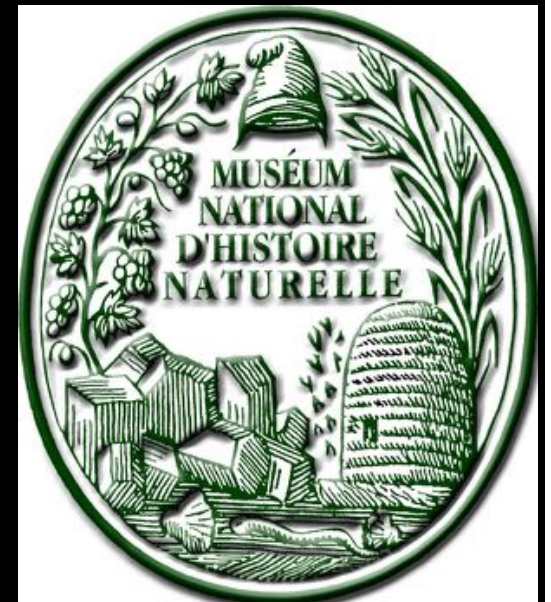


Les orchidées et leurs mycorhizes

M.-A. SELOSSE

Muséum nat. d'Histoire naturelle, Paris

Universités de Gdansk (Pologne) & Viçosa (Brésil)



Les réseaux mycorhiziens

Mycohétérotrophie et réseaux

Mixotrophie et réseaux mycorhiziens

La difficile transition vers l'hétérotrophie

... due à un attachement fatal à la lumière ?

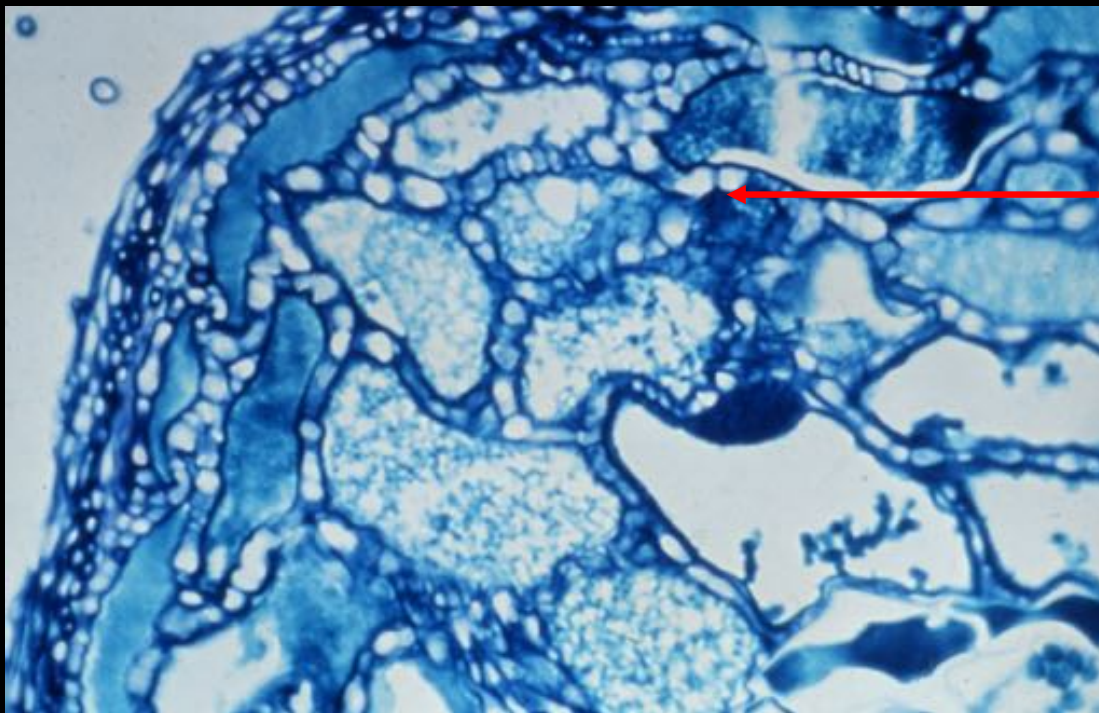
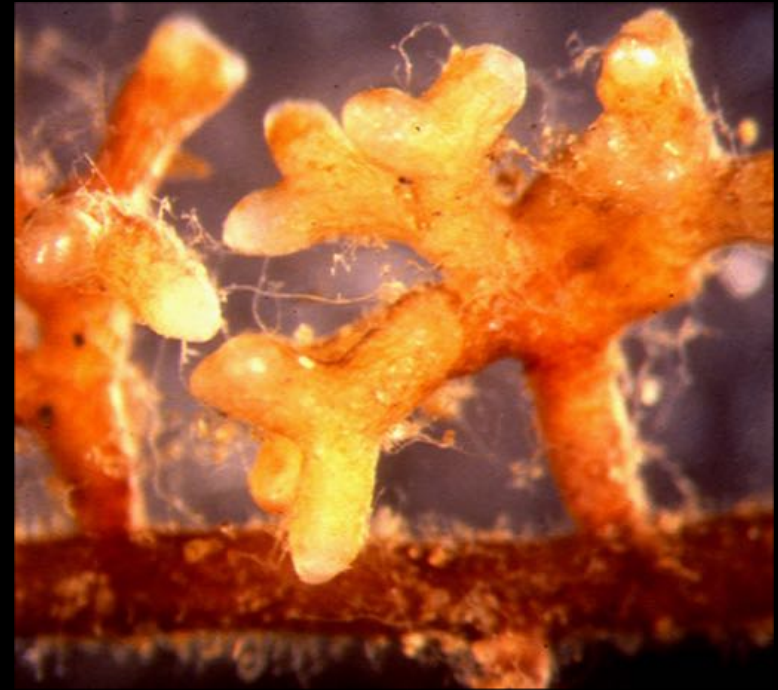
MYCORRHIZAL SYMBIOSIS

... a symbiosis between
two partners



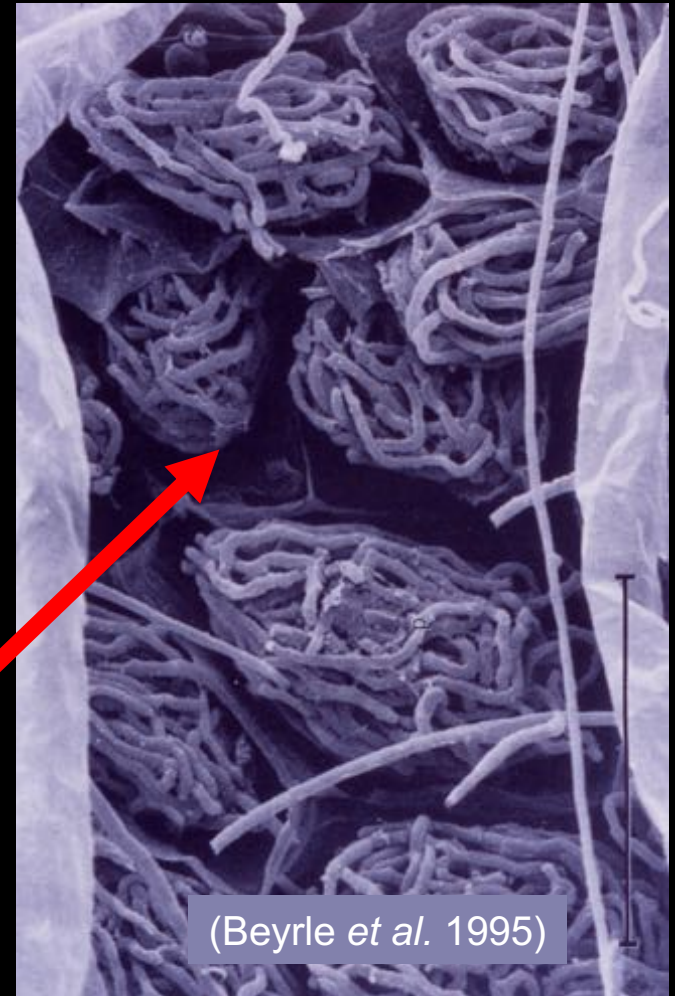
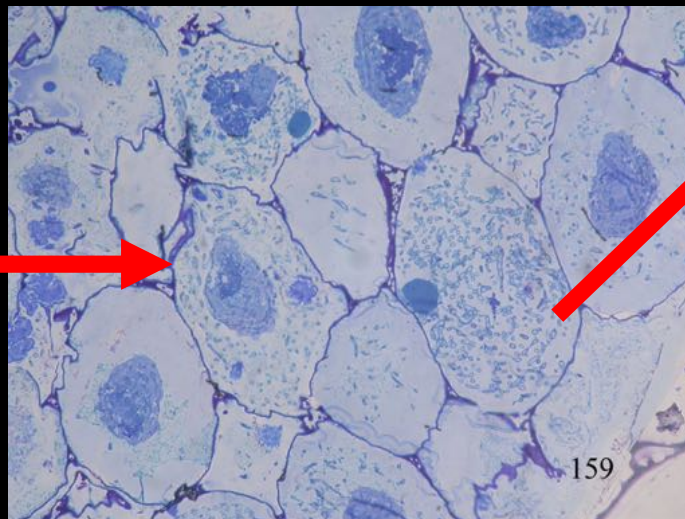
ECTO- MYCORRHIZAE

Asco- and Basidiomycetes on most trees in temperate regions



ORCHID MYCORRHIZAE

Intracellular pelotons formed within root cells, usually with « rhizoctonias »



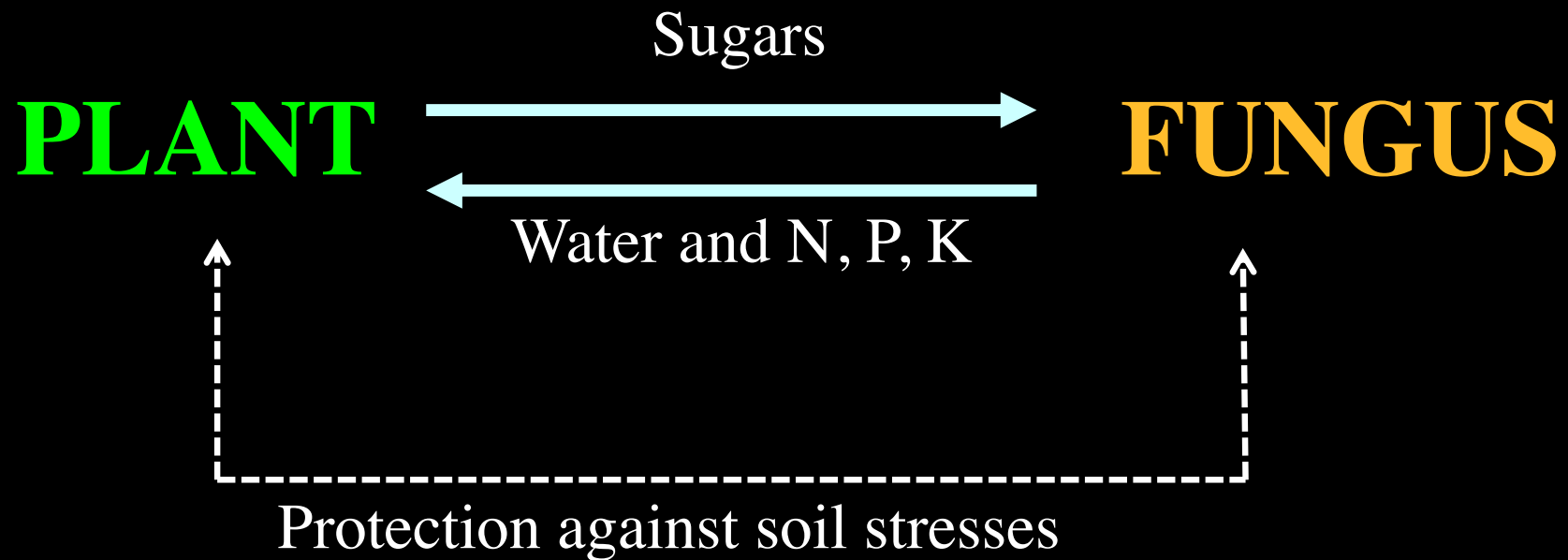


Orchid germination, usually involving rhizoctonias

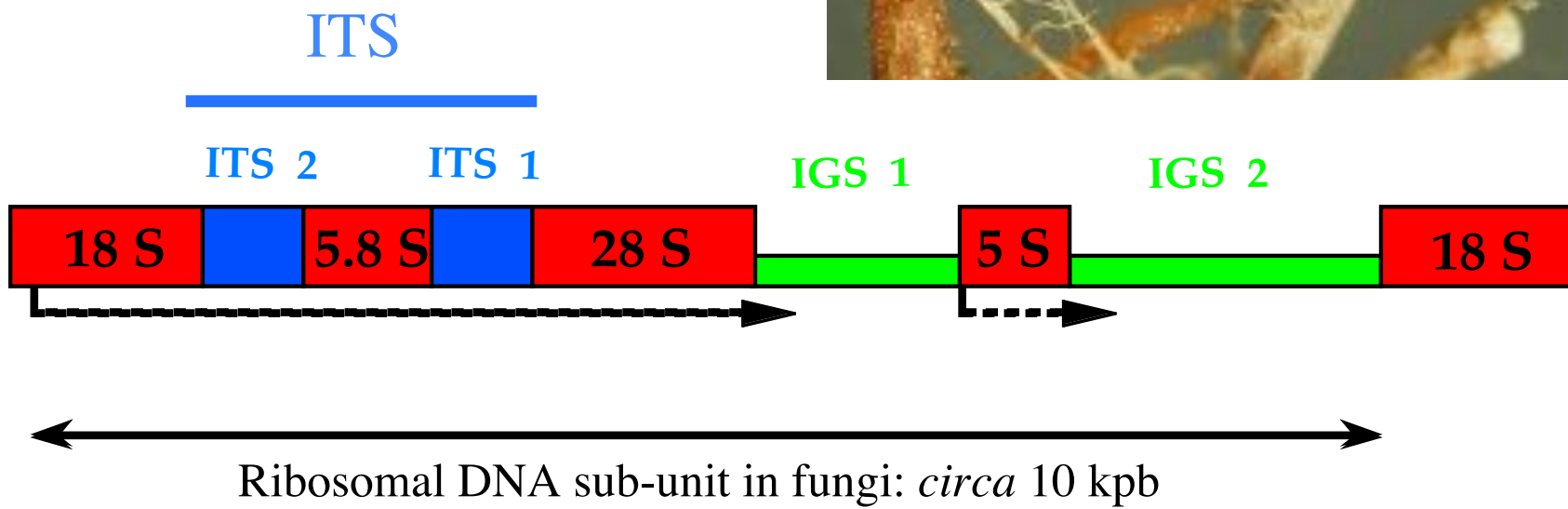
MYCORRHIZAL SYMBIOSIS

>85% of plants associate with soil fungi

Reciprocal nutrient exchanges + protection:



ITS BARCODING



Les réseaux mycorhiziens

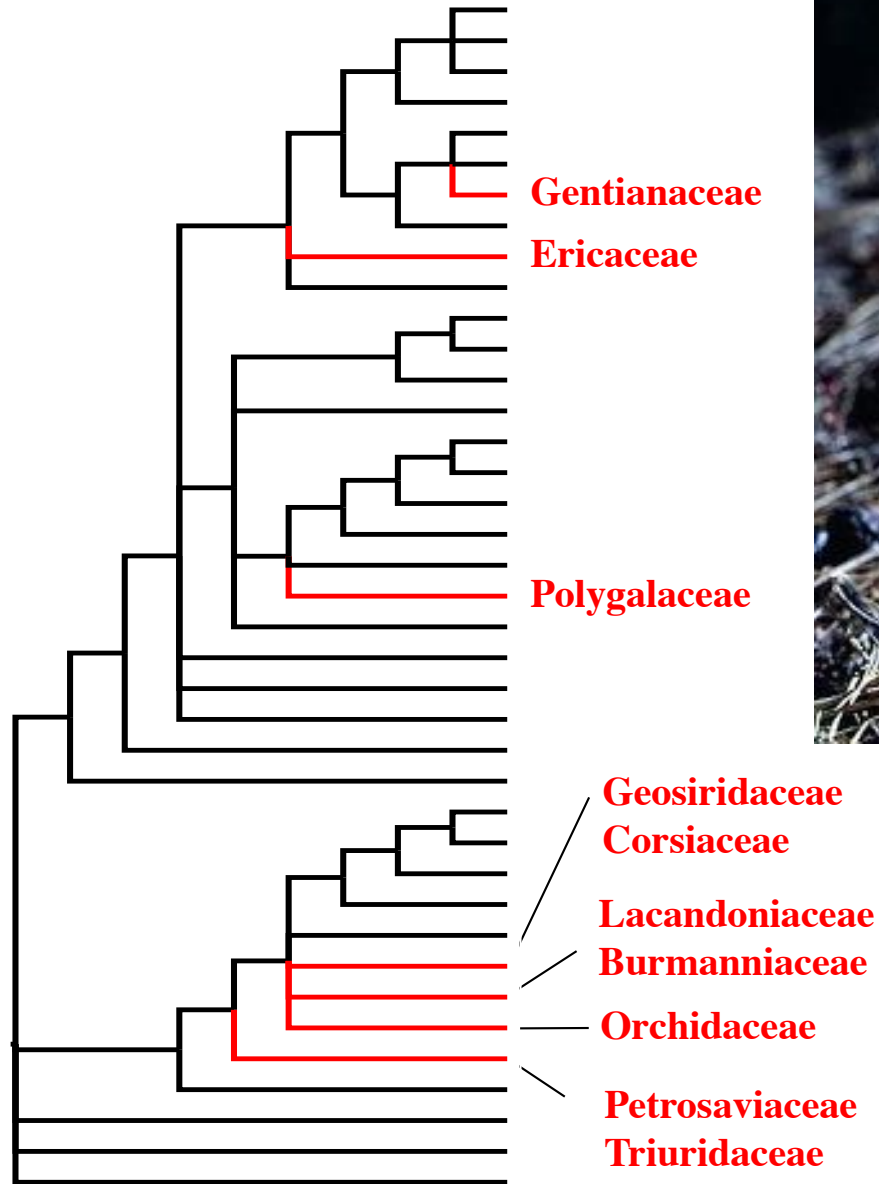
Mycohétérotrophie et réseaux

Mixotrophie et réseaux mycorhiziens

La difficile transition vers l'hétérotrophie

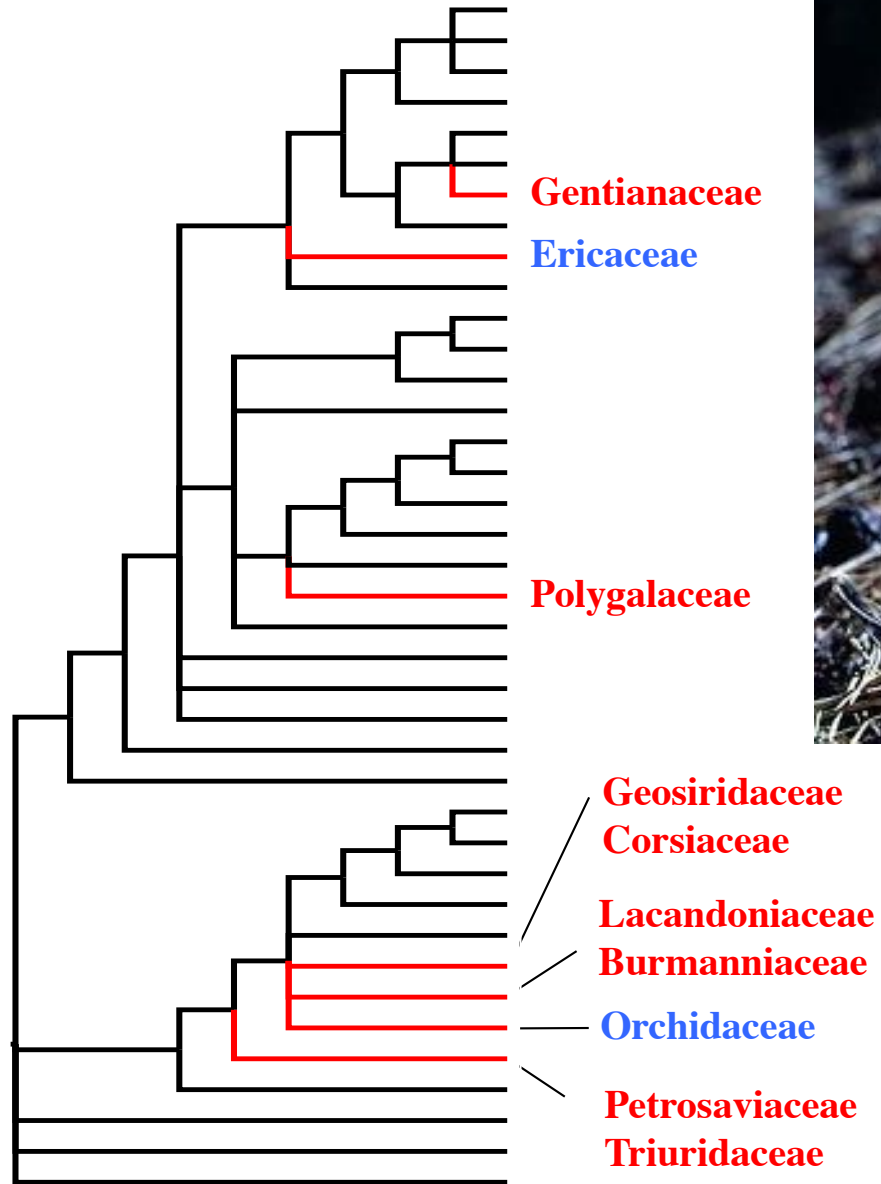
... due à un attachement fatal à la lumière ?

Mycoheterotrophs



(from Brundett, 2004)

Mycoheterotrophs



(from Brundett, 2004)

Neottia nidus-avis

Selosse *et al.*, 2002, *Mol. Ecol.* 11, 1831

McKendrick *et al.*, 2002, *New Phytol.* 145, 523

Selosse *et al.*, 2002, *New Phytol.* 155, 183

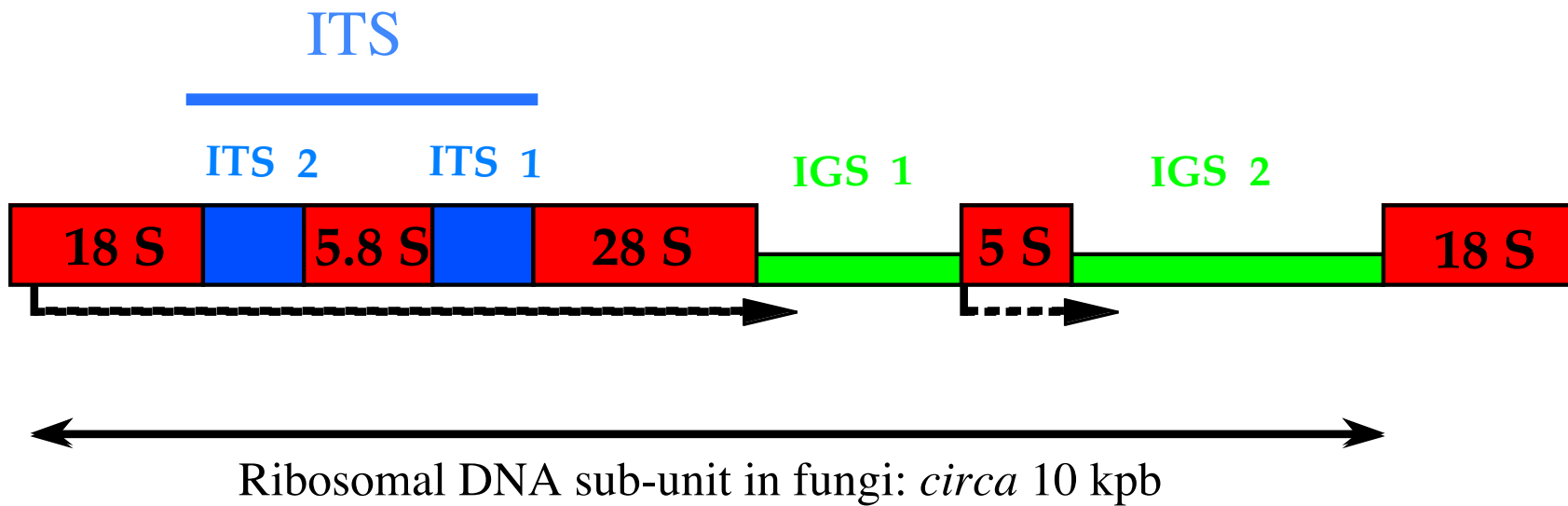


ITS BARCODING



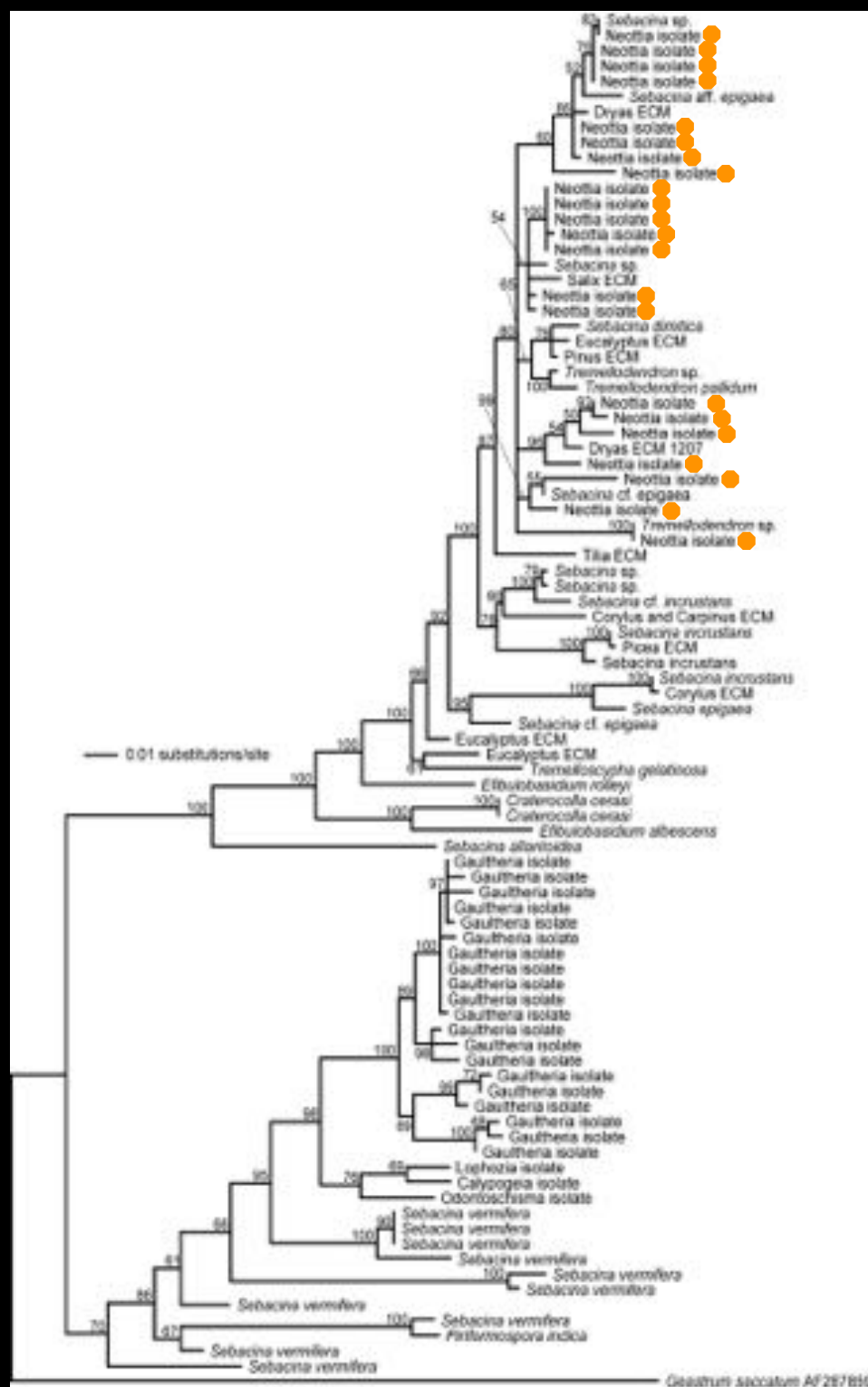
Taxonomic resolution :

