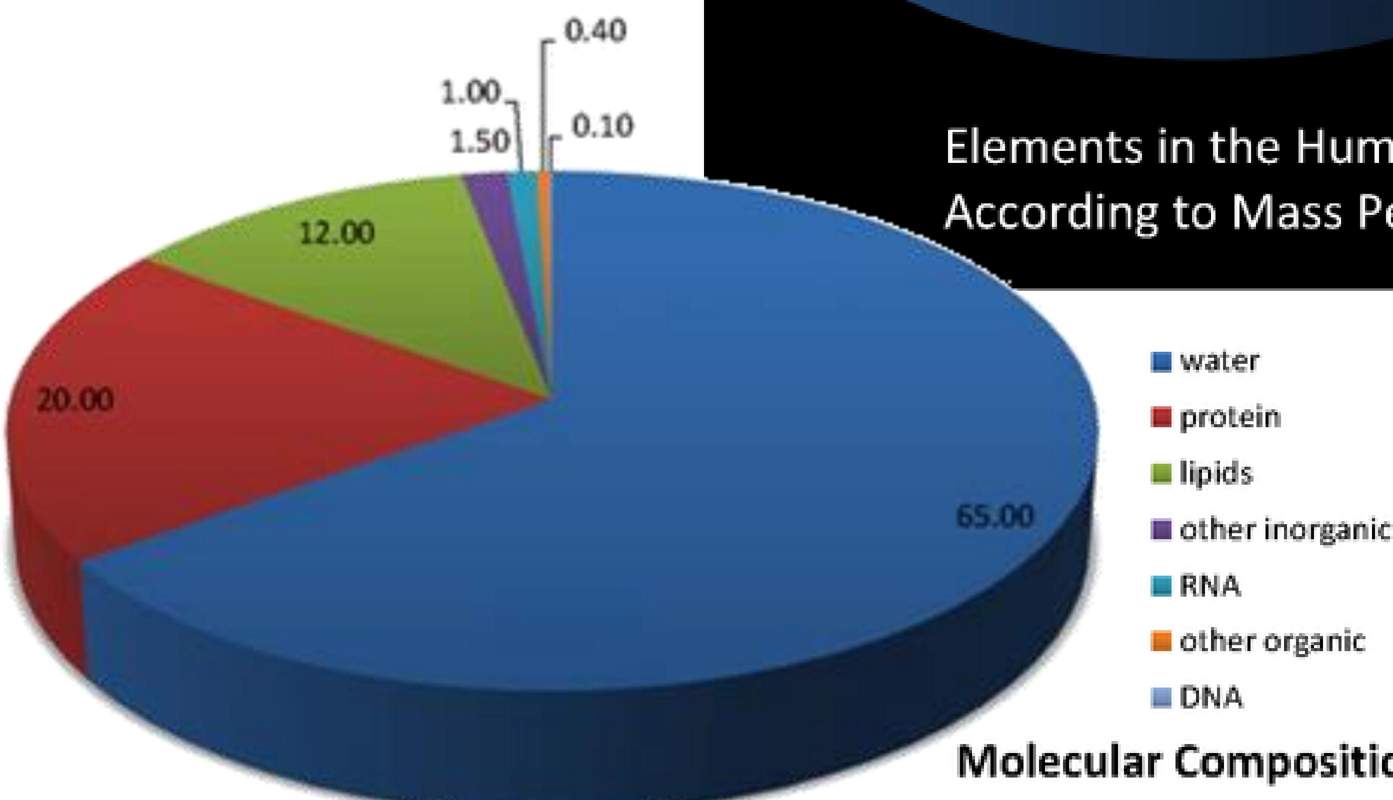


S P N H C O

Corpo umano



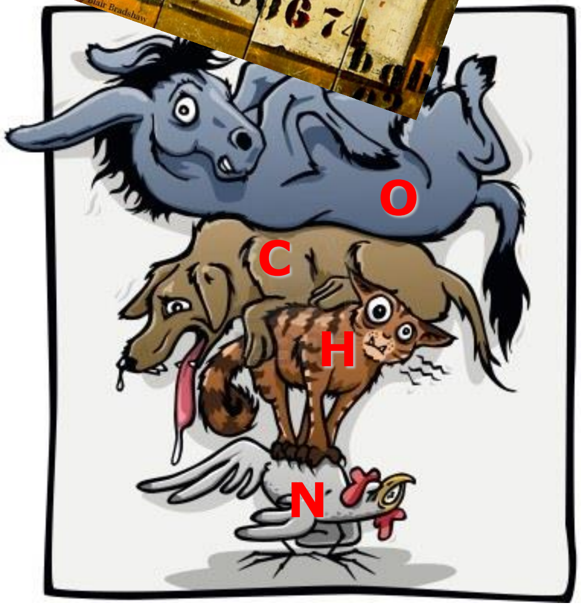
Elements in the Human Body According to Mass Percent



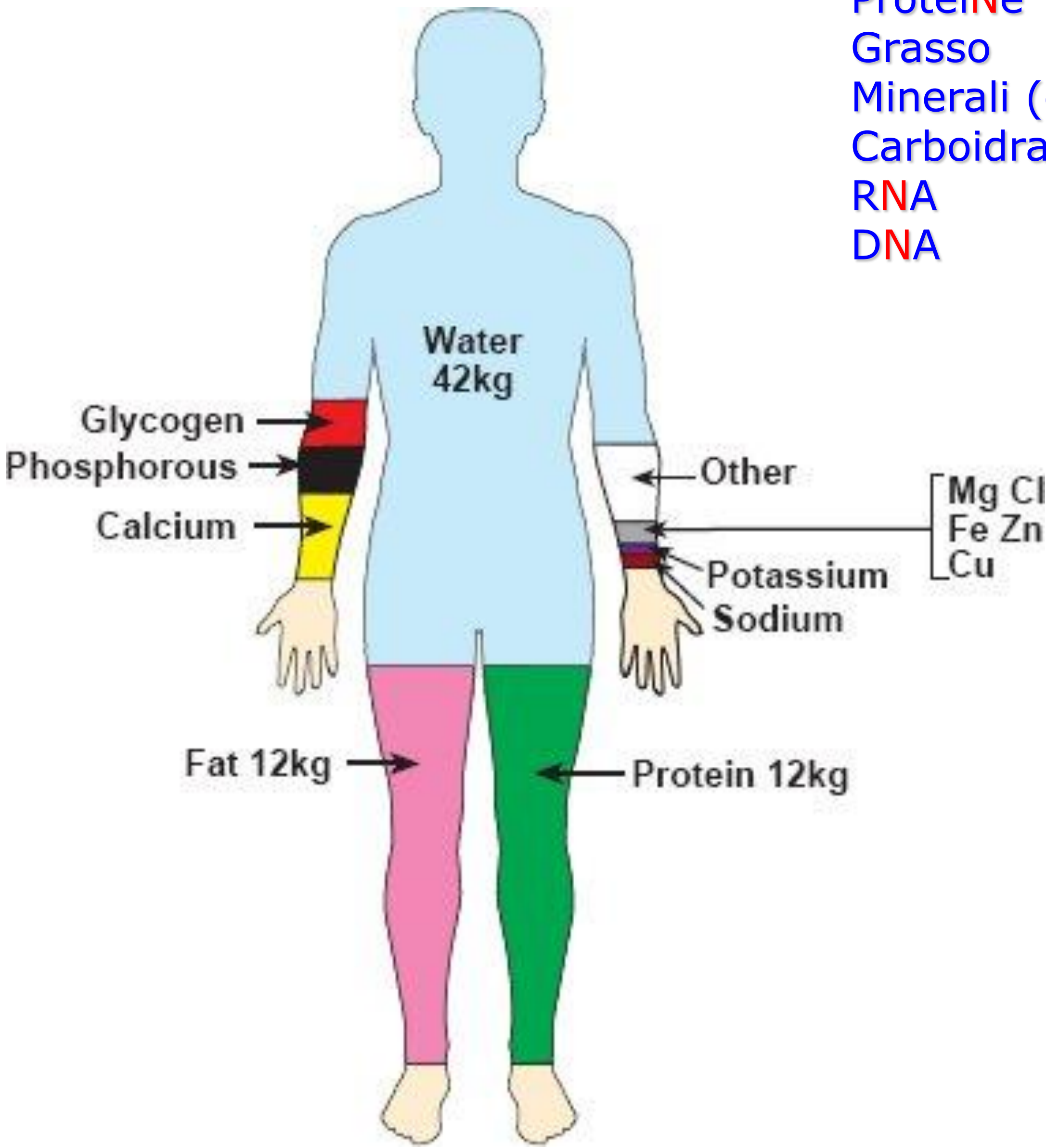
Molecular Composition of a Human Cell

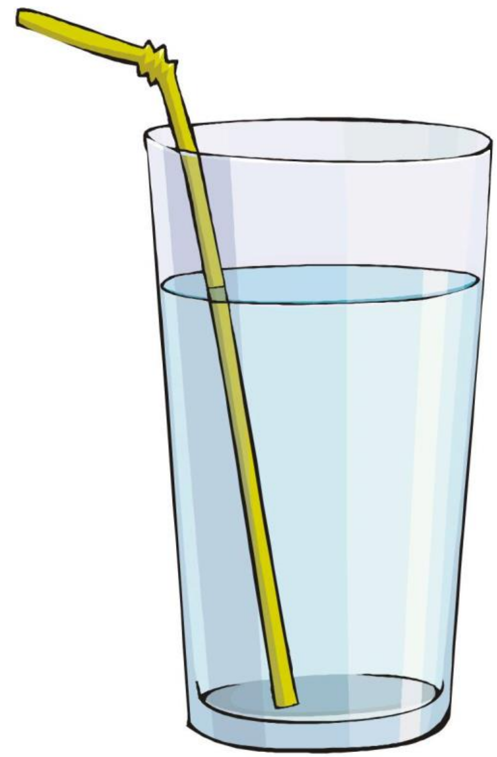
Element	Mass of element in a 70-kg person
oxygen	43 kg
carbon	16 kg
hydrogen	7 kg
nitrogen	1.8 kg
calcium	1.0 kg
phosphorus	780 g
potassium	140 g
sulfur	140 g
sodium	100 g
chlorine	95 g
magnesium	19 g
iron	4.2 g
fluorine	2.6 g
zinc	2.3 g

Element	Mass of element in a 70-kg person
oxygen	43 kg
carbon	16 kg
hydrogen	7 kg
nitrogen	1.8 kg



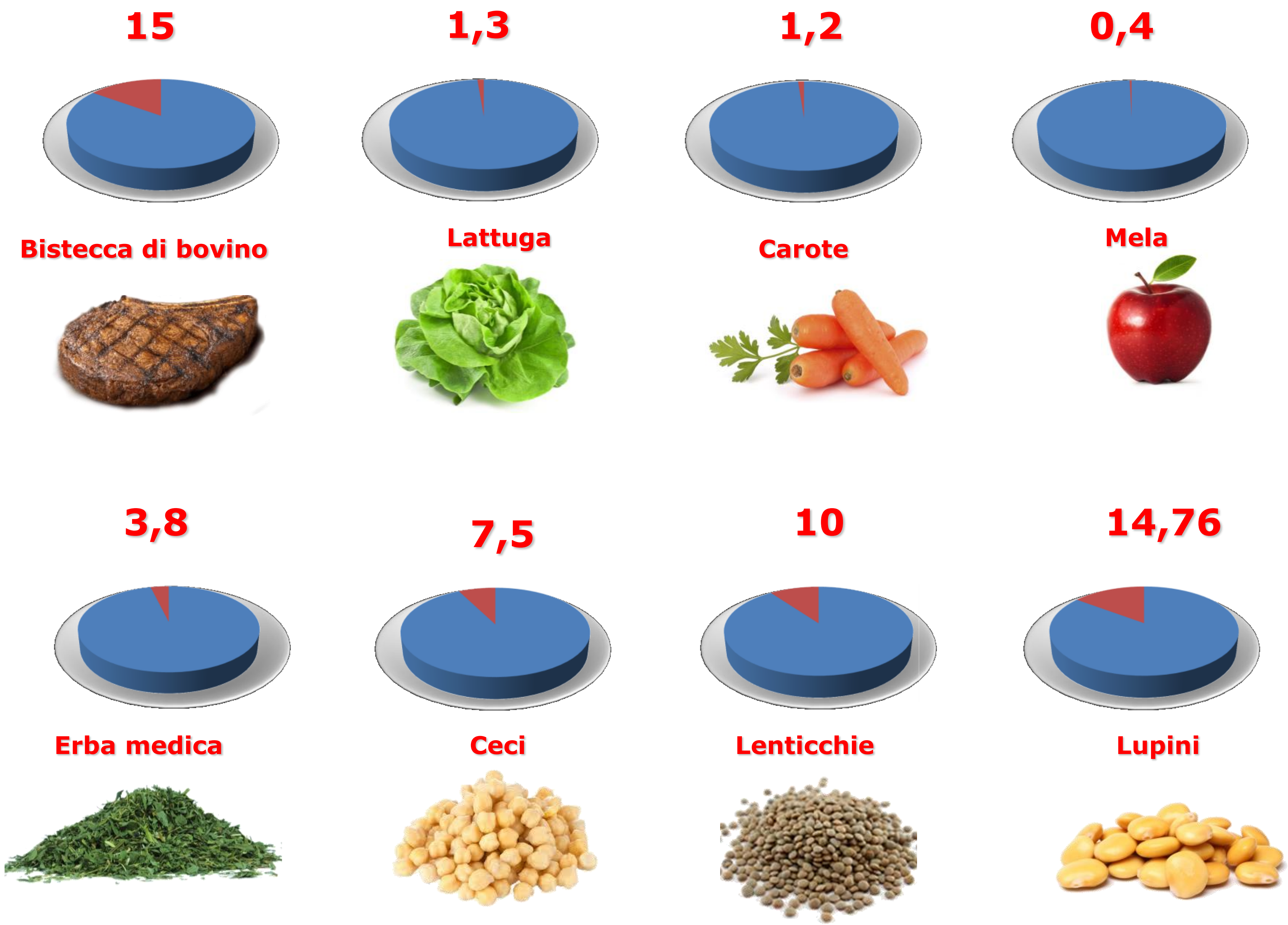
Acqua	**42 kg**
ProteiNe	**12 kg**
Grasso	**12 kg**
Minerali (ossei e non)	**3,5 kg**
Carboidrati	**0,5 kg**
RNA	**0,9 kg**
DNA	**0,2 kg**



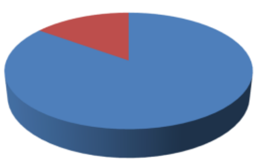



rapporto Carbonio / Azoto
(>)
25/1
15/1
(<)
10/1
(<)
10/1

Grammi di proteina su 100 g di alimento peso fresco

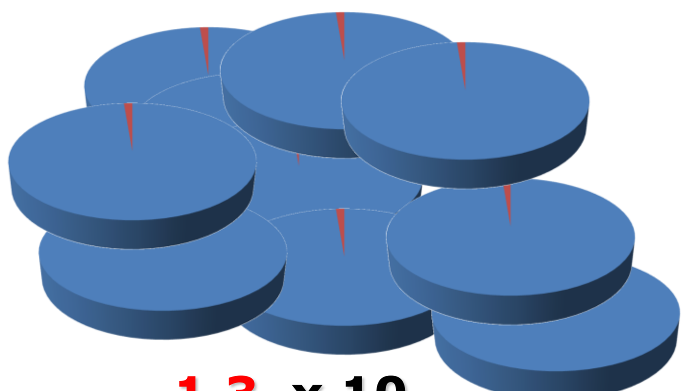


Bistecca di bovino



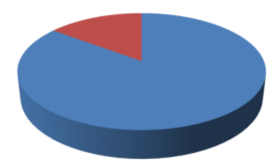
15

Lattuga

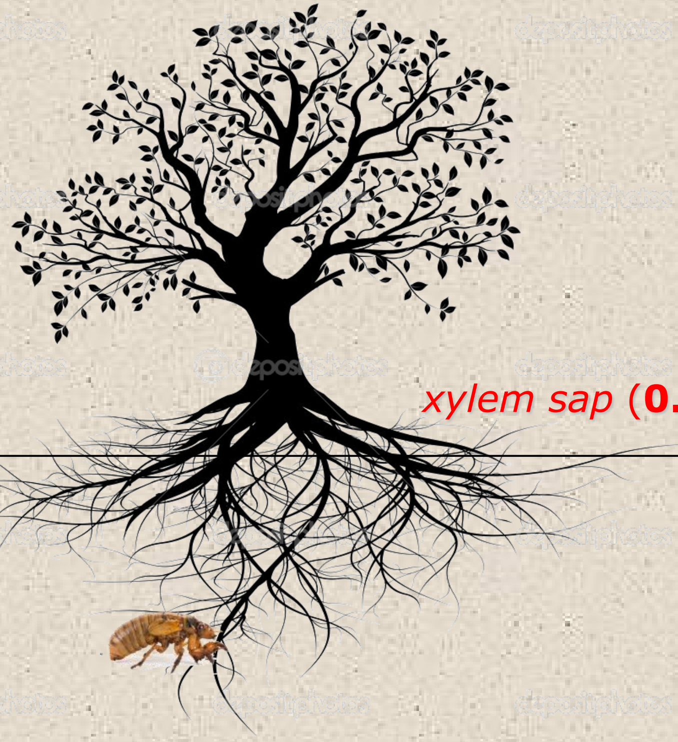


1,3 x 10

Lupini



14,76

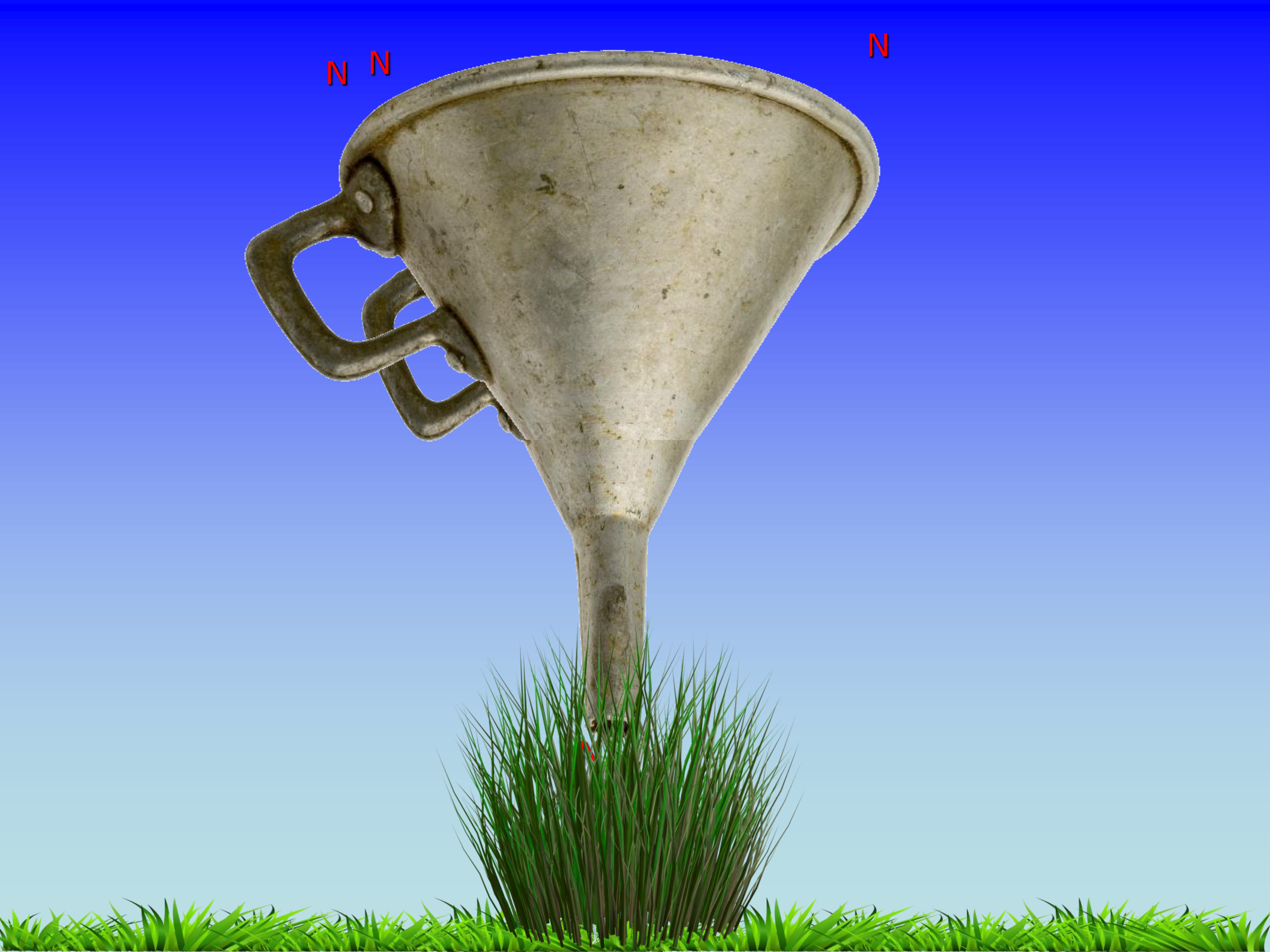


xylem sap (**0.0002-0.1% N** w/v)