- Genus / order
- Species / sub-species
- Sub-species / genet



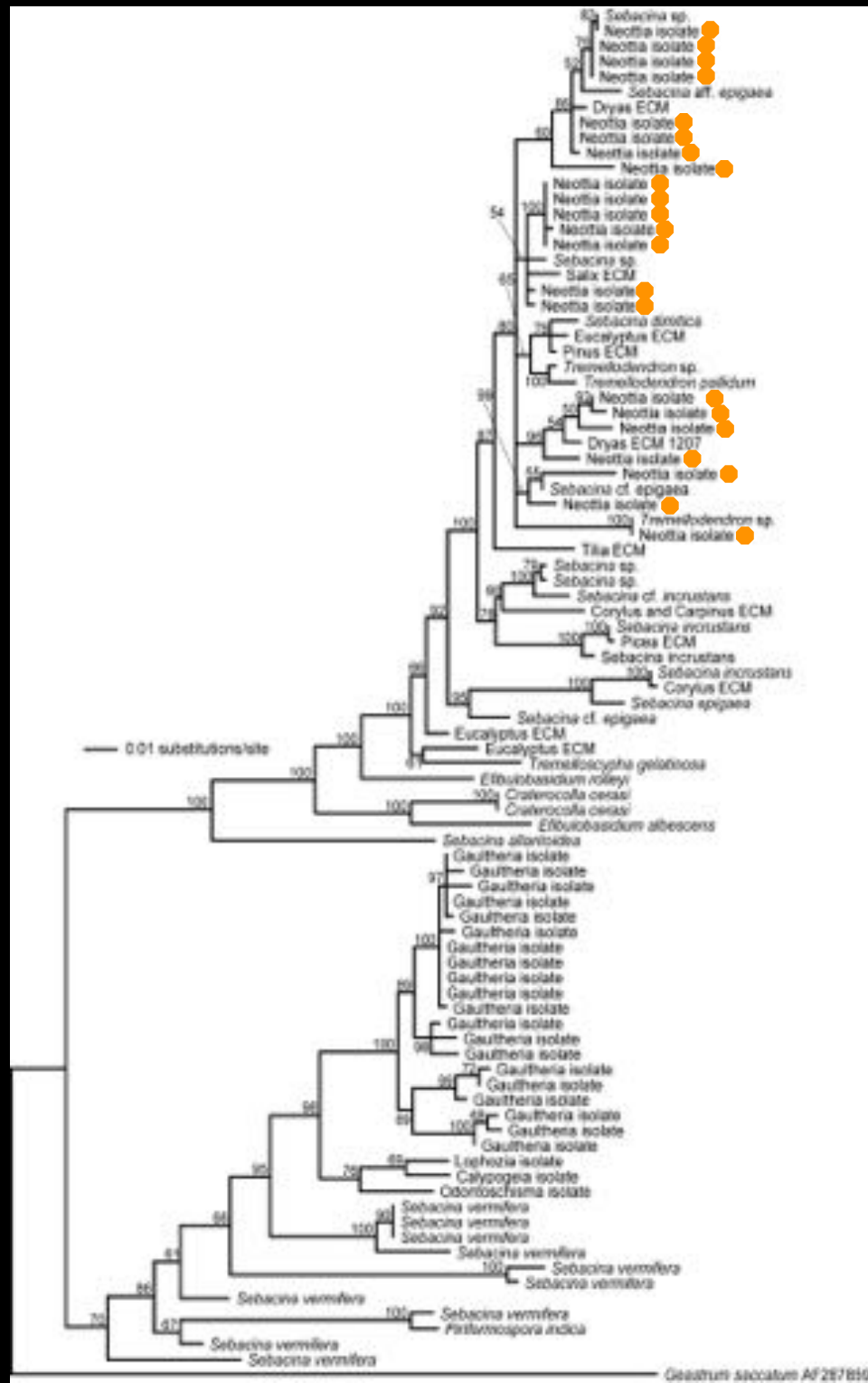
Neottia *nidus-avis*

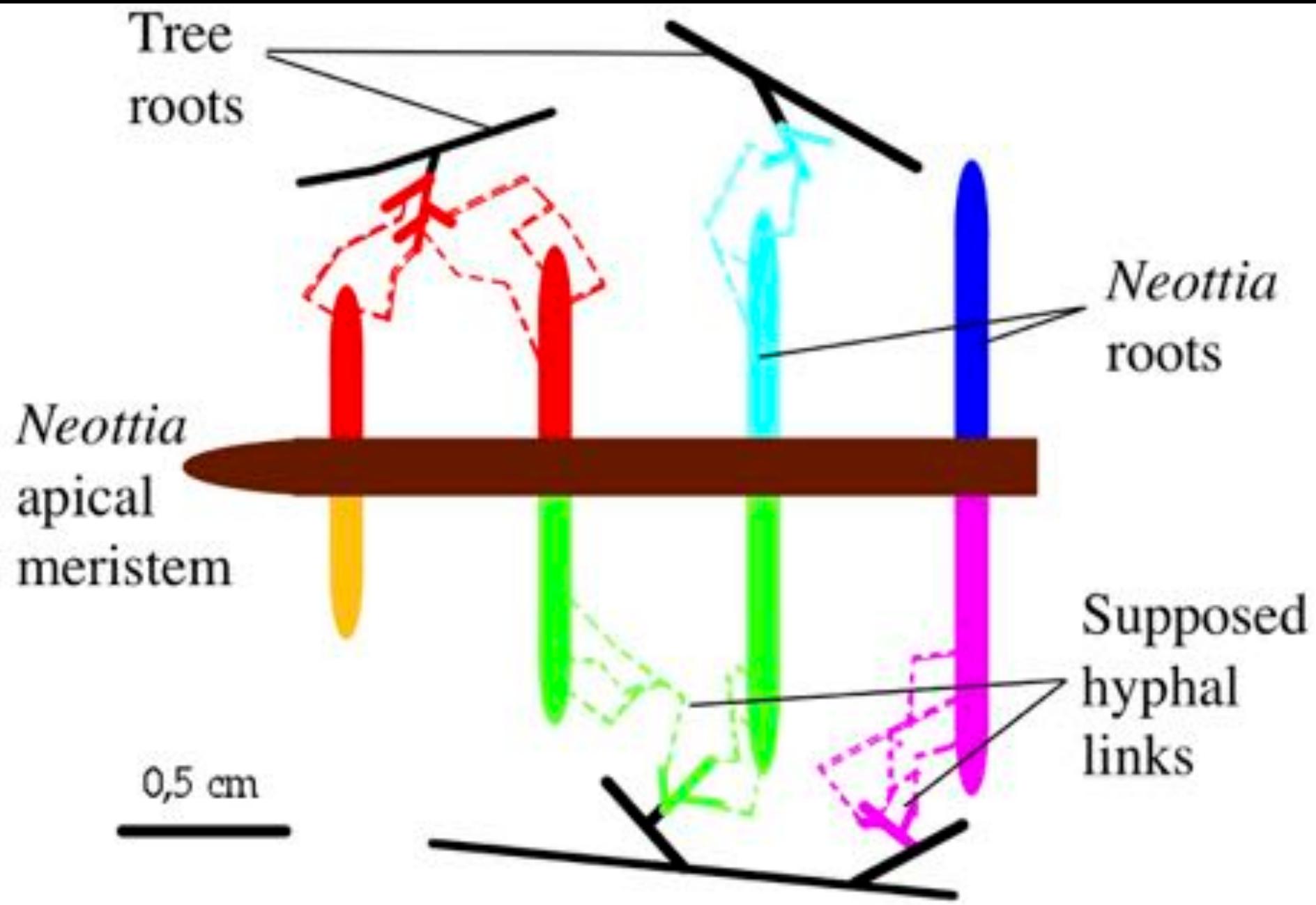
associated with Sebaciniales...



Neottia nidus-avis

associated with Sebaciniales...
themselves ectomycorrhizal
on nearby tree roots



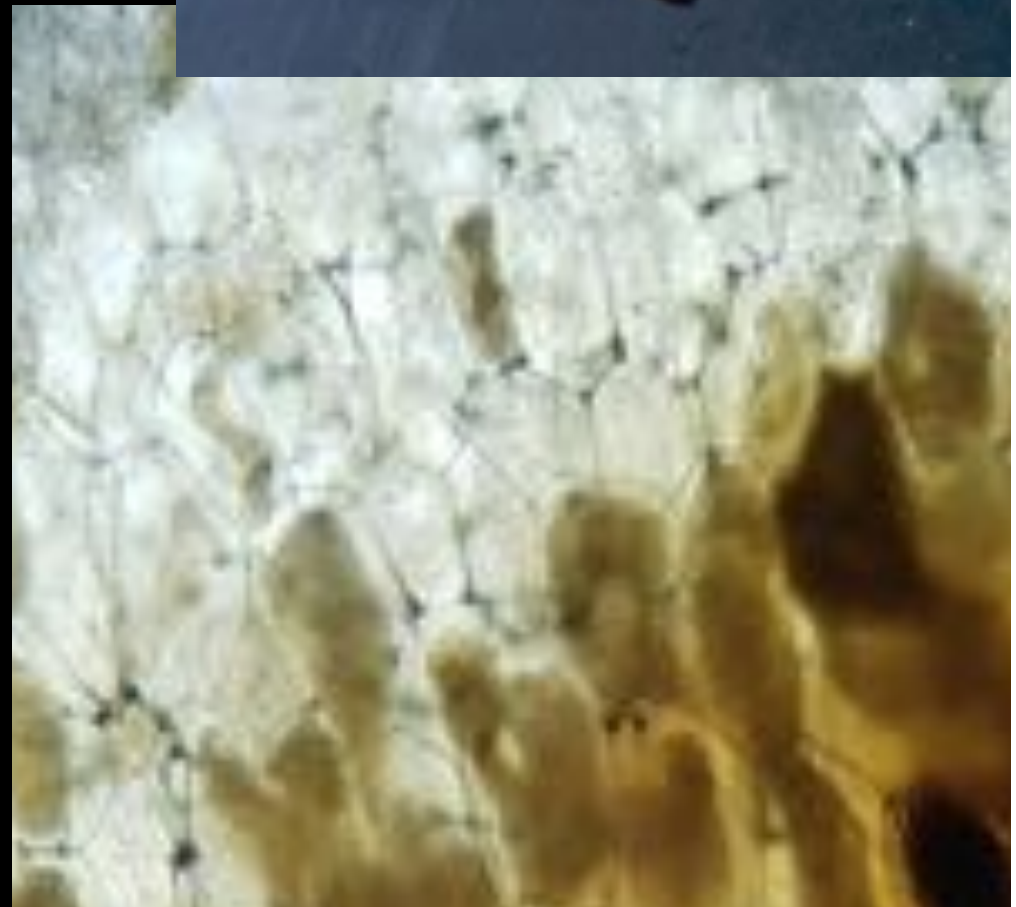


Selosse *et al.*, 2002

Epipogium aphyllum



Epipogium aphyllum



Epipogium aphyllum

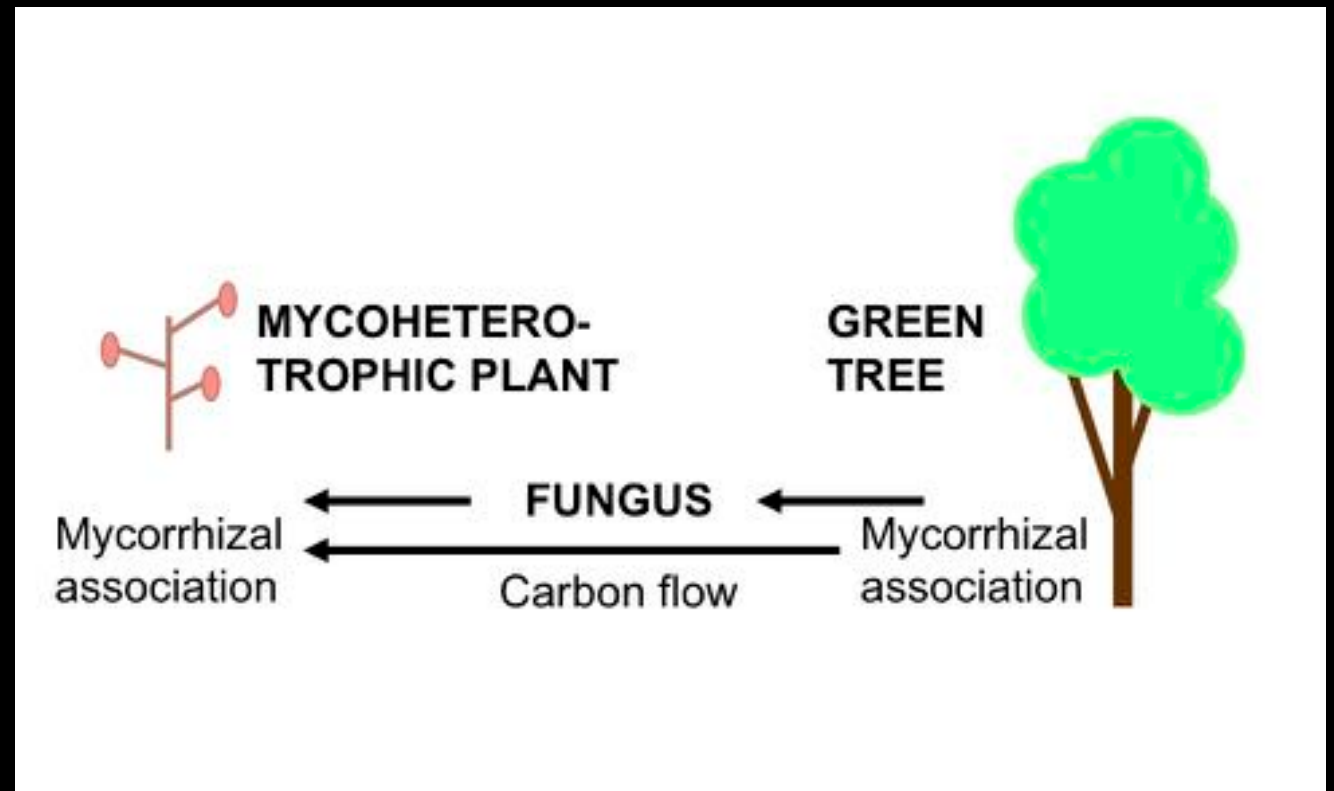
... with *Inocybe* species

Roy *et al.*, *Annals Bot.*, 2009.



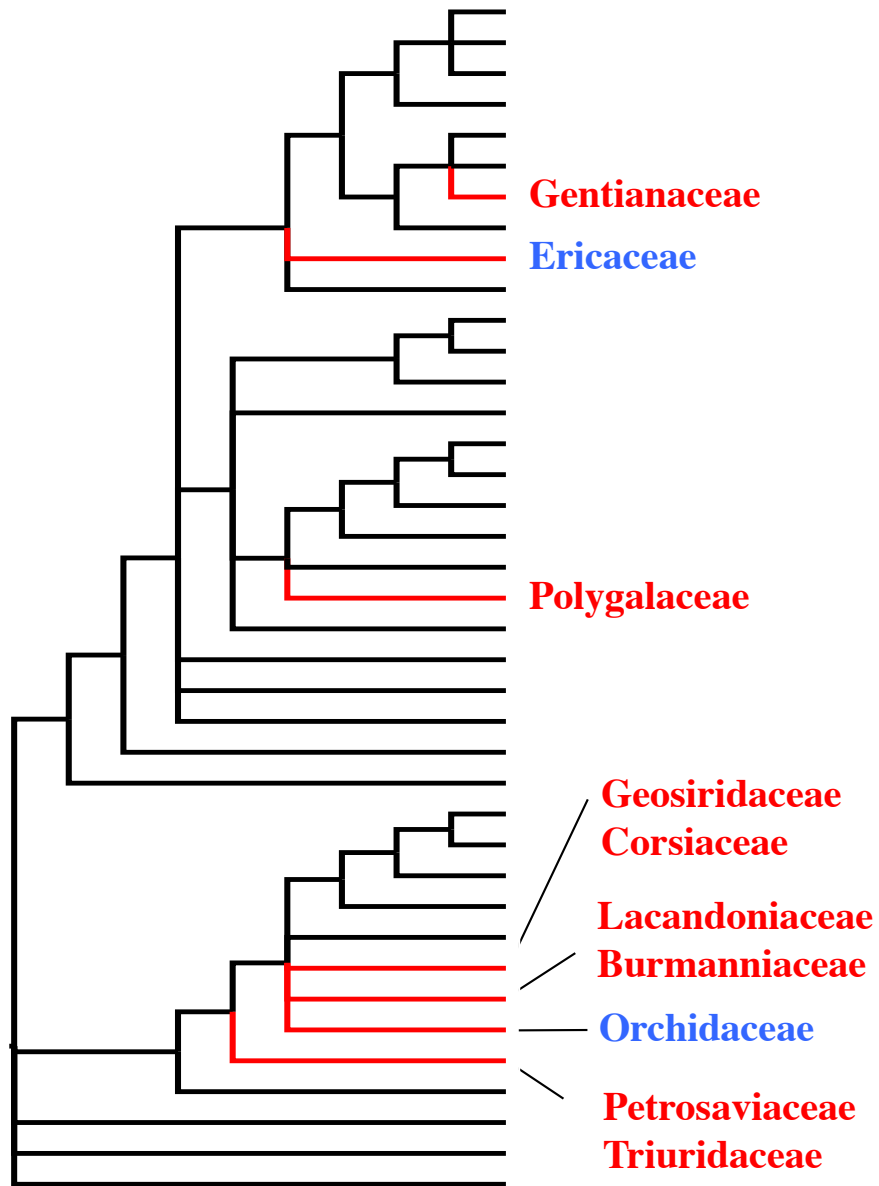
MYCOHETEROTROPHY

Exploiting the mycorrhizal network...



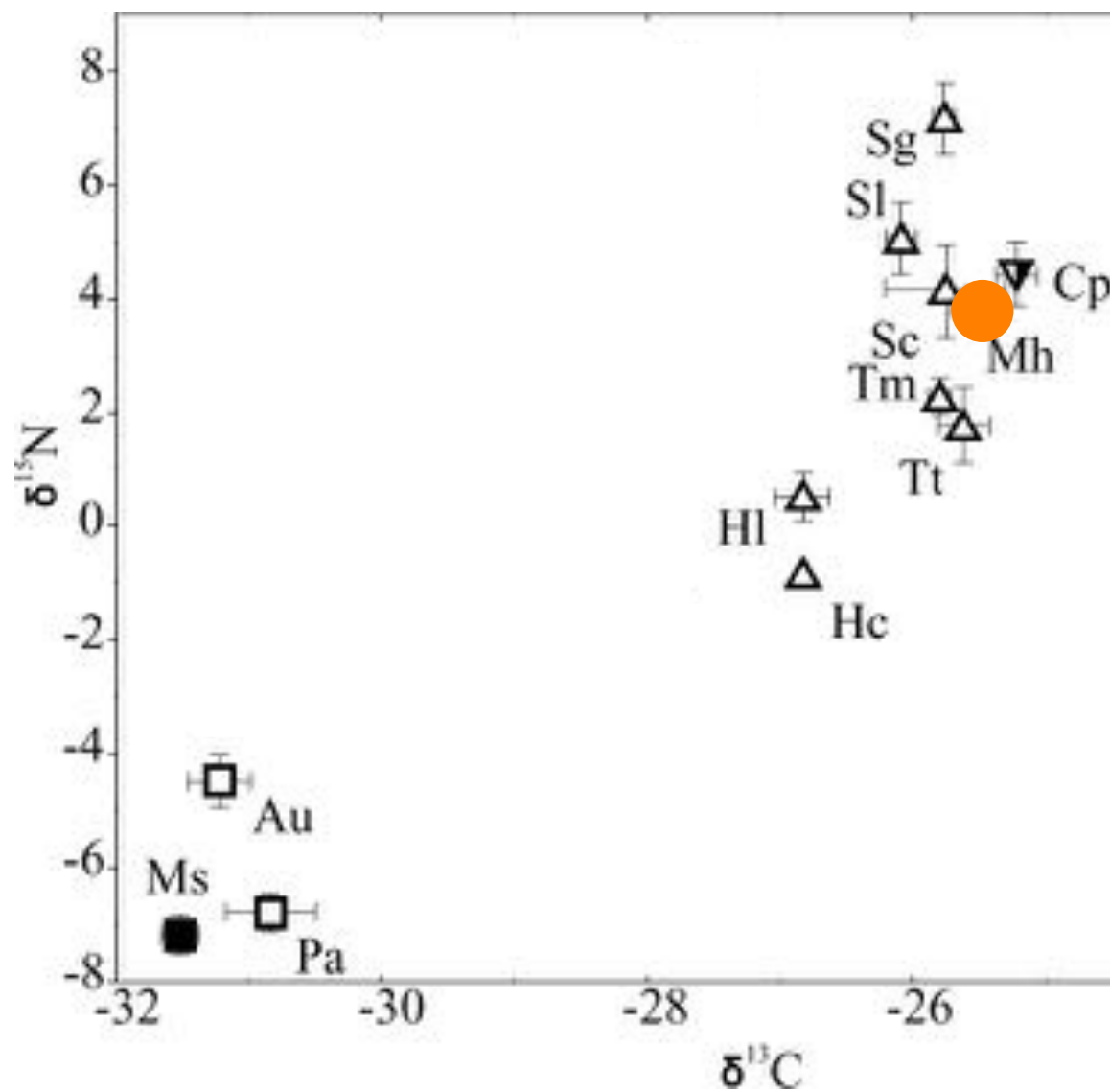
... as reflected in their spontaneous stable isotopes (^{13}C and ^{15}N) abundances

Mycoheterotrophs



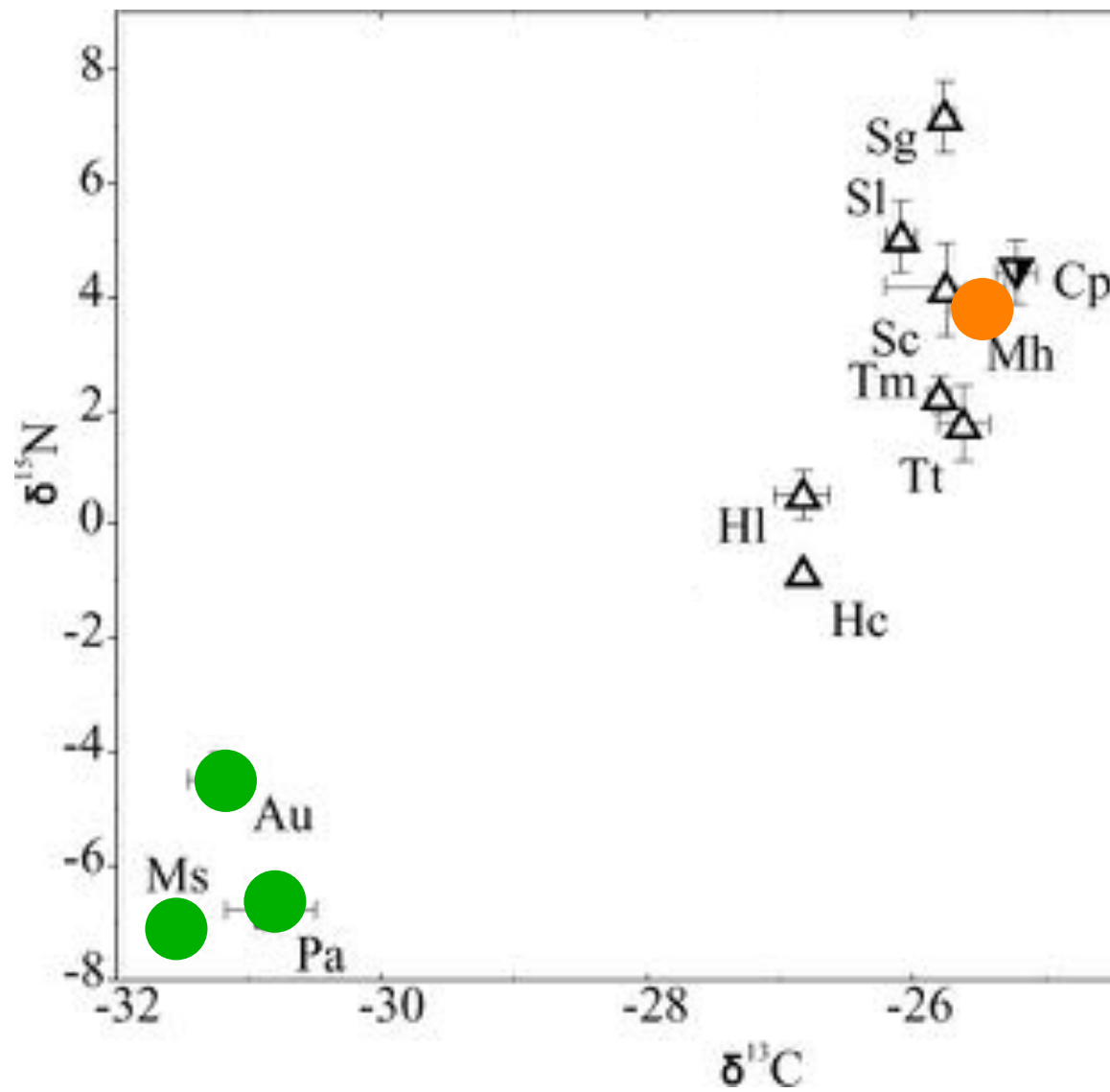
(from Brundett, 2004)