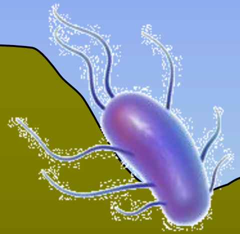
.....17 years

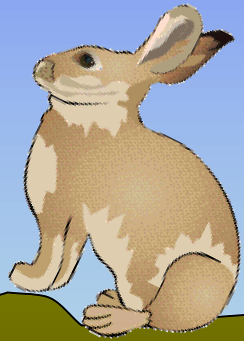

WHY DOES THE
WORLD STAY GREEN?
Nutrition and survival of plant-eaters
TCR White


N
N
N
N
N
N


N
N
N

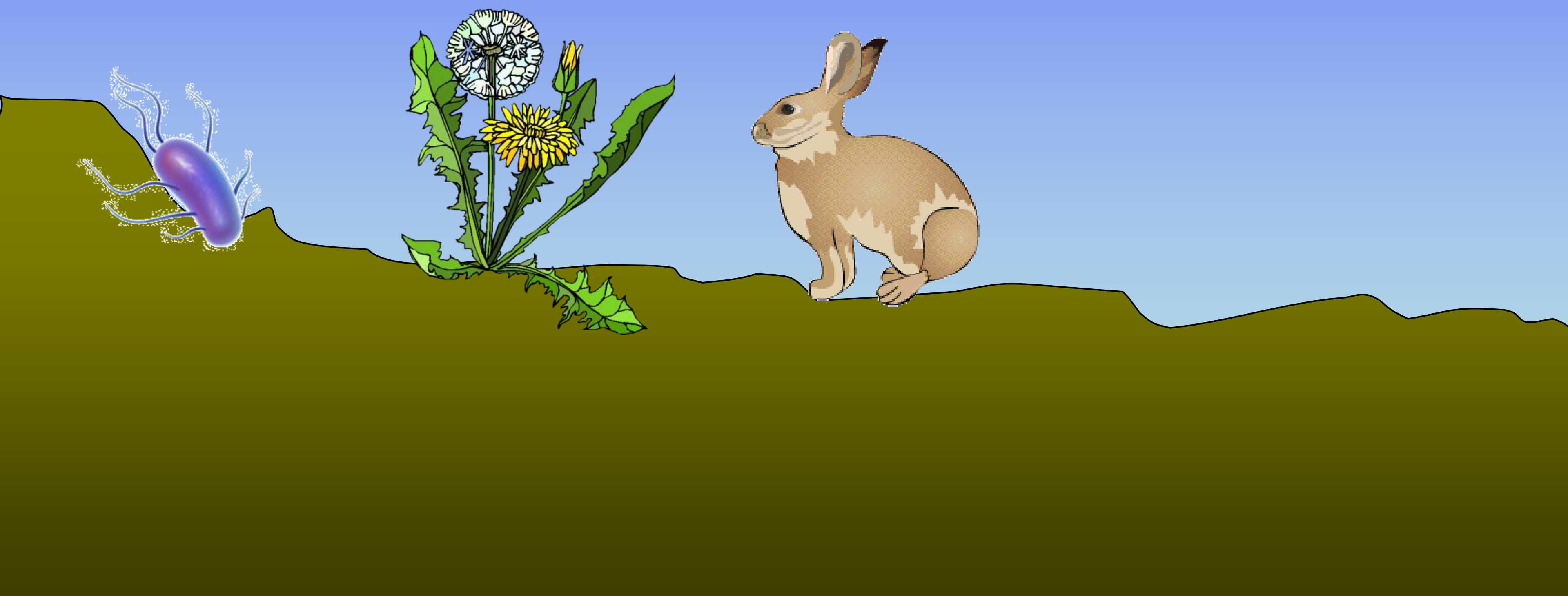
N_2 *78 %*

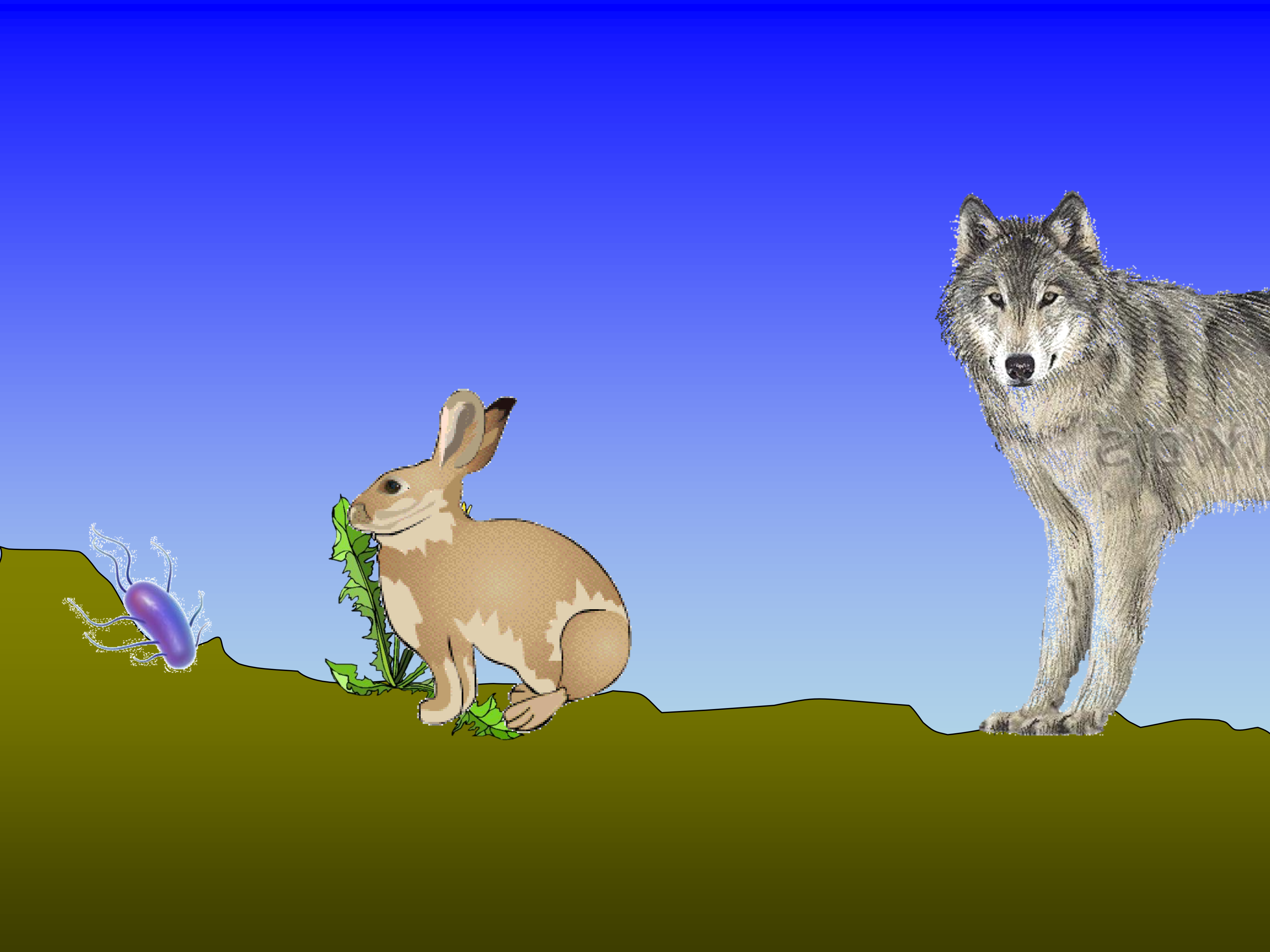




$NH_4^+ \rightarrow NO_3^-$

Azoto proteico






Hedysarum naudinianum


Hedysarum coronarium
(sulla)


Scorpiurus muricatus
Hedysarum spinosissimum

Castelsardo

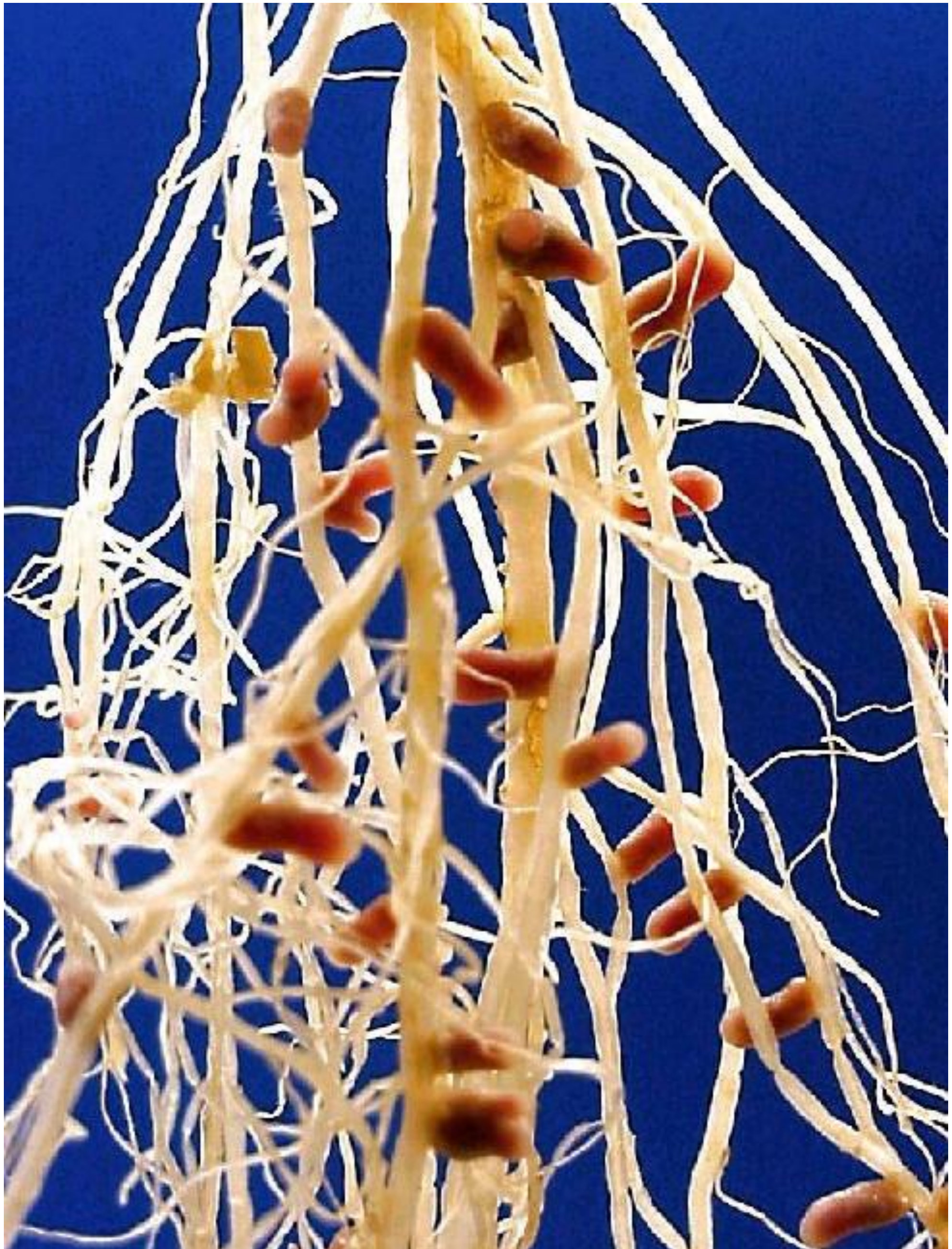
-The 191 legume species growing in the island of Sardinia -


Hedysarum glomeratum


Ornithopus compressus

Ornithopus pinnatus


Tetragonolobus purpureus

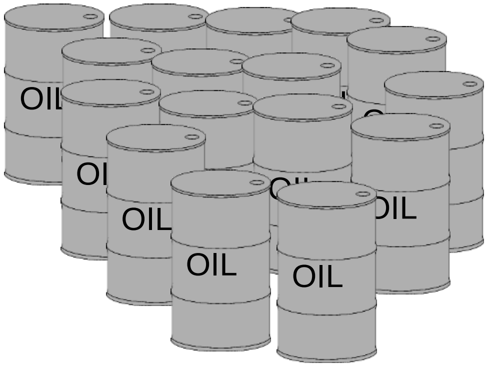


Azotofissazione Biologica

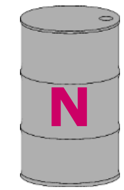
$$N_2 \xrightarrow{225\ kcal/mole} NH_4^+$$

(~~350°C 100 Mpa~~)