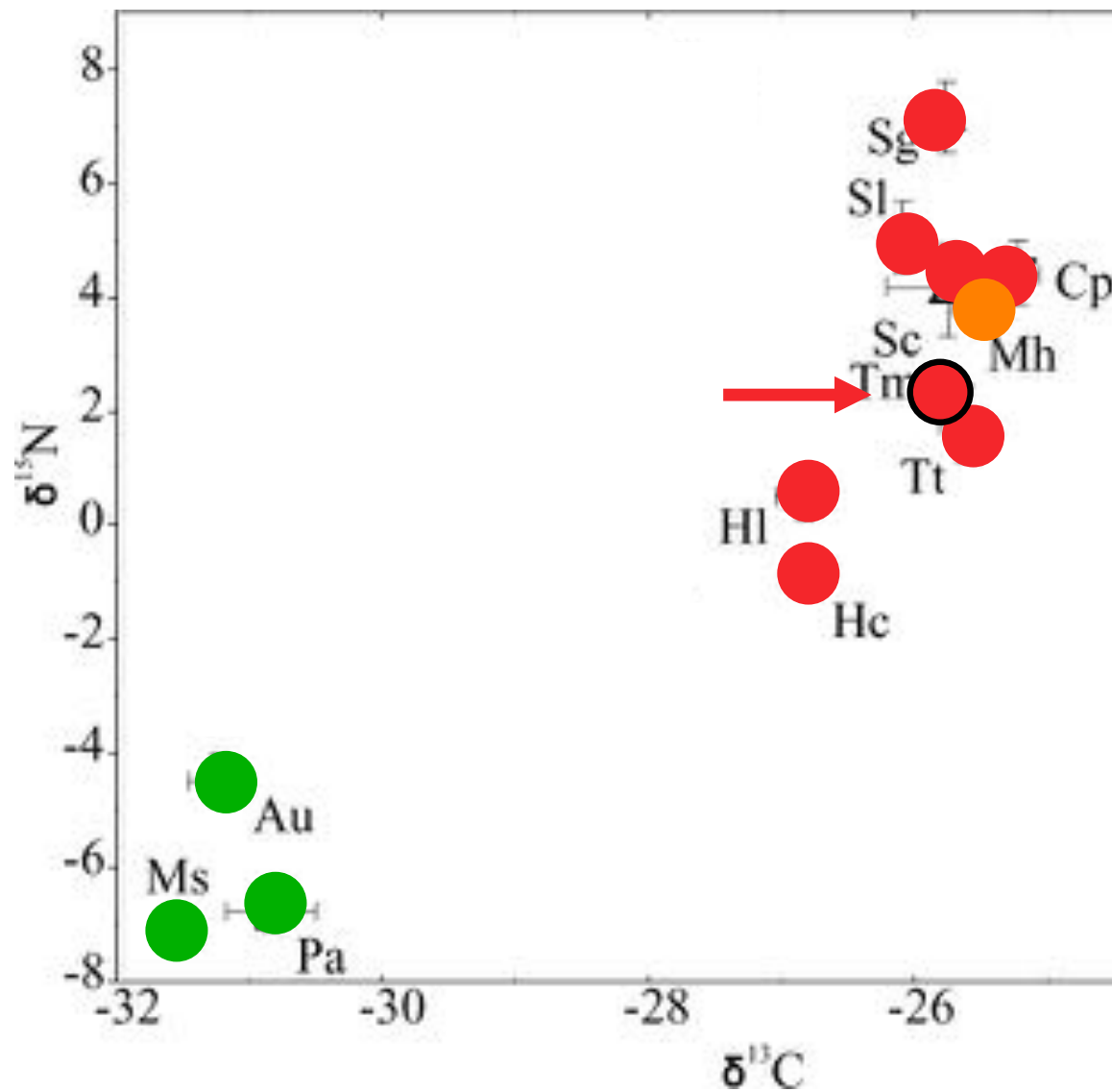


Mycoheterotrophic Ericaceae:
Hypopitys monotropa

Tedersoo *et al.*,
 2007, *Oecologia*



Autotrophs: *Arctostaphylos uva-ursi*, *Picea abies*, *Melampyrum sylvaticum*

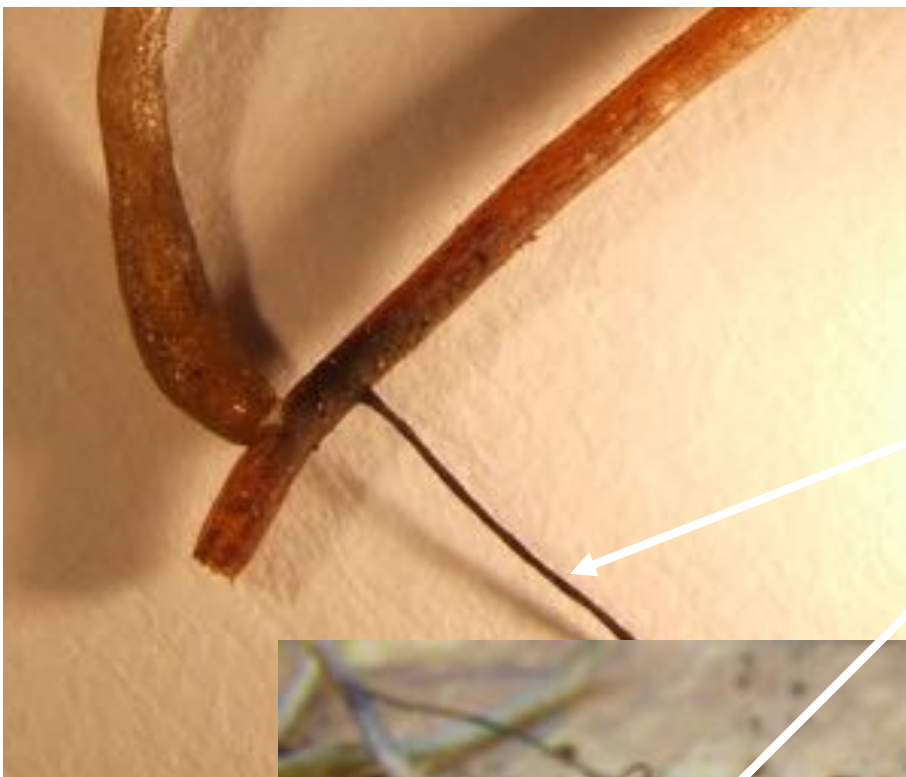
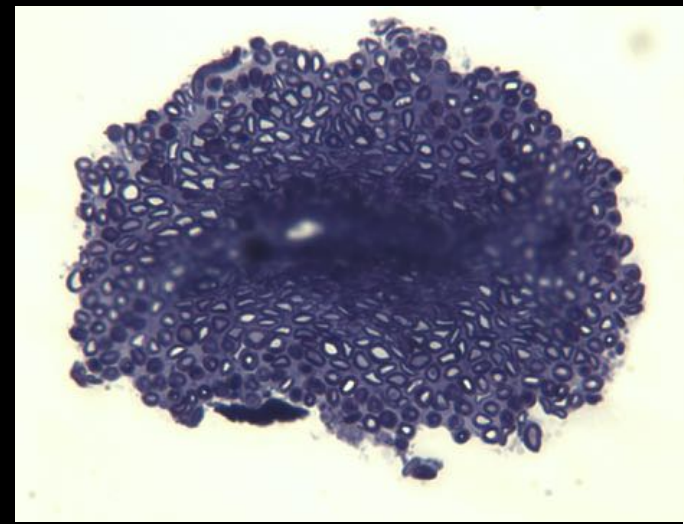


Ectomycorrhizal fungi, including *Tricholoma myomyces*, mycorrhizal on *H. monotropa*



Wulfschlaegelia calcrata

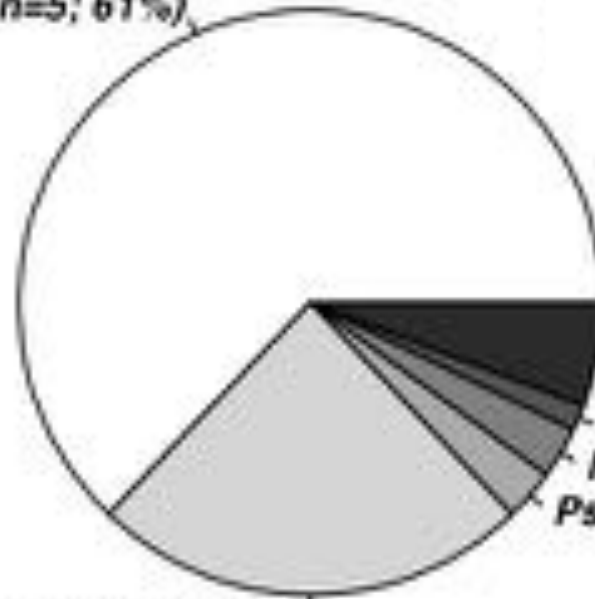
Rhizomorphs
(groups of hyphae)



In 14 individuals from 4 sites: only saprotrophic fungi !



Mycenoid sp. (n=5; 61%)



Ascomycetes (n=2; 6%)

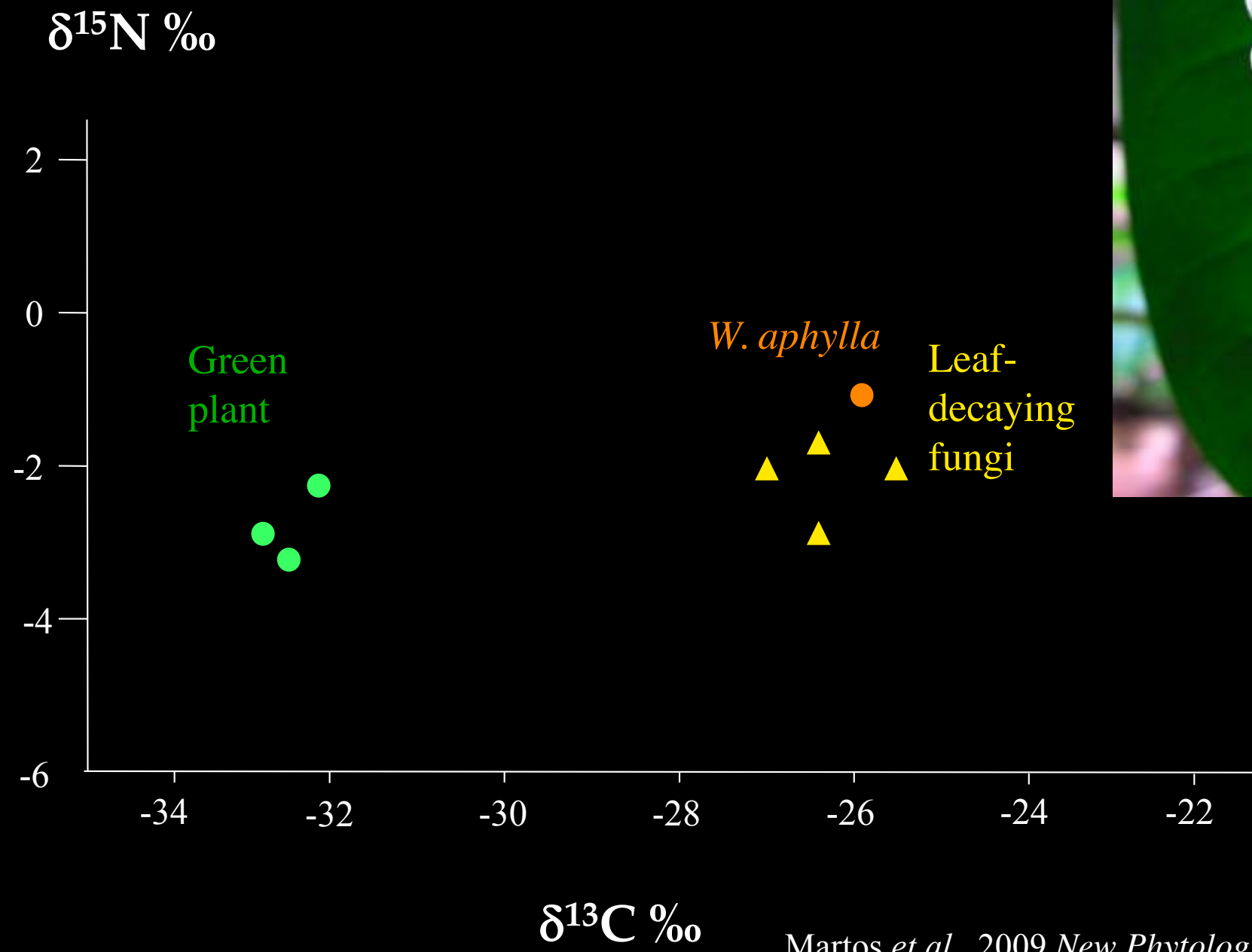
Porpomyces sp. (n=1; 2%)

Helicogloea sp. (n=1; 3%)

Psathyrella sp. (n=1; 3%)

Gymnoid sp. (n=2; 25%)

All are white rot and litter decaying fungi
No strict specificity at individual level



Les réseaux mycorhiziens

Mycohétérotrophie et réseaux

Mixotrophie et réseaux mycorhiziens

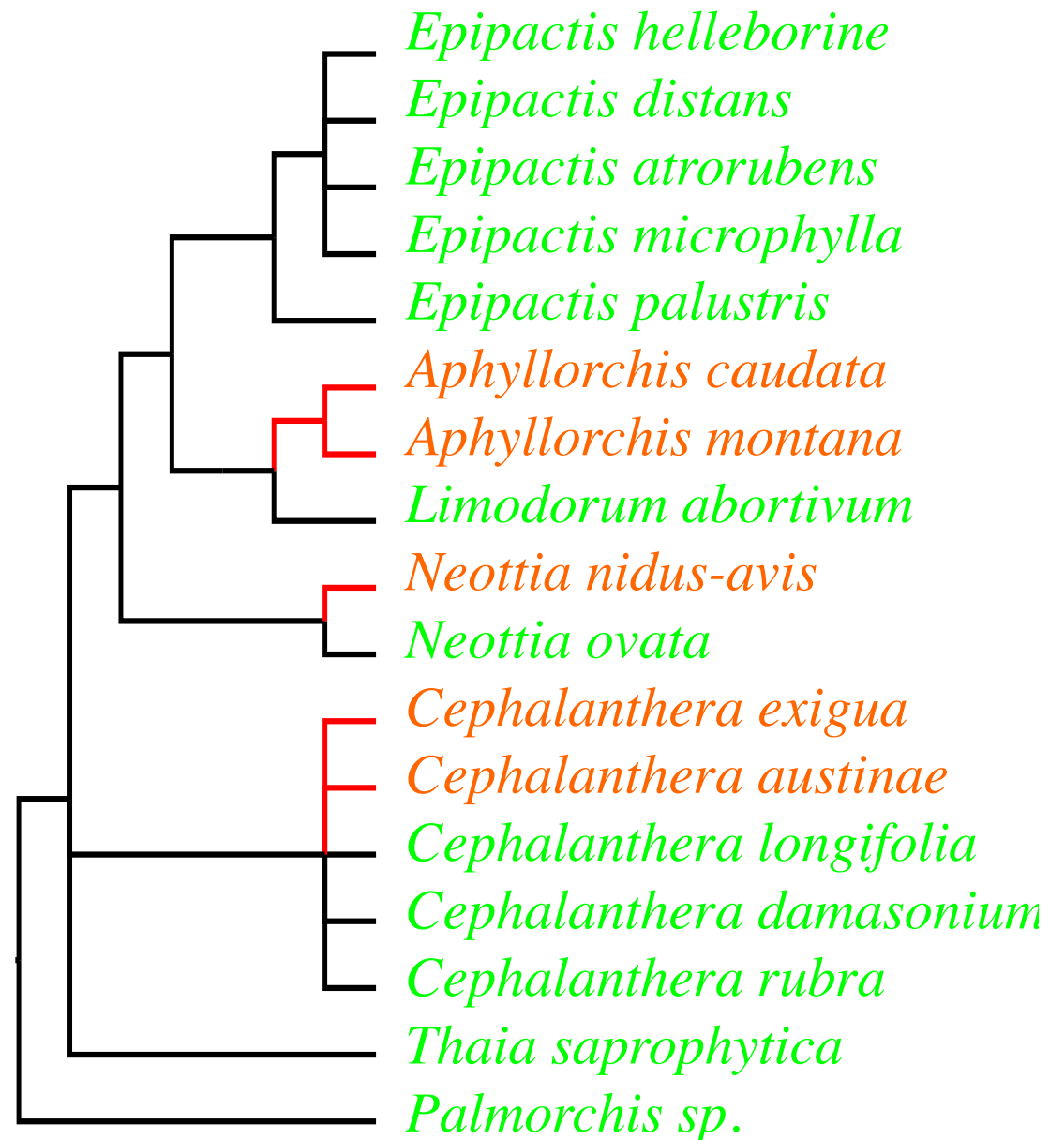
La difficile transition vers l'hétérotrophie

... due à un attachement fatal à la lumière ?

Green orchids related to mycoheterotrophs

E.g. the Neottieae tribe,
mycoheterotrophy
arose repeatedly, by
convergent evolution
among **green species**

... that associate with
ectomycorrhizal fungi



Limodorum abortivum



Russula delica group

Girlanda *et al.*, *Mol.
Ecol.*, 2006

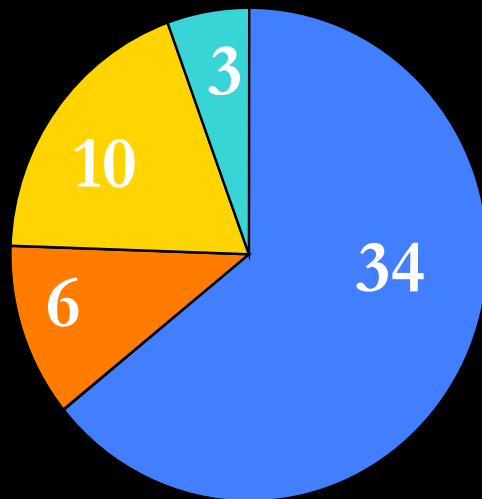
Cephalanthera damasonium
& *Cephalanthera longifolia*



Cephalanthera damasonium & *Cephalanthera longifolia*

C. longifolia

Julou *et al.*, 2005



« Rhizoctonias »

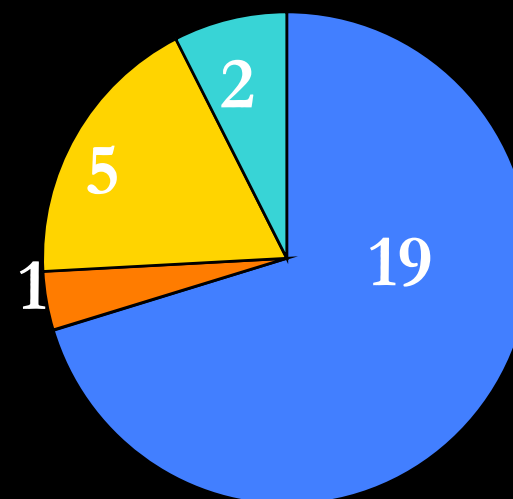
Saprophytes

Ectomycorrhizal fungi

Plant endophytes or parasites

C. damasonium

Abadie *et al.*, 2006



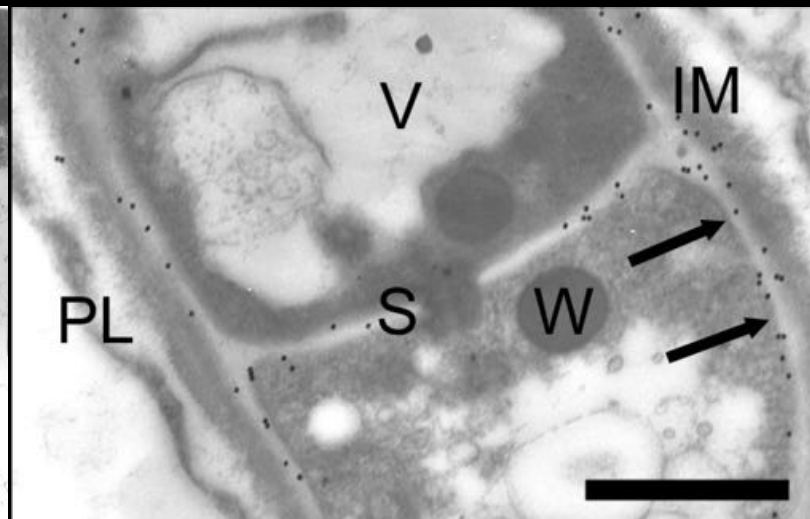
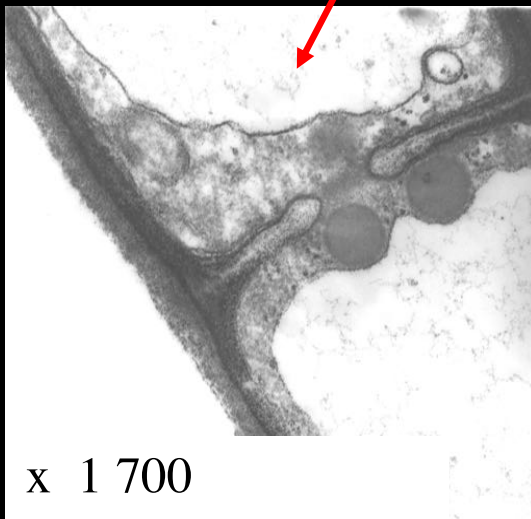
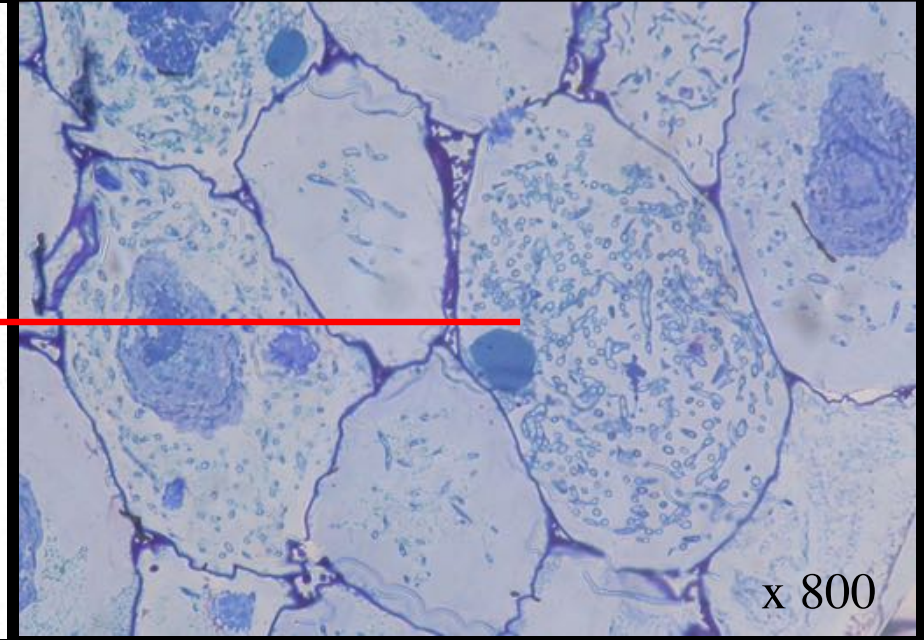
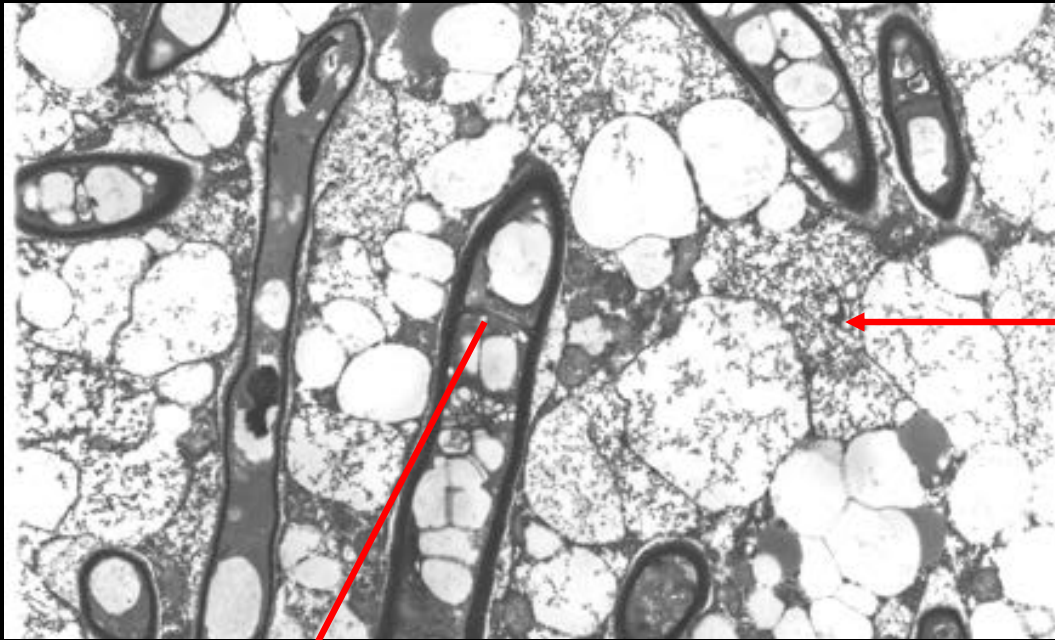
Epipactis microphylla



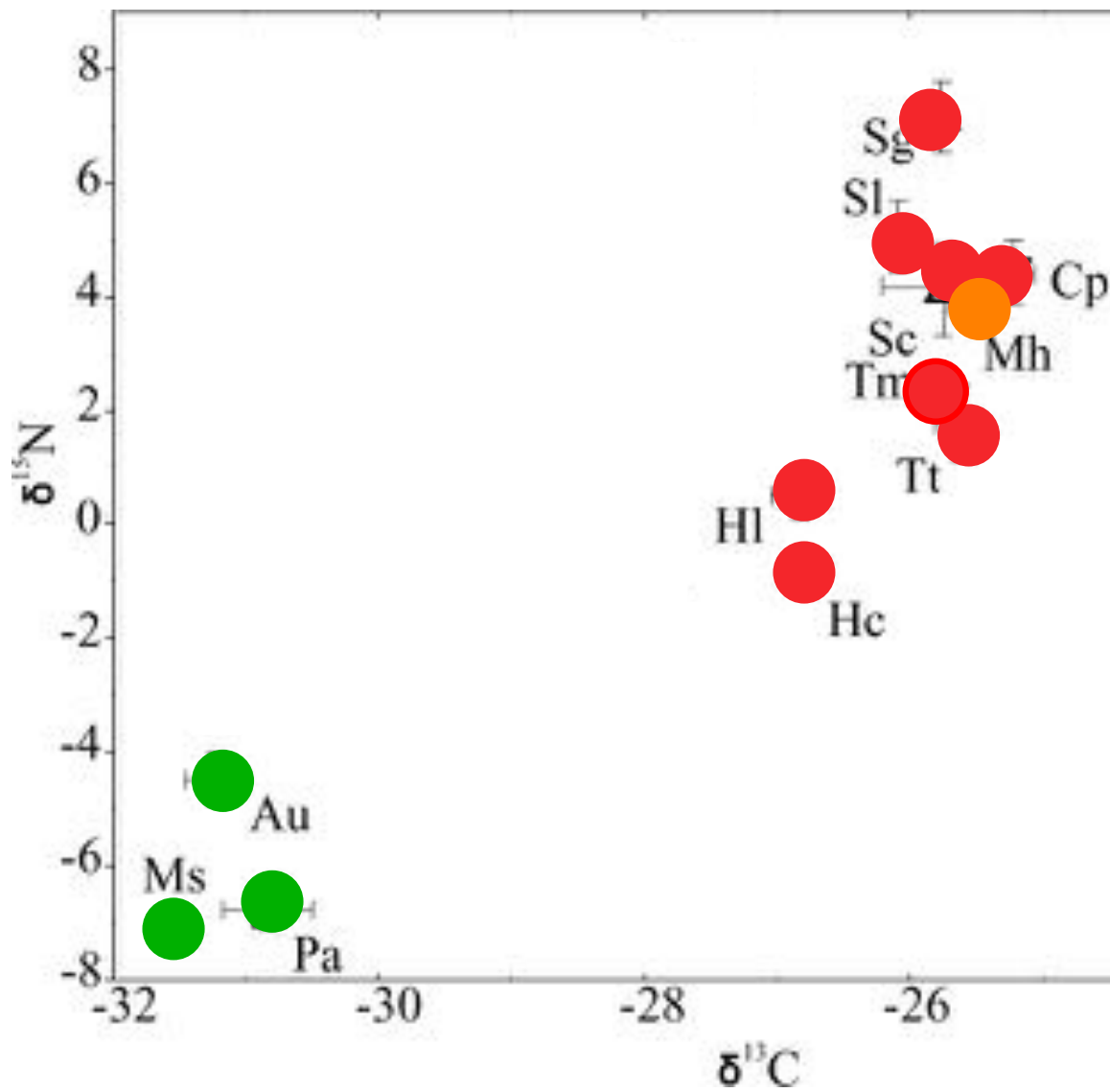
Diverse ectomycorrhizal
fungi, mainly **truffles** spp.

Selosse *et al.*, *Microb.
Ecol.*, 2006

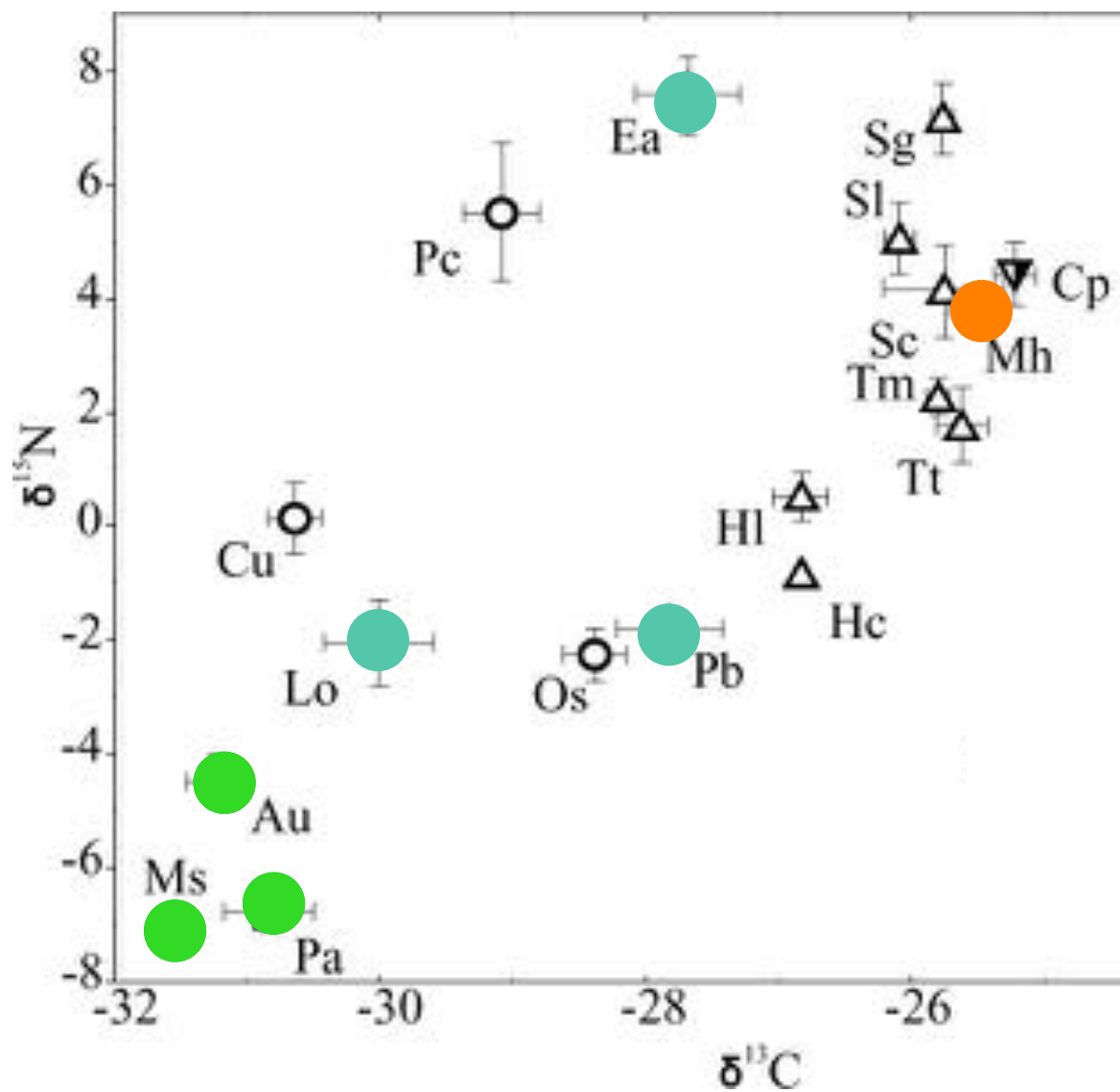
Epipactis microphylla associates with truffles



Microscopy and antibodies demonstrate that **truffles are mycorrhizal** (Selosse *et al.*, 2006 *Microbial. Ecol.*)