Temperatura ambiente
Pressione atmosferica

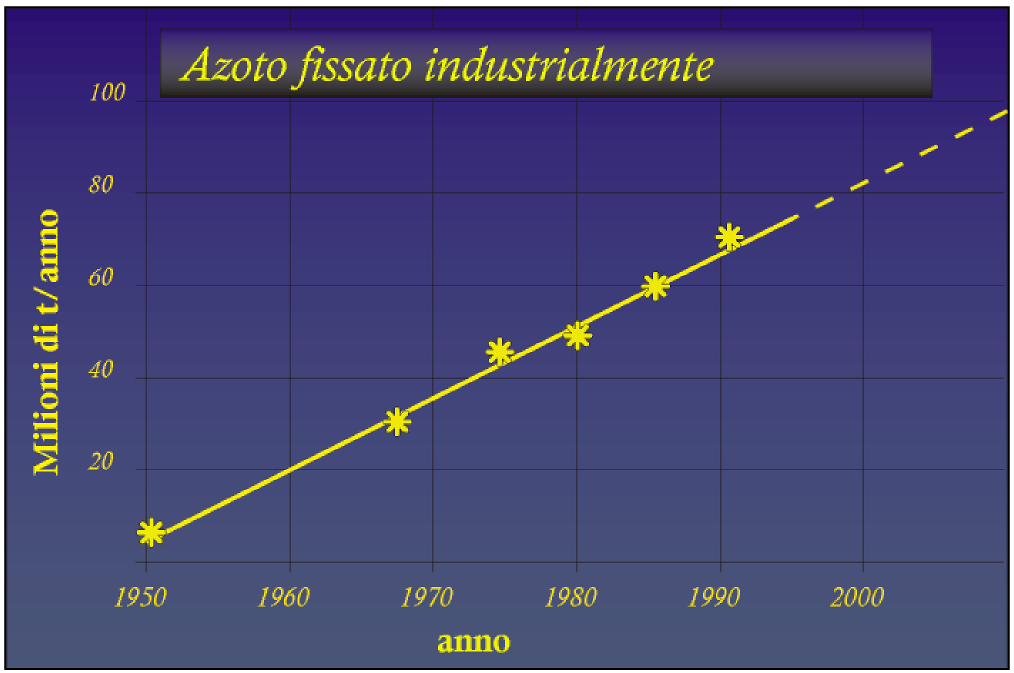


Fissazione Industriale dell'AZOTO



17 quintali* → *1 quintale

$$N_2 + 3H_2 = 2NH_3$$



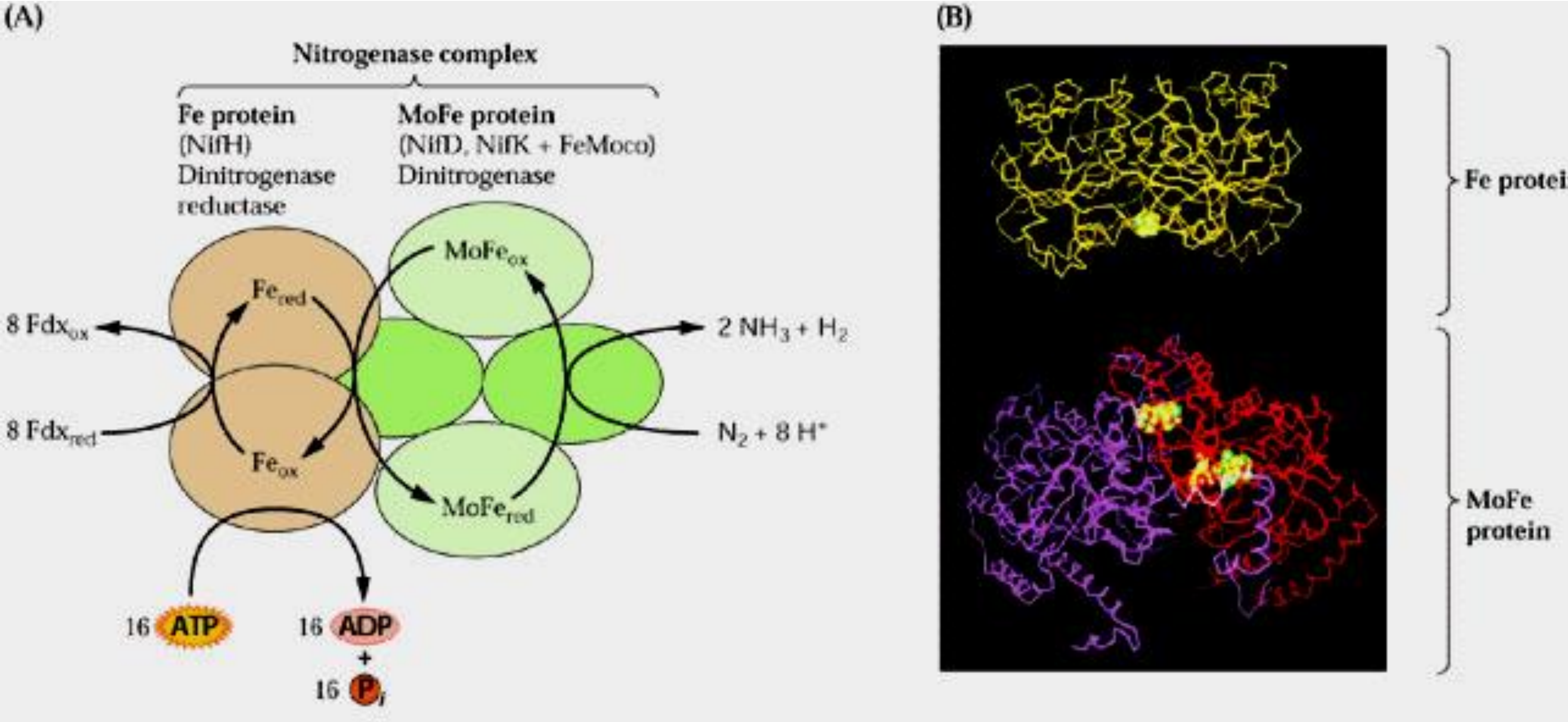
Processo Haber-Bosch

Per ottenere **100 Kg NH_3** occorrono

- **1700 Kg** di petrolio-equivalenti
  (nafta, olio combustibile, metano)
- Produzione **idrogeno gassoso**
- Pressioni elevate (**350-1000 ATM**)
- Temperature elevate (**300-500 °C**)
- **Catalizzatori** chimici

Mentre **i microrganismi azotofissatori** producono da pochi Kg fino a **700-1000 Kg di N fissato/anno/ettaro**

- **a temperatura ambiente**
- **a pressione atmosferica**



NITROGENASI *complesso enzimatico*

$$N_2 + 8H^+ + 8e^- + 16ATP \rightarrow 2NH_3 + 16ADP + 16Pi + H_2$$

- una mole di H_2 si forma per ogni mole di N_2 ridotto
- almeno 4 molecole di ATP vengono consumate per ogni coppia di e^- trasferiti
  - *per ridurre N_2 ad NH_3*
  - *o per formare H_2 in assenza di N_2*
- la ferridossina ridotta serve come riducente nella reazione

Azotofissatori liberi

Eterotrofi (organotrofi) pochi Kg N fissato/ettaro

substrati organici come fonte di Energia

Azotobacter	***aerobio, suoli neutri o alcalini***
Beijerinckia ***Derxia***	***aerobi, più spesso in suoli acidi (tropici)***
Klebsiella	***anaerobio facoltativo, ambienti umidi, suolo, radice, superficie foliare***
Clostridium	***anaerobio, substrati organici come donatori di elettroni***
Desulfovibrio ***Desulfotomaculum***	***anaerobi, substrati organici come donatori di elettroni, S riduttori***

Azotofissatori liberi

<u>Autotrofi (fototrofi)</u> 1-10 Kg N fissato/ettaro

luce come fonte di Energia

Anabaena	***Cianobatteri***
Nostoc	***Cianobatteri***
Calothrix	***Cianobatteri***
Rhodospirillum	***solfobatteri fotosintetici***
Chlorobium	***solfobatteri fotosintetici***
Chromatium	***solfobatteri fotosintetici***

Azotofissatori simbiotici


<u>Eterotrofi (organotrofi)</u> 5-140 Kg N fissato/Ha
fotosintati della pianta come fonte di energia

Azotobacter / Paspalum (Graminaceae)
Azospirillum / Digitaria (Graminaceae)
anche canna da zucchero e sorgo, anche ormoni


Rhizobium / Leguminose
alcuni beta-proteobatteri / Leguminose
Bradyrhizobium /Leguminose

Bradyrhizobium /Parasponia, Trema (Ulmaceae)

Frankia (Attinomiceti) / 137 specie di angiosperme arboree
(Alnaceae, Betulaceae, Rosaceae, Coriariaceae, Rhamnaceae,
Eleagnaceae, Myricaceae etc.)







Azotofissatori simbiotici

Autotrofi (Fototrofi)   5-30 Kg N fissato/Ha

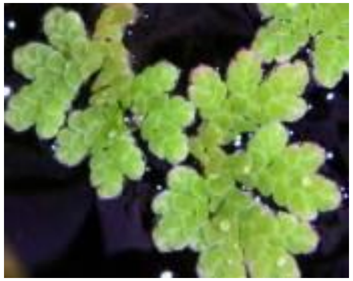
luce come prevalente fonte di energia

Nostoc / Cycas (Gimnosperme)

Nostoc / Peltigera (Licheni)

Halosiphon /Sphagnum (Muschi)

Anabaena /Azolla (Felci)



Noduli di leguminose da granella

Glycine max (soja)

Cicer arietinum (cece)

Arachis hypogaea (arachide)

Noduli di leguminose da foraggio

Lotus corniculatus (ginestrino)

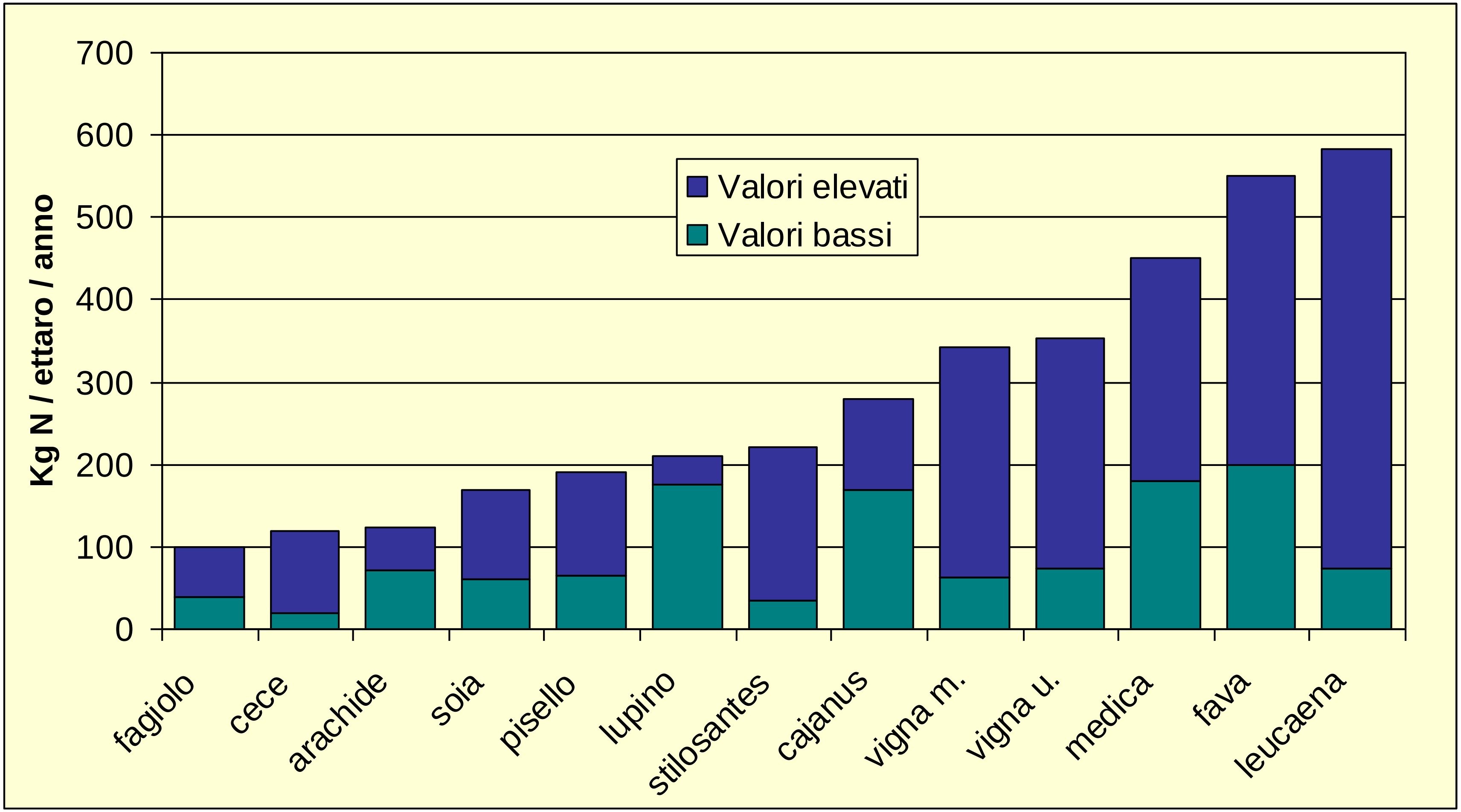
Trifolium repens (trifoglio)

Onobrychis viciaefolia (lupinella)

Cyanopsis tetragonoloba (guar)

 *Azoto totale fissato nella biosfera*	Area (ha · 10^6)	Kg N_2 fissato /anno /ha	Input totale /anno (t · 10^6)
FISSAZIONE BIOLOGICA			**122**
Piante leguminose	250	80	20
Piante non leguminose	1015	8	8
Praterie	6000	5	30
Risaie	135	30	4
Foreste	4000	3	12
Terre non coltivabili	2000	6	12
Corpi idrici	36000	1	36
FISSAZIONE ATMOSFERICA			**7**
FISSAZIONE INDUSTRIALE			**25**

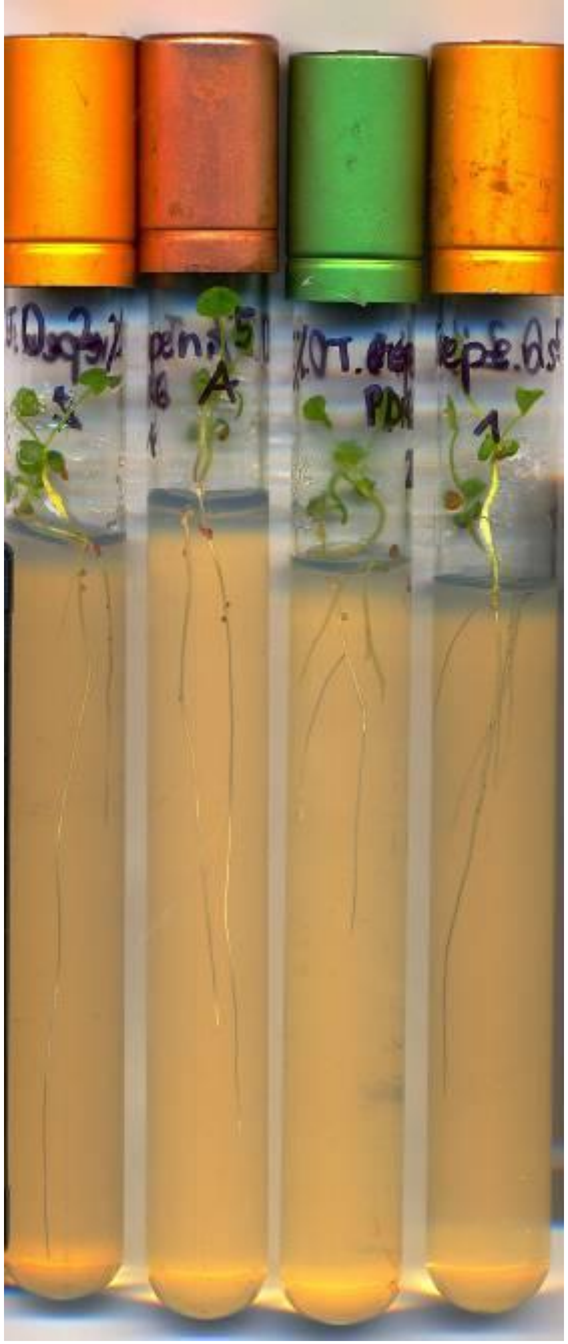
Stime relative alle quantità di azoto fissato da alcune leguminose