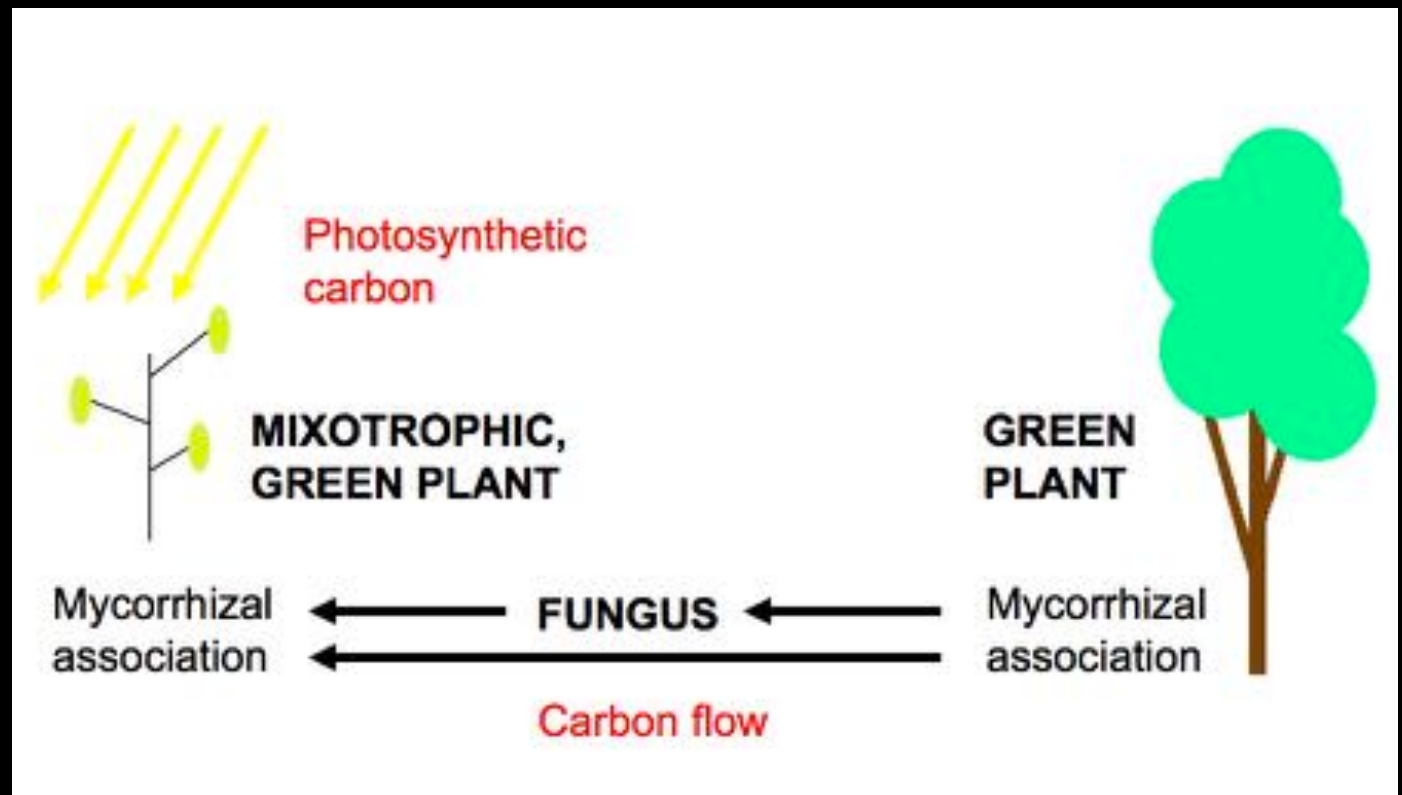
Tedersoo *et al.*, 2007, *Oecologia*



« Green » orchids: *Listera ovata*,
Platanthera bifolia, *Epipactis atrorubens*

MIXOTROPHY

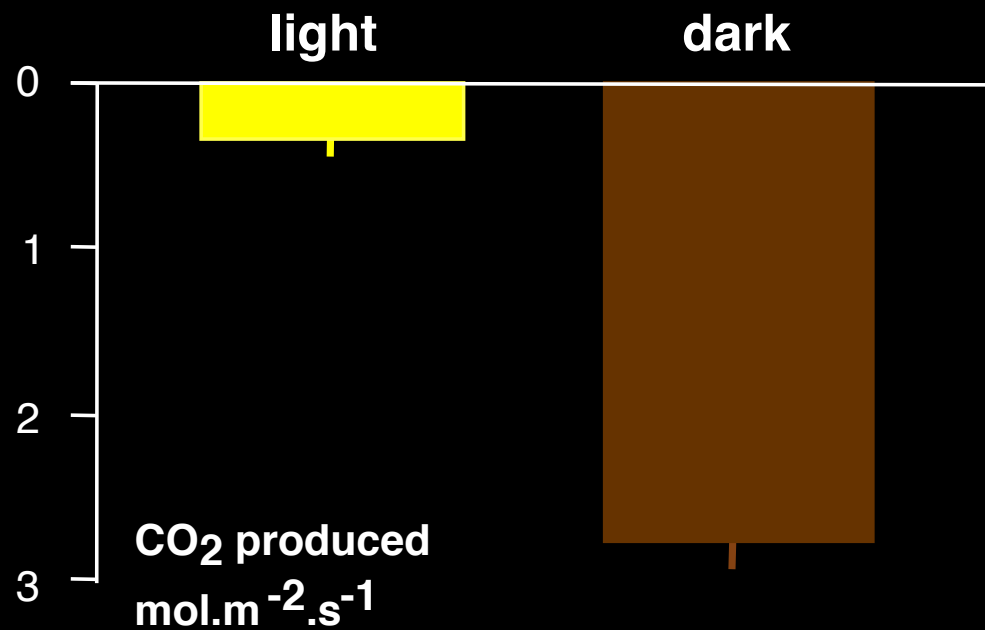
Exploiting both the mycorrhizal network & photosynthesis...



... as reflected in their ^{13}C & ^{15}N abundances

Limodorum abortivum

A green orchid photosynthesizing
below the compensation point



Girlanda *et al.*, *Mol. Ecol.*, 2006

In **mixotrophic** orchid species, achlophyllous variants, the **albinos**,

... survive as mycoheterotrophs!

Cephalanthera damasonium

(P. Pernot & F. Dusak)





Epipactis purpurata

(A. Hasenfratz)



M. Roy



A. Soulié

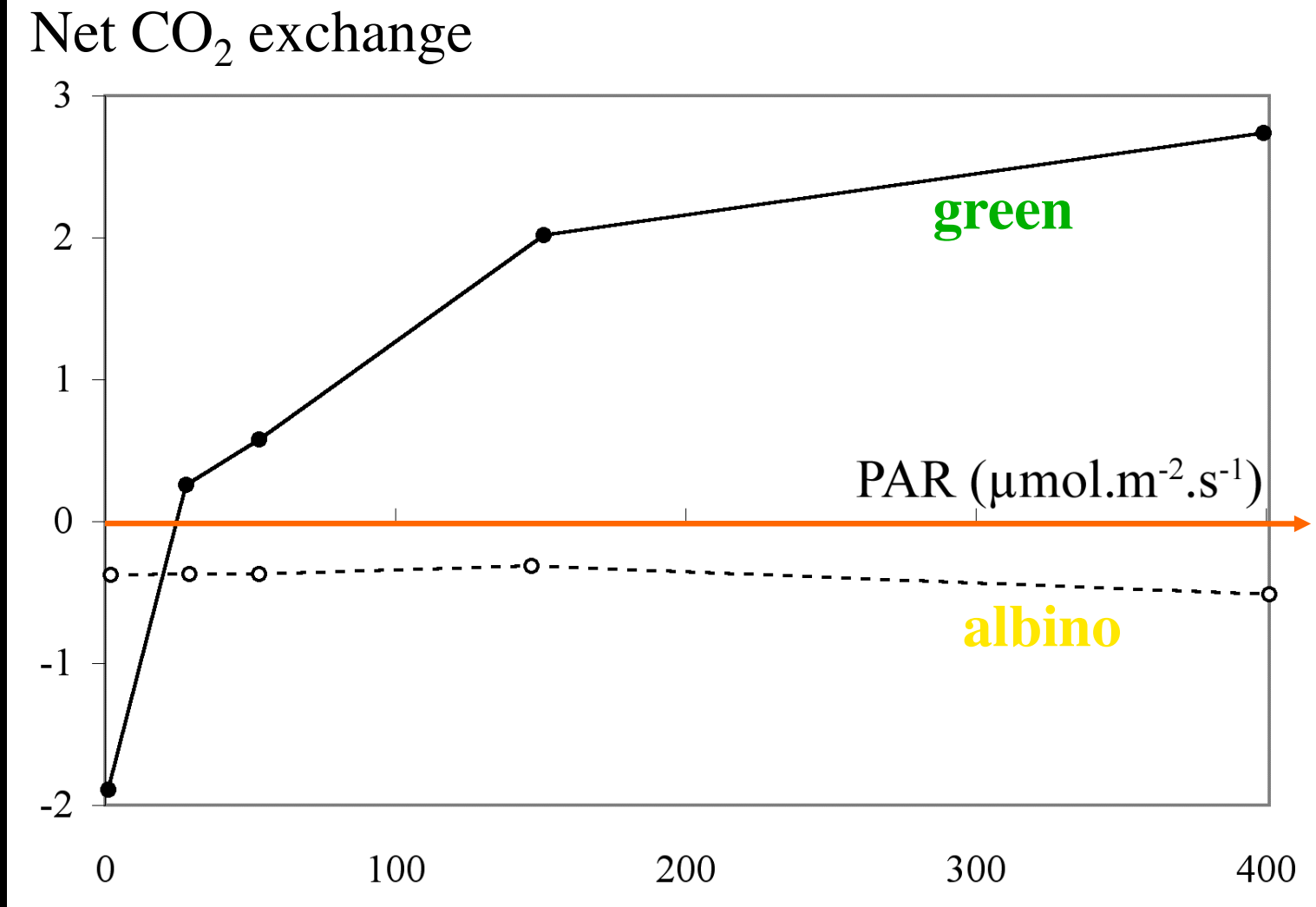


J.C. Claessens



S. Acker

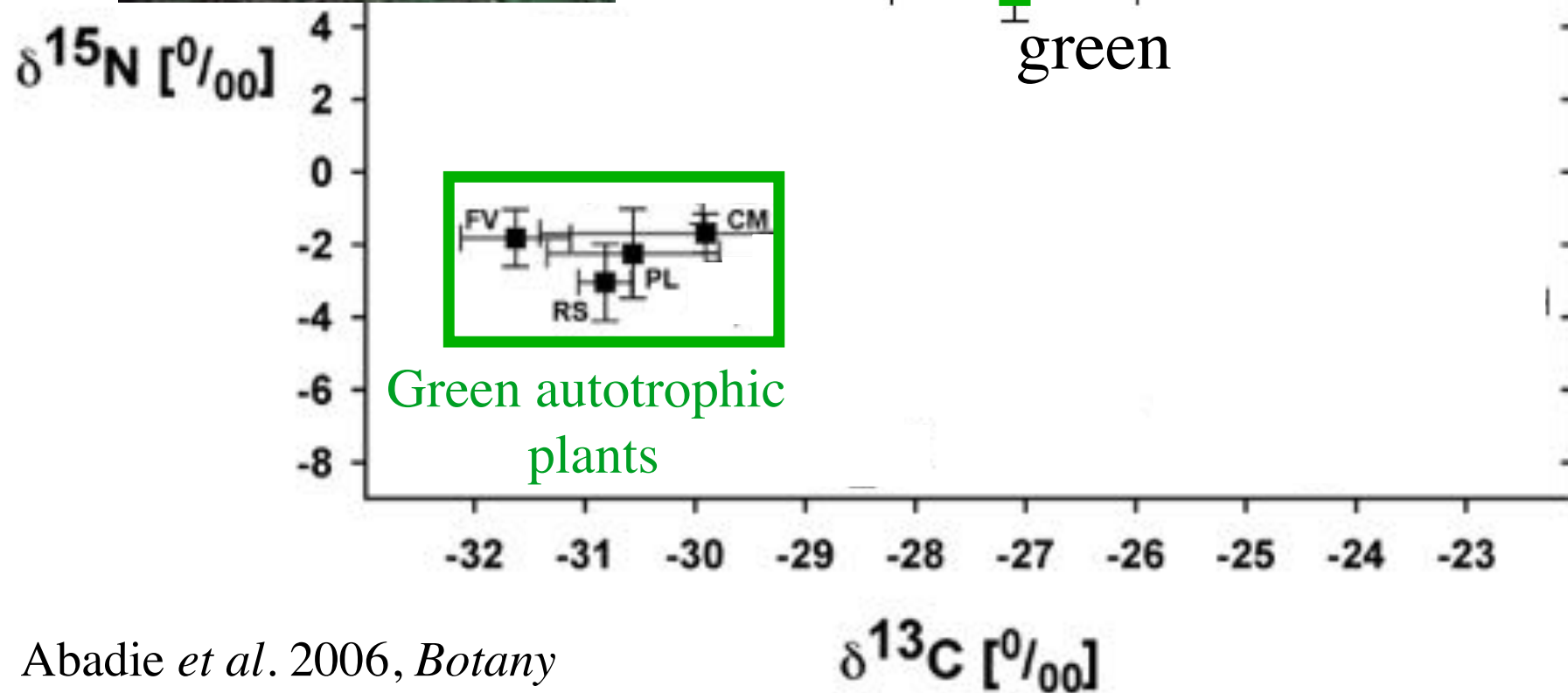
Gas exchange in *C. damasonium*



PAR = photosynthetic active radiations

Julou *et al.* 2005, *New Phytol.*

Isotopes in *C. longifolia*



Trends in Plant Science



Green plants that feed on fungi

Some green plants

- are mixotrophic and
use mycorrhizal
networks

Selosse & Roy, 2009

Trends in Plant Science



Green plants that feed on fungi

Some green plants

- are mixotrophic and use mycorrhizal networks
- are predisposed to evolve mycoheterotrophy

Selosse & Roy, 2009

Trends in Plant Science



Some green plants

- are mixotrophic and use mycorrhizal networks

- are predisposed to evolve mycoheterotrophy

- ... with albinos as transitions?

Selosse & Roy, 2009

Les réseaux mycorhiziens

Mycohétérotrophie et réseaux

Mixotrophie et réseaux mycorhiziens

La difficile transition vers l'hétérotrophie

... due à un attachement fatal à la lumière ?

Many mutation could abolish greenness, yet
albinos remain very rare
in mixotrophic orchid
populations...

Why then?