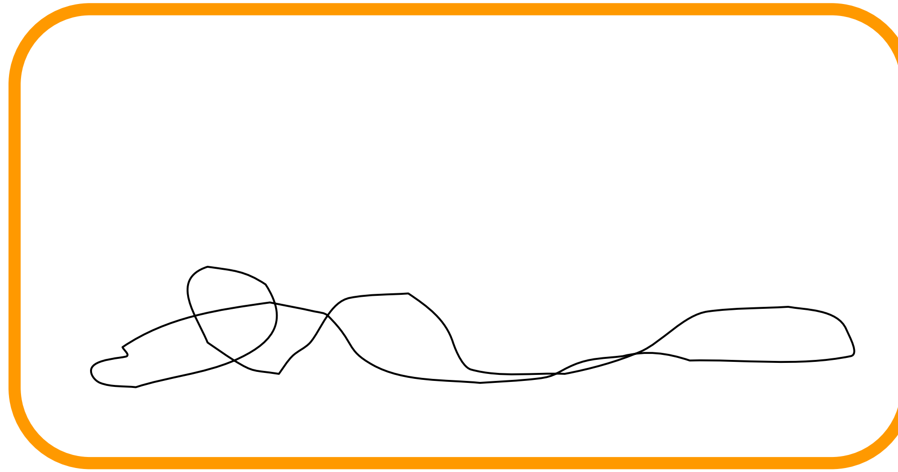


L'azotofissazione simbiotica **costa** comunque alla pianta, in termini **di C fotosintato, dal 15 al 30%** *per fissazione di* N_2 *e assimilazione di* NH_4^+

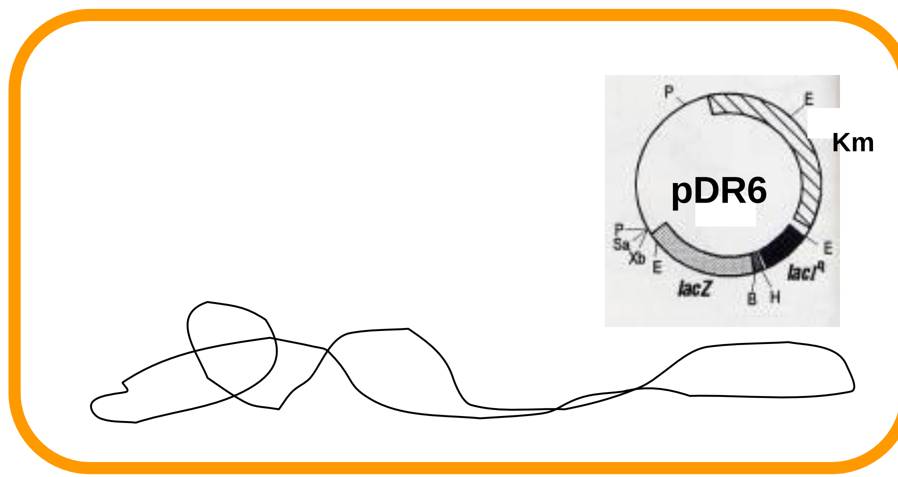

Non-inoculation
Inoculation with Y629


Uninoculated
Inoculated

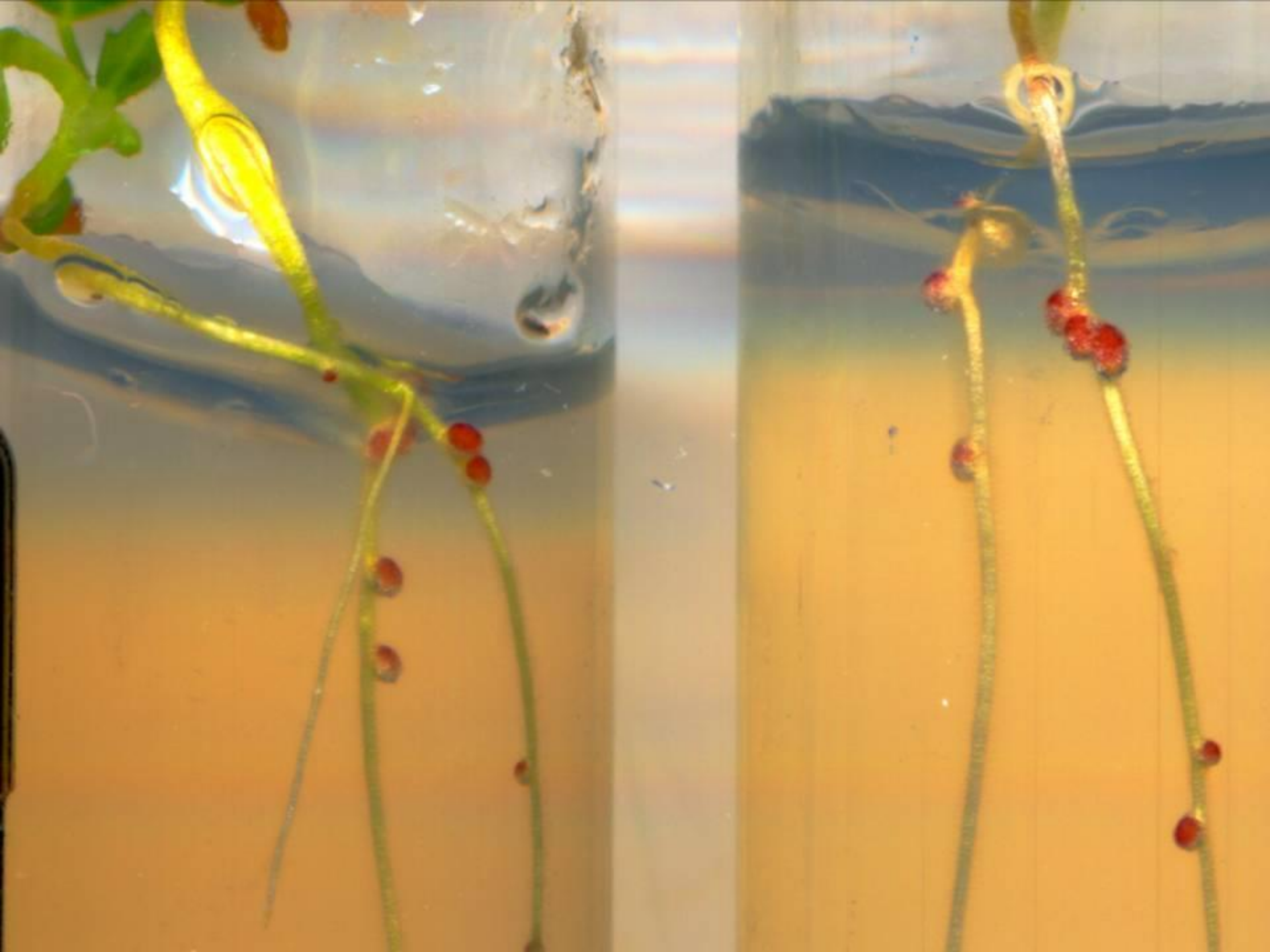


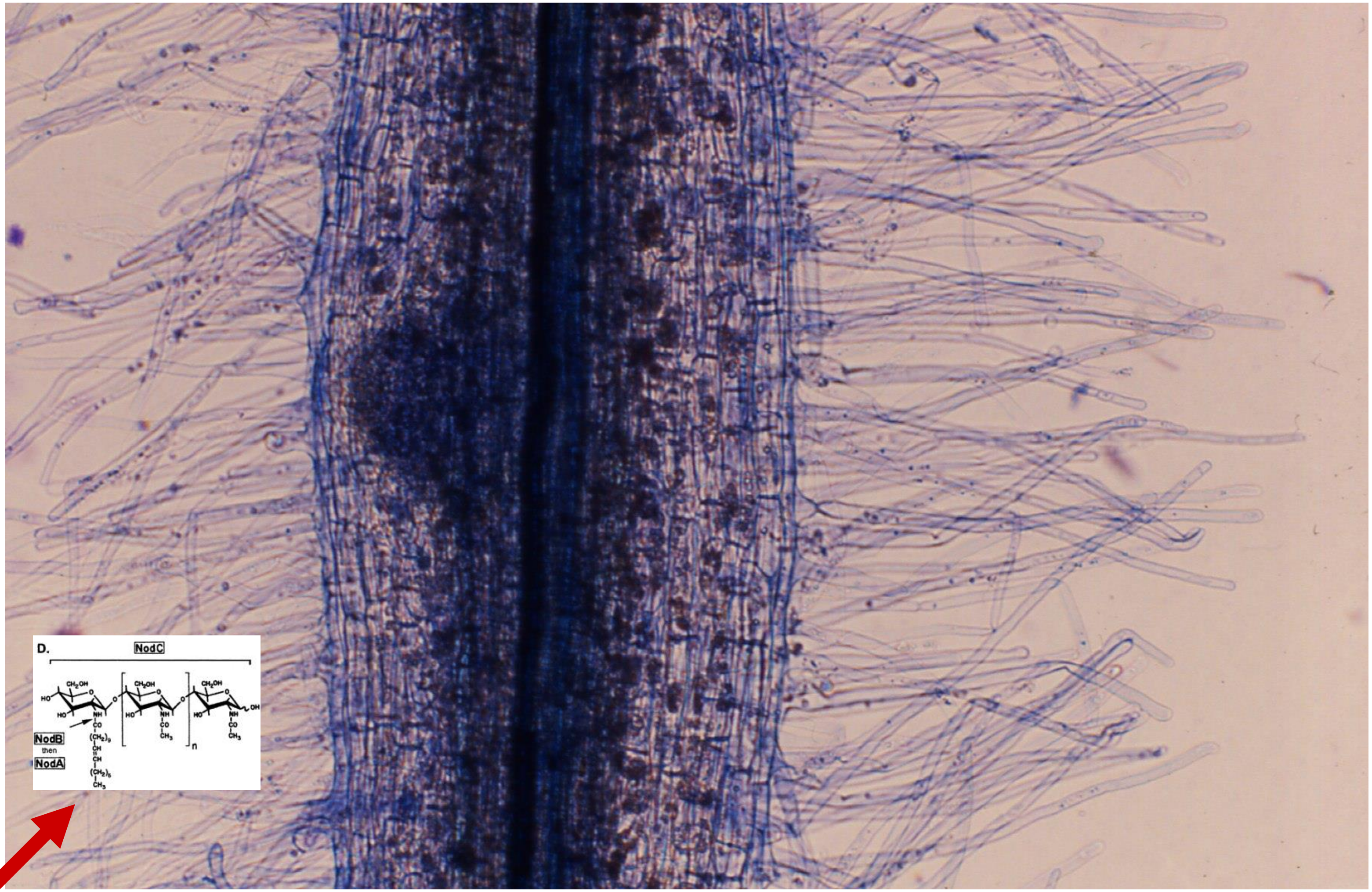


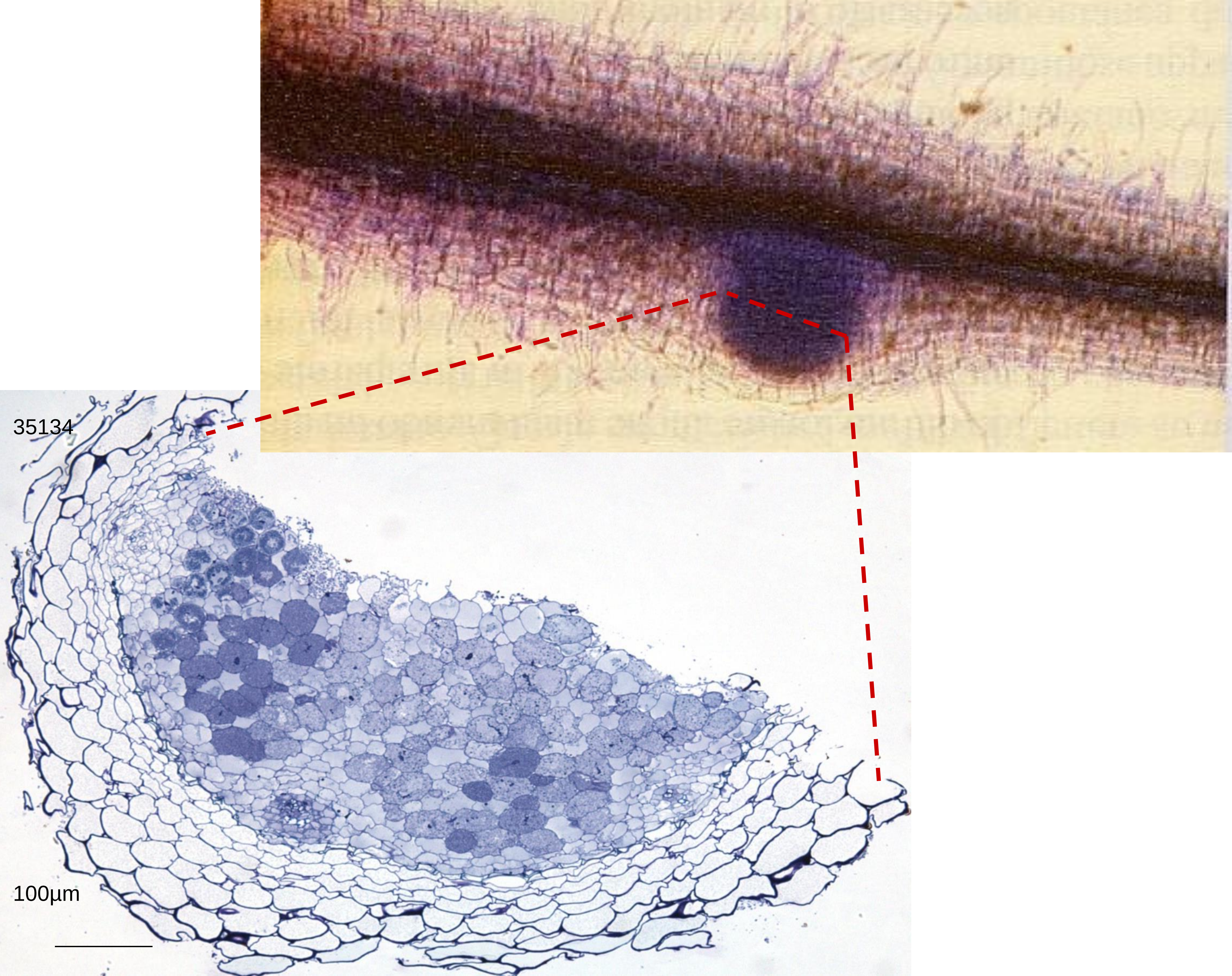
R. l. bv. trifolii

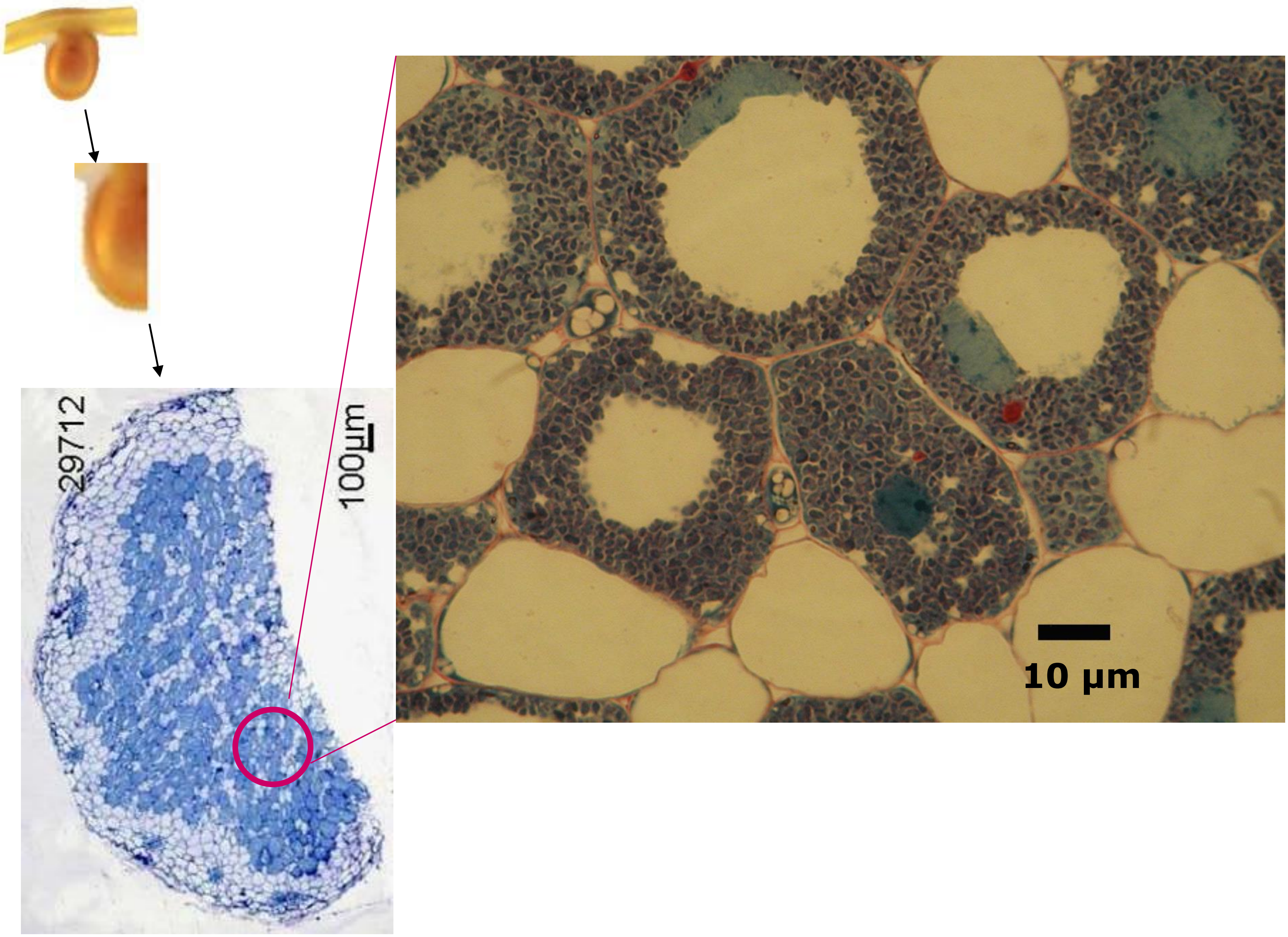


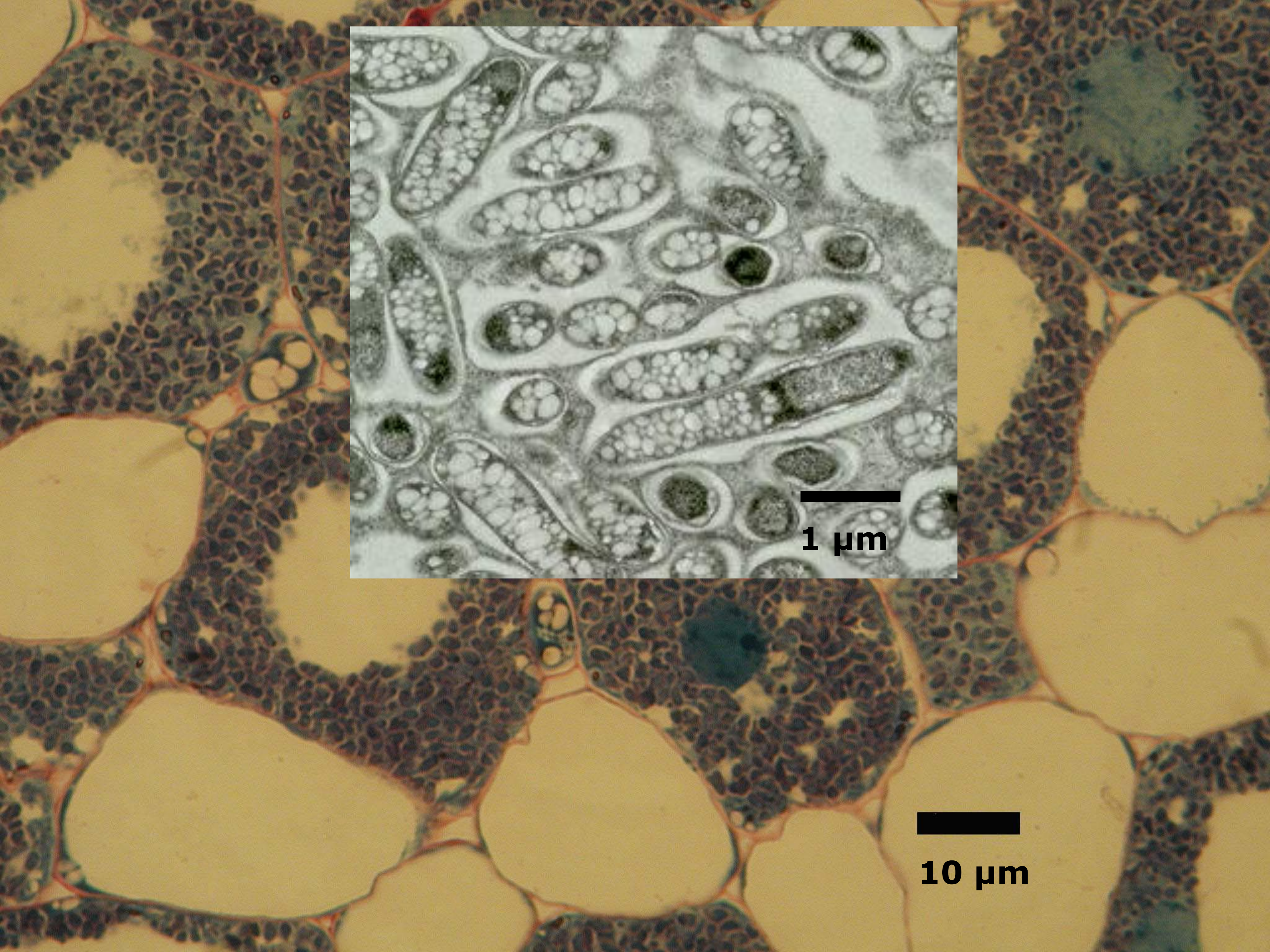
R. l. bv. trifolii pDR6

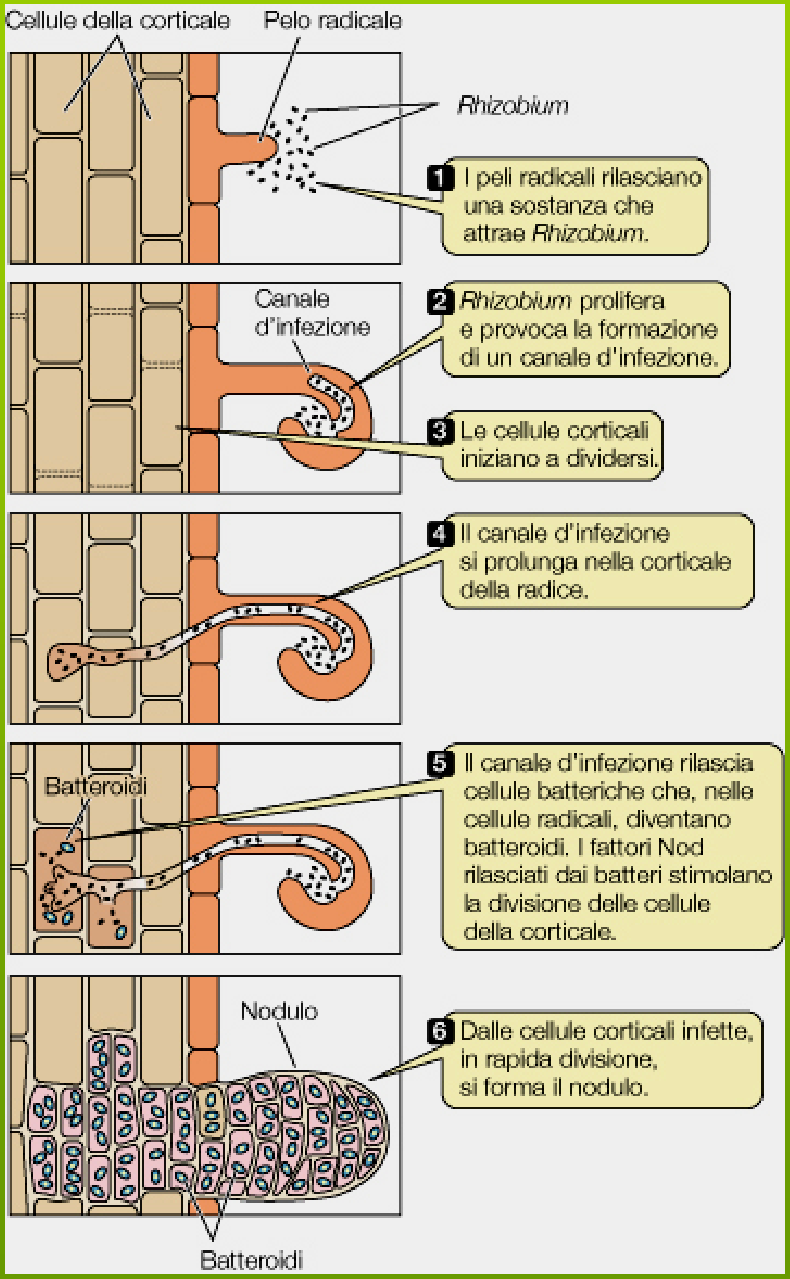


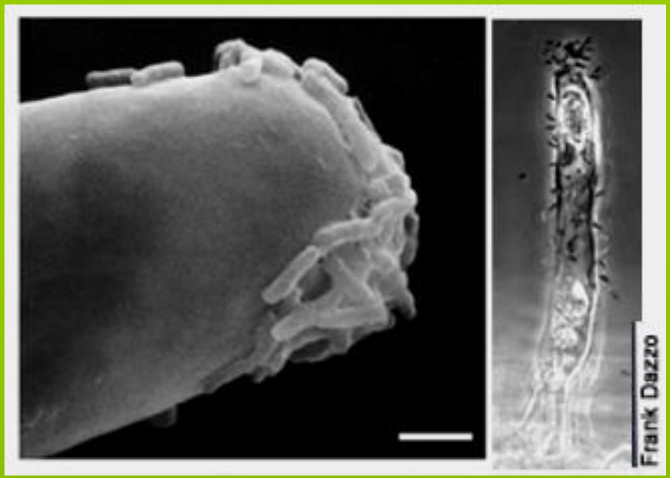

D.
NodC
NodB
then
NodA


35134
100µm

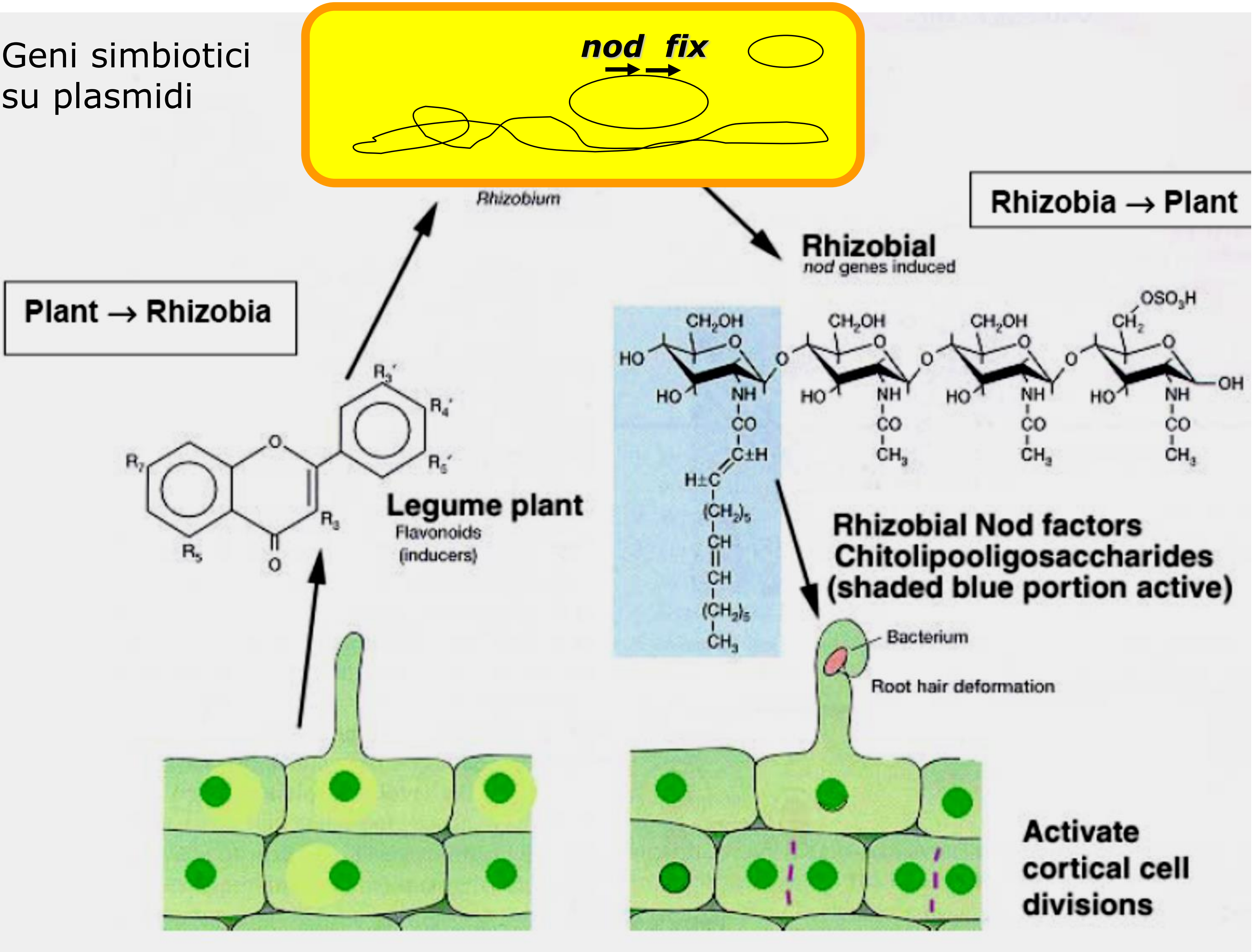

29712
100µm
10 µm


1 µm
10 µm


Cellule della corticale
Pelo radicale
Rhizobium
1 I peli radicali rilasciano una sostanza che attrae Rhizobium.
Canale d'infezione
2 Rhizobium prolifera e provoca la formazione di un canale d'infezione.
3 Le cellule corticali iniziano a dividersi.
4 Il canale d'infezione si prolunga nella corticale della radice.
Batteroidi
5 Il canale d'infezione rilascia cellule batteriche che, nelle cellule radicali, diventano batteroidi. I fattori Nod rilasciati dai batteri stimolano la divisione delle cellule della corticale.
Nodulo
6 Dalle cellule corticali infette, in rapida divisione, si forma il nodulo.
Batteroidi

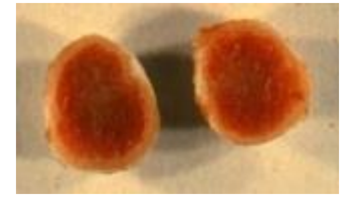

Frank Dazzo

Geni simbiotici
su plasmidi



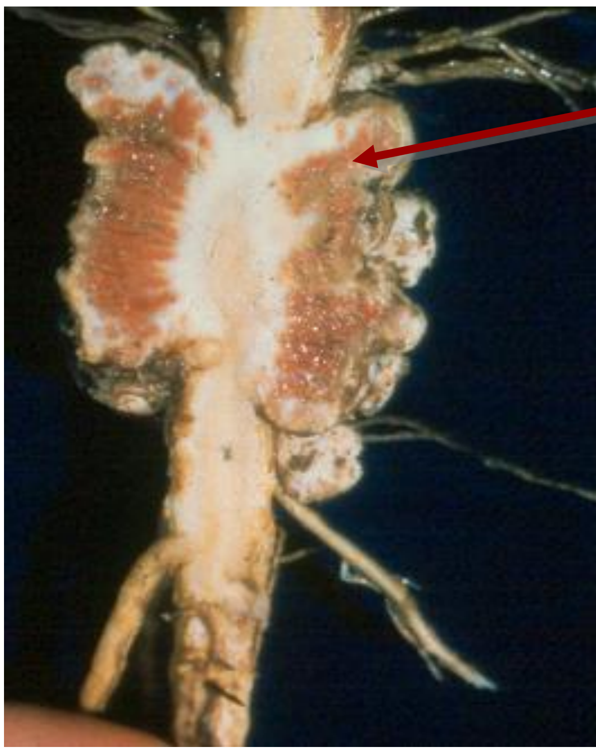
La NITROGENASI

è sensibile all'ossigeno molecolare



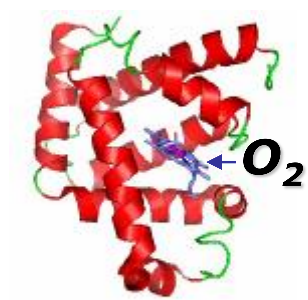
Rizobi → aerobi, simbionti (*Leguminosae*)

compartimentalizzazione → **noduli (tubercoli) radicali**

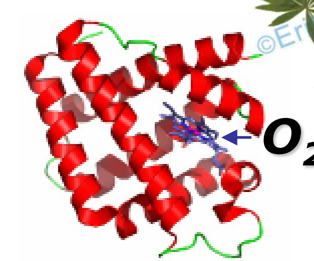


Leg-emoglobina

Proteina con eme e ferro con funzione di legare ossigeno, in analogia con emoglobine e mioglobine animali



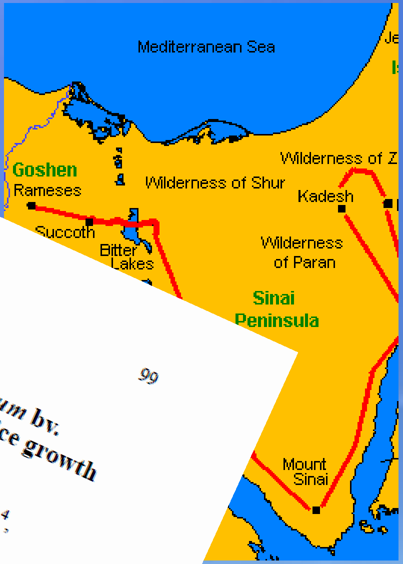
Mioglobina di capodoglio



Leg-emoglobina di lupino


Più di 140 Kg di azoto atmosferico fissato per ettaro e per anno,
Grazie alla simbiosi tra batteri e leguminose.

Rizobi nelle gran



Plant and Soil **194**: 99–114, 1997.

Natural endophytic association between *Rhizobium leguminosarum* bv. *trifoli* and rice roots and assessment of its potential to promote rice growth

Youssef G. Yanni[1], R.Y. Rizk[1], V. Corich[2], A. Squartini[2], K. Ninke[3,4], S. Philip-Hollingsworth[3], G. Orgambide[3], F. de Bruijn[3,4,5], J. Stoltzfus[5], D. Buckley[3,4], T.M. Schmidt[3,4], P.F. Mateos[6], J.K. Ladha[7] and Frank B. Dazzo[3,4,8]

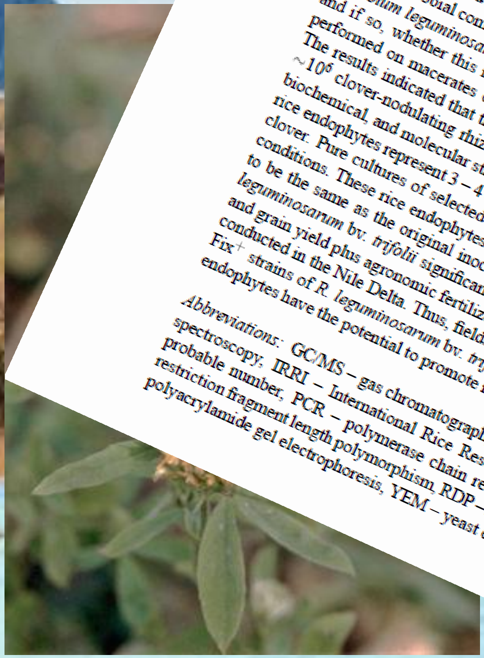
*[1]Sakha Agricultural Research Station, Kafr El-Sheikh, 33717 A. R. Egypt; [2]Dipt. di Biotecnologie Agrarie, Universita Degli Studi di Padova, Padova, Italy; [3]Dept. of Microbiology, [4]Center for Microbial Ecology and [5]Plant Research Laboratory, Michigan State University, East Lansing, MI 48824, U.S.A.; [6]Dept. de Microbiologia y Genetica, Universidad de Salamanca, Salamanca, Spain and [7]International Rice Research Institute, P.O. Box 933, 1099 Manila, Philippines. [8]Address all correspondence to Dr. Frank B. Dazzo, Dept. of Microbiology, Michigan State University, East Lansing, MI 48824, USA**

Key words: association, clover, endophyte, PGPR, *Rhizobium leguminosarum* bv. *trifolii*, rice, root, symbiosis

Abstract

For over 7 centuries, production of rice (*Oryza sativa* L.) in Egypt has benefited from rotation with Egyptian berseem clover (*Trifolium alexandrinum*). The nitrogen supplied by this rotation replaces 25–33% of the recommended rate of fertilizer-N application for rice production. This benefit to the rice cannot be explained solely by an increased availability of fixed N through mineralization of N- rich clover crop residues. Since rice normally supports a diverse microbial community of internal root colonists, we have examined the possibility that the clover symbiont, *Rhizobium leguminosarum* bv. *trifolii* colonizes rice roots endophytically in fields where these crops are rotated, and if so, whether this novel plant-microbe association benefits rice growth. MPN plant infection studies were performed on macerates of surface-sterilized rice roots inoculated on *T. alexandrinum* as the legume trap host. The results indicated that the root interior of rice grown in fields rotated with clover in the Nile Delta contained ~10^6 clover-nodulating rhizobial endophytes g^{-1} fresh weight of root. Plant tests plus microscopical, cultural, biochemical, and molecular structure studies indicated that the numerically dominant isolates of clover-nodulating rice endophytes represent 3–4 authentic strains of *R. leguminosarum* bv. *trifolii* that were Nod$^+$ Fix$^+$ on berseem clover. Pure cultures of selected strains were able to colonize the interior of rice roots grown under gnotobiotic conditions. These rice endophytes were reisolated from surface-sterilized roots and shown by molecular methods to be the same as the original inoculant strains, thus verifying Koch's postulates. Two endophytic strains of *R. leguminosarum* bv. *trifolii* significantly increased shoot and root growth of rice in growth chamber experiments, and grain yield plus agronomic fertilizer N-use efficiency of Giza-175 hybrid rice in a field inoculation experiment conducted in the Nile Delta. Thus, fields where rice has been grown in rotation with clover since antiquity contain Fix$^+$ strains of *R. leguminosarum* bv. *trifolii* that naturally colonize the rice root interior, and these true rhizobial endophytes have the potential to promote rice growth and productivity under laboratory and field conditions.

Abbreviations: GC/MS – gas chromatography-mass spectrometry, ^{1}H-NMR – proton nuclear magnetic resonance spectroscopy, IRRI – International Rice Research Institute, LSD – least significant difference, MPN – most probable number, PCR – polymerase chain reaction, PGPR – plant growth promoting rhizobacteria, RFLP – restriction fragment length polymorphism, RDP – ribosomal database project, SDS-PAGE – sodium dodecylsulfate-polyacrylamide gel electrophoresis, YEM – yeast extract mannitol



Trifolium alexandrinum

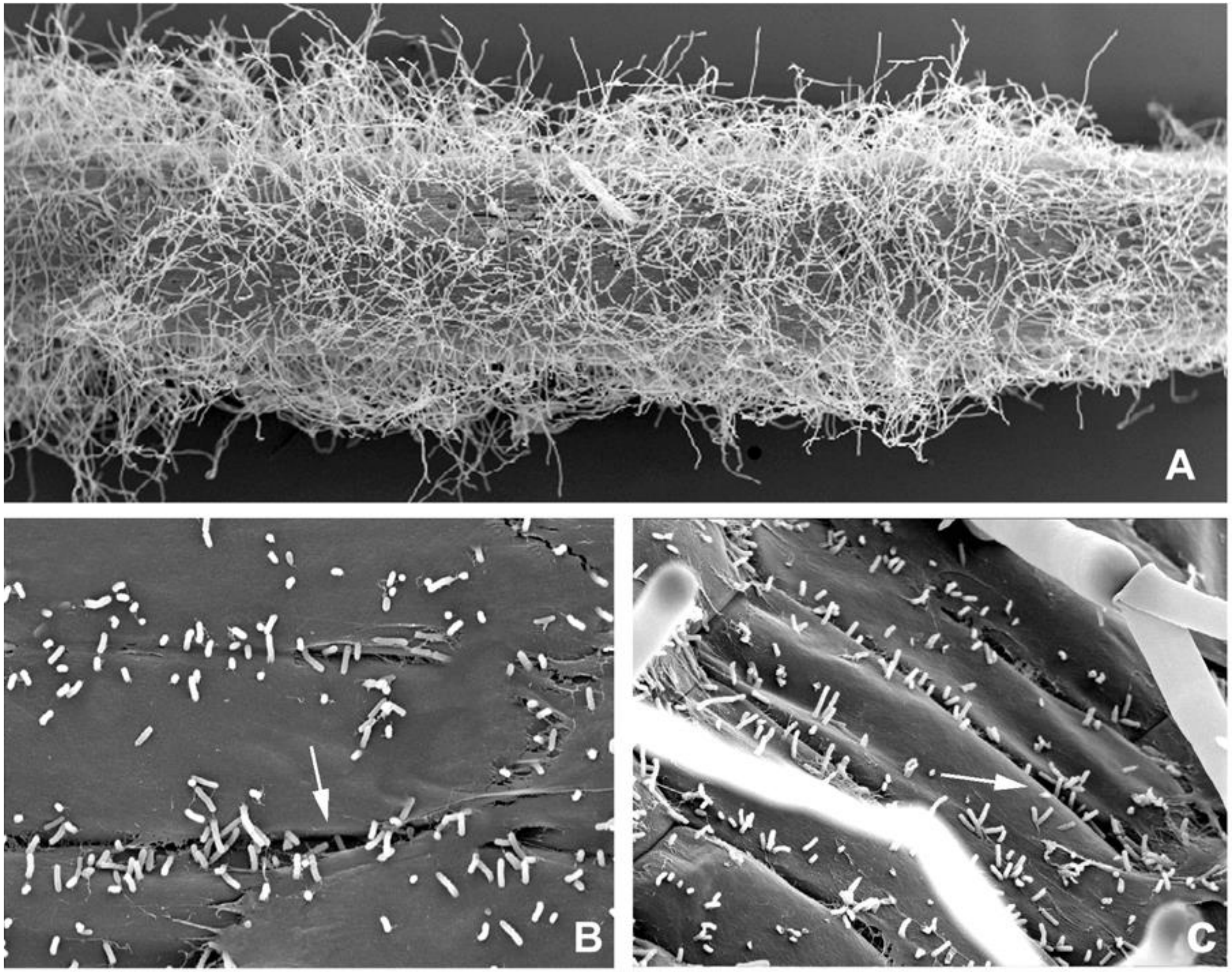


Fig. 12. Scanning electron microscopy of Sakha 102 rice roots colonized by *Rhizobium leguminosarum* bv. trifolii strain E11. (*A*) Low-magnification micrograph showing a 4-mm rootlet segment with numerous root hairs and 'windows' between them providing opportunity to view the rhizoplane surface. (*B*, *C*) Higher-magnification micrographs showing the colonization of the epidermal surface by the bacteria. Arrows point to localized sites where the bacteria have entered small crevices at junctions between epidermal cells.

Asia Pacific J Clin Nutr 2004;13 (2):217-220 217

Original Article

Legumes: the most important dietary predictor of survival in older people of different ethnicities

Irene Darmadi-Blackberry MB, PhD[1], Mark L Wahlqvist AO, MD[2], Antigone Kouris-Blazos PhD[2], Bertil Steen MD, PhD[3], Widjaja Lukito MD, PhD[4], Yoshimitsu Horie PhD[5] and Kazuyo Horie BSc[6]

[1]*Public Health Division, National Ageing Research Institute, Melbourne, Australia*
[2]*Asia Pacific Health & Nutrition Centre, Monash Asia Institute, Monash University, Australia*
[3]*Department of Geriatric Medicine, Goteborg University, Goteborg, Sweden*
[4]*SEAMEO TROPMED, University of Indonesia, Jakarta, Indonesia*
[5]*School of Humanities and Social Sciences, Nagoya City University, Nagoya, Japan*
[6]*Faculty of Home Economics, Aichi Gakusen, Okazaki, Japan*

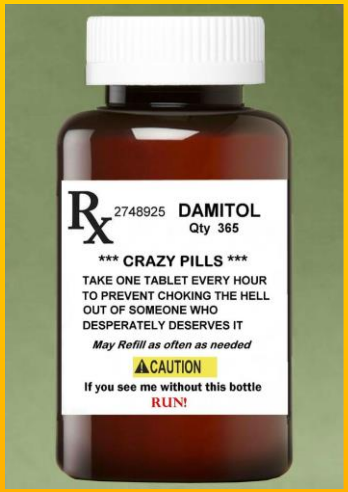
To identify protective dietary predictors amongst long-lived elderly people (N=785), the *"Food Habits in Later Life"* (FHILL) study was undertaken among five cohorts in Japan, Sweden, Greece and Australia. Between 1988 and 1991, baseline data on food intakes were collected. There were 785 participants aged 70 and over that were followed up to seven years. Based on an alternative Cox Proportional Hazard model adjusted to age at enrolment (in 5-year intervals), gender and smoking, the legume food group showed 7-8% reduction in mortality hazard ratio for every 20g increase in daily intake with or without controlling for ethnicity (RR 0.92; 95% CI 0.85-0.99 and RR 0.93; 95% CI 0.87-0.99, respectively). Other food groups were not found to be consistently significant in predicting survival amongst the FHILL cohorts.

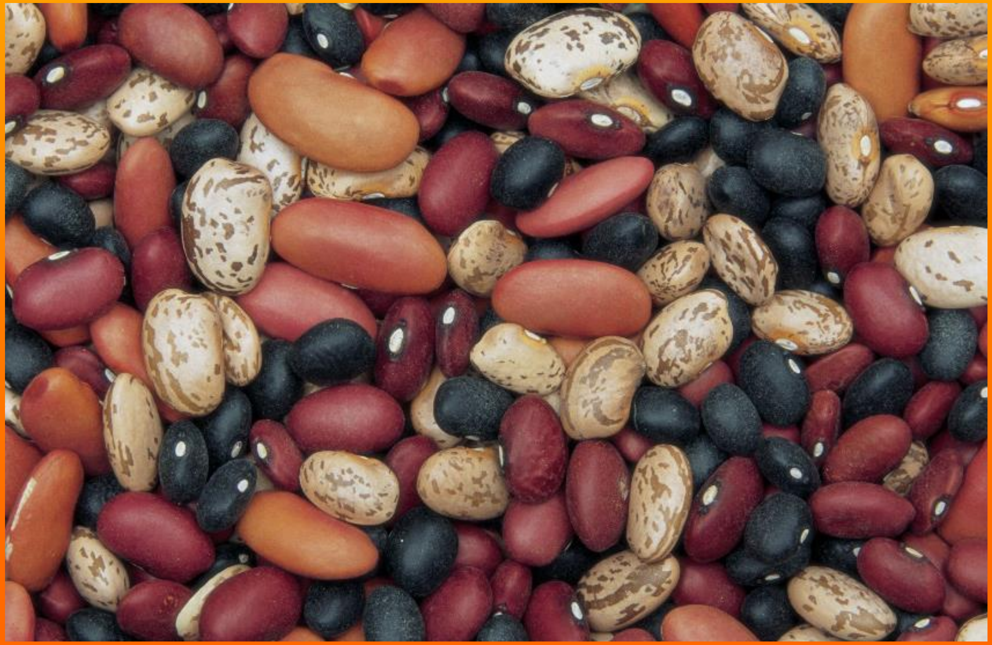
Key words: legumes, diet, survival, FHILL, mortality, food intake, elderly, Australia, Greece, Japan, Sweden, fish, fat ra

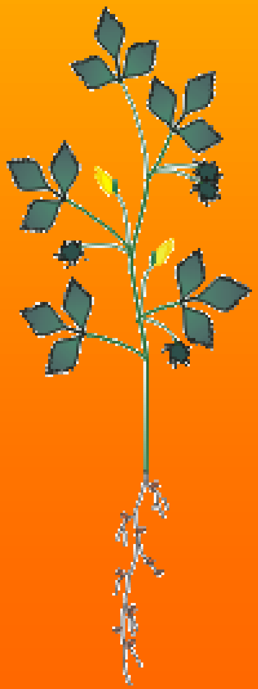
Introduction

It is becoming apparent that people of various nations with different food cultures can have comparable life expectancy and morbidity rates.[1] The '*Food Habits in Later Life*' (FHILL)[2] is a cross-cultural study conducted under the auspices of the International Union of Nutritional Sciences (IUNS) and the World Health Organization (WHO). The FHILL studies have concentrated on food intake and food intake patterns as differentiators and common denominators in health susceptibility and for sur-

We have previously demonstrated that a plant bas (similar to the traditional mediterranean diet) includes fish is the most important predictor of s amongst FHILL cohorts *(in press)*. This resu significant after accounting for other non-nut variables such as smoking status, gender, exercise status, social activity, activities of daily livi memory status in one Cox Proportional Hazard Details of this analysis are discussed elsewhere *(in press)*.


Rx 2748925 DAMITOL
Qty 365
*** CRAZY PILLS ***
TAKE ONE TABLET EVERY HOUR
TO PREVENT CHOKING THE HELL
OUT OF SOMEONE WHO
DESPERATELY DESERVES IT
May Refill as often as needed
CAUTION
If you see me without this bottle
RUN!






Grazie per l’attenzione