Roy *et al.* 2013,
Ecol. Monographs
83: 95–117



A 4-year monitoring of two
Cephalanthera damasonium
populations, with 20-70 albinos

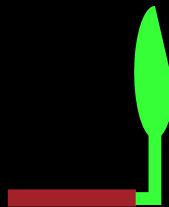
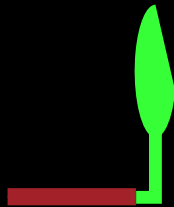
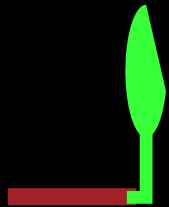


Dormancy...

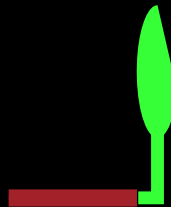
Year n

n+1

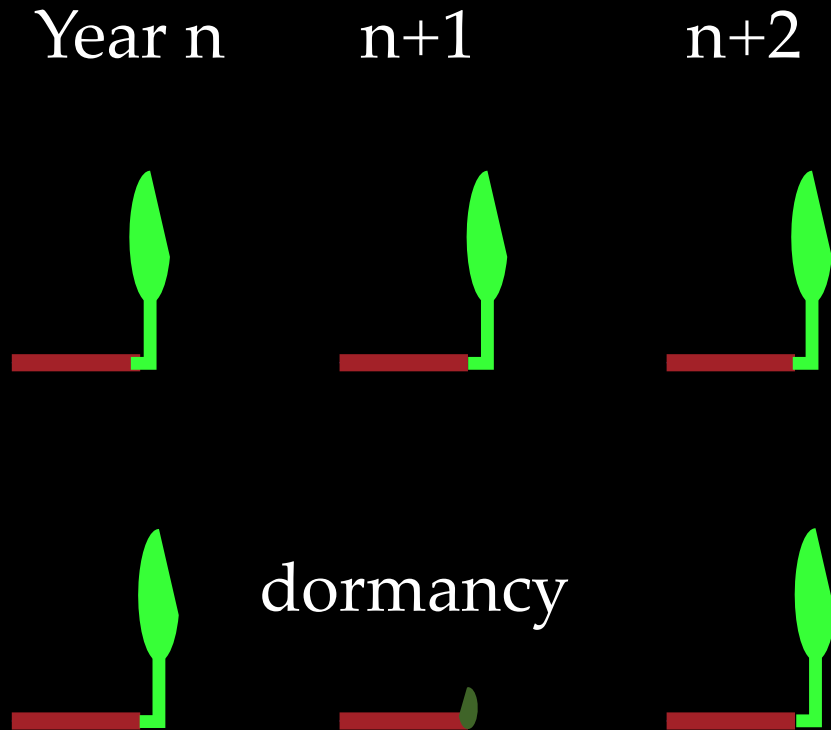
n+2



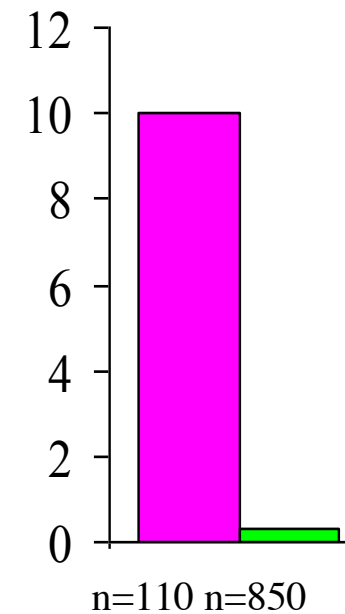
dormancy



Dormancy: more frequent!



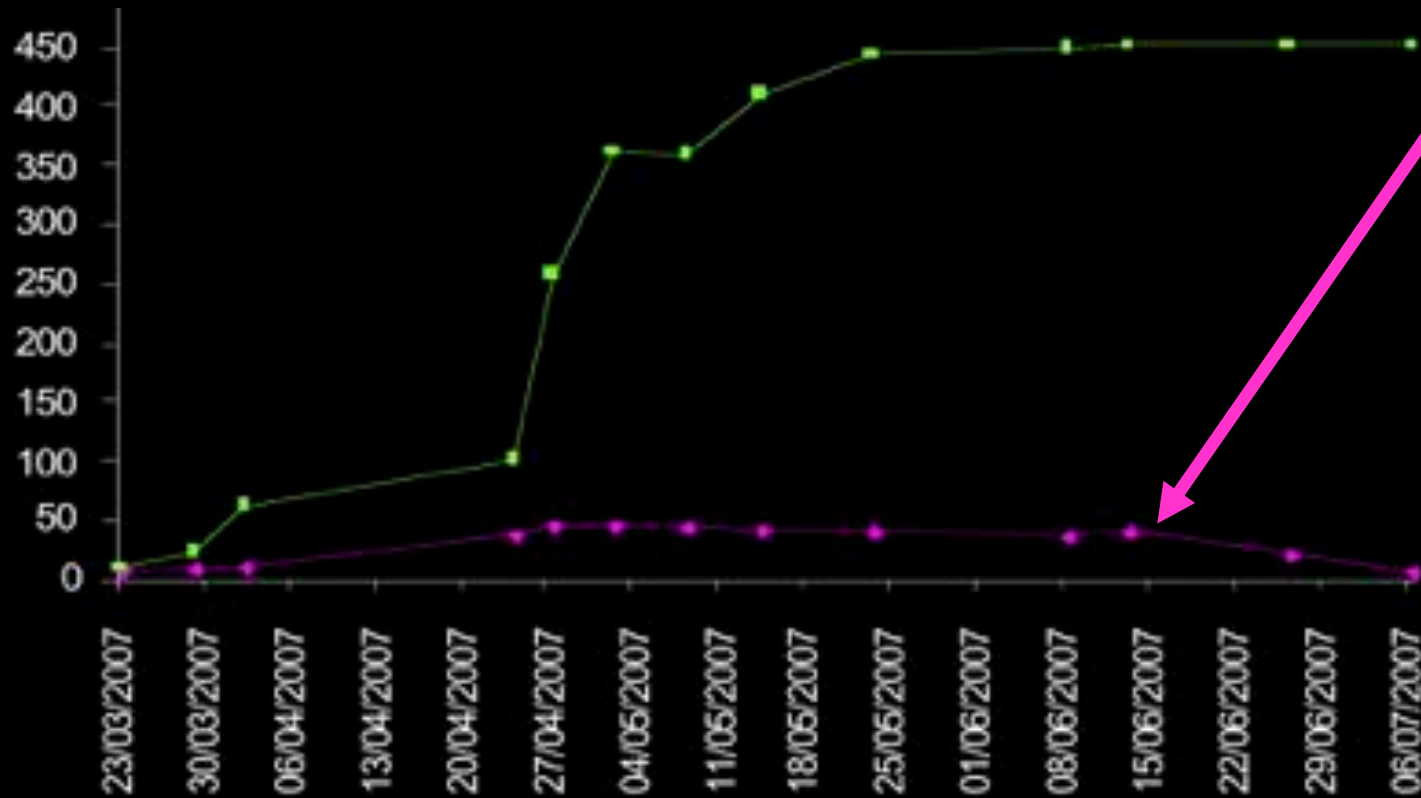
% of dormant individuals in 2007



Albinos were 16.5x more often dormant than **green** individuals

Shoot survival...

Number of shoots



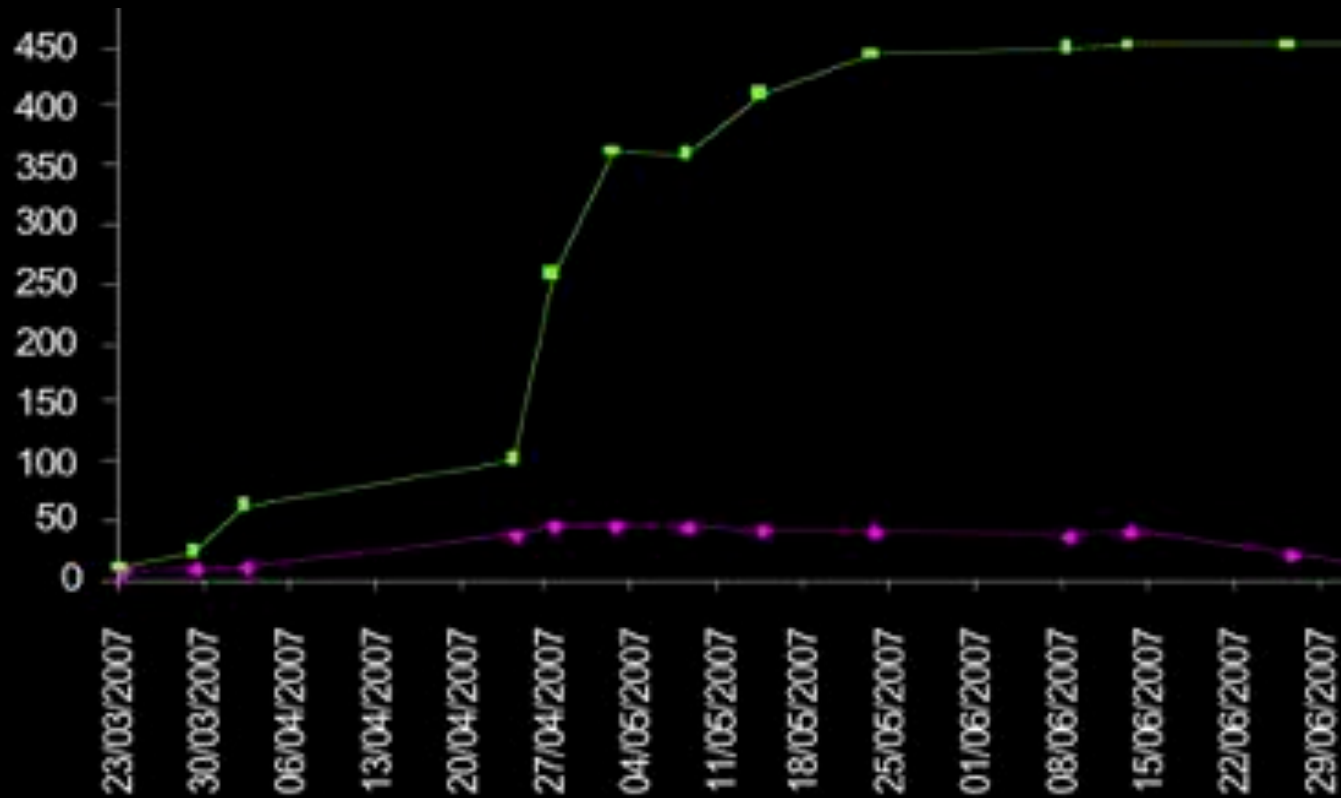
Apparition
of shoots

Flowering time

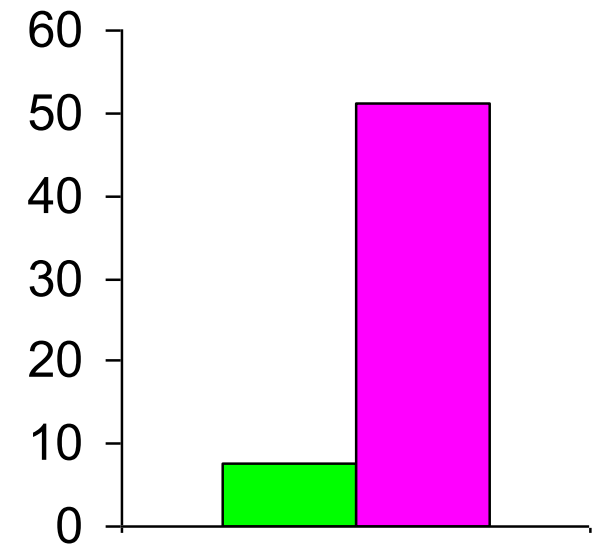


Shoot survival: lower!

Number of shoots



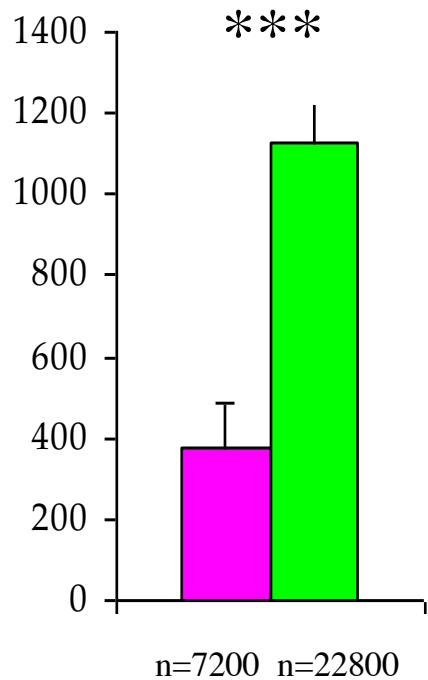
% shoots dried in late June 2007



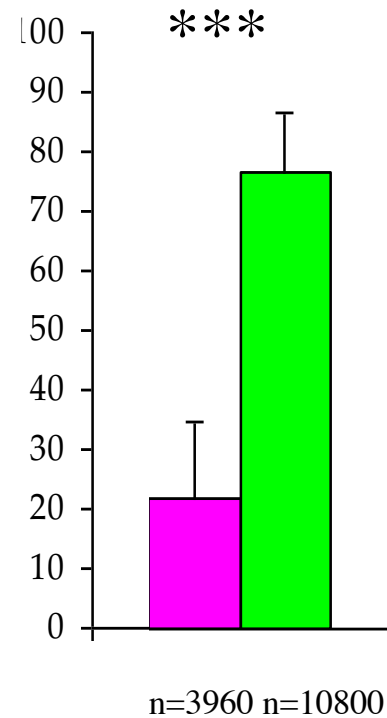
51% **albino** shoots dry in June (vs 7% for **green** ones) > no seeds!

Seed production and quality: lower...

Number of seeds
per fruits (x10)



% of seeds germinating *in situ*
in seeds pockets (3 months)



3 x 3 less viable seeds in **albinos**!

Comparison **green ind.** / **albinos**

Patterns of dormancy **6.8x more**

Patterns of shoot survival **16.5x less**

Seed production **9x less**

= 10^3 x lower fitness



Roy *et al.* 2013, *Ecol.*
Monographs **83**: 95–117

Comparison **green ind.** / **albinos**

Patterns of dormancy **6.8x more**

Patterns of shoot survival **16.5x less**

Seed production **9x less**

**= 10^3 x lower
fitness**

**WHY DID ALBINOS
FAIL THE TRANSITION ?**



Les réseaux mycorhiziens

Mycohétérotrophie et réseaux

Mixotrophie et réseaux mycorhiziens

La difficile transition vers l'hétérotrophie

... due à un attachement fatal à la lumière ?

Allocation of
mycoheterotrophic C
versus photosynthetic
C in **mixotrophic**
orchids

based on $\delta^{13}\text{C}$

(and N content)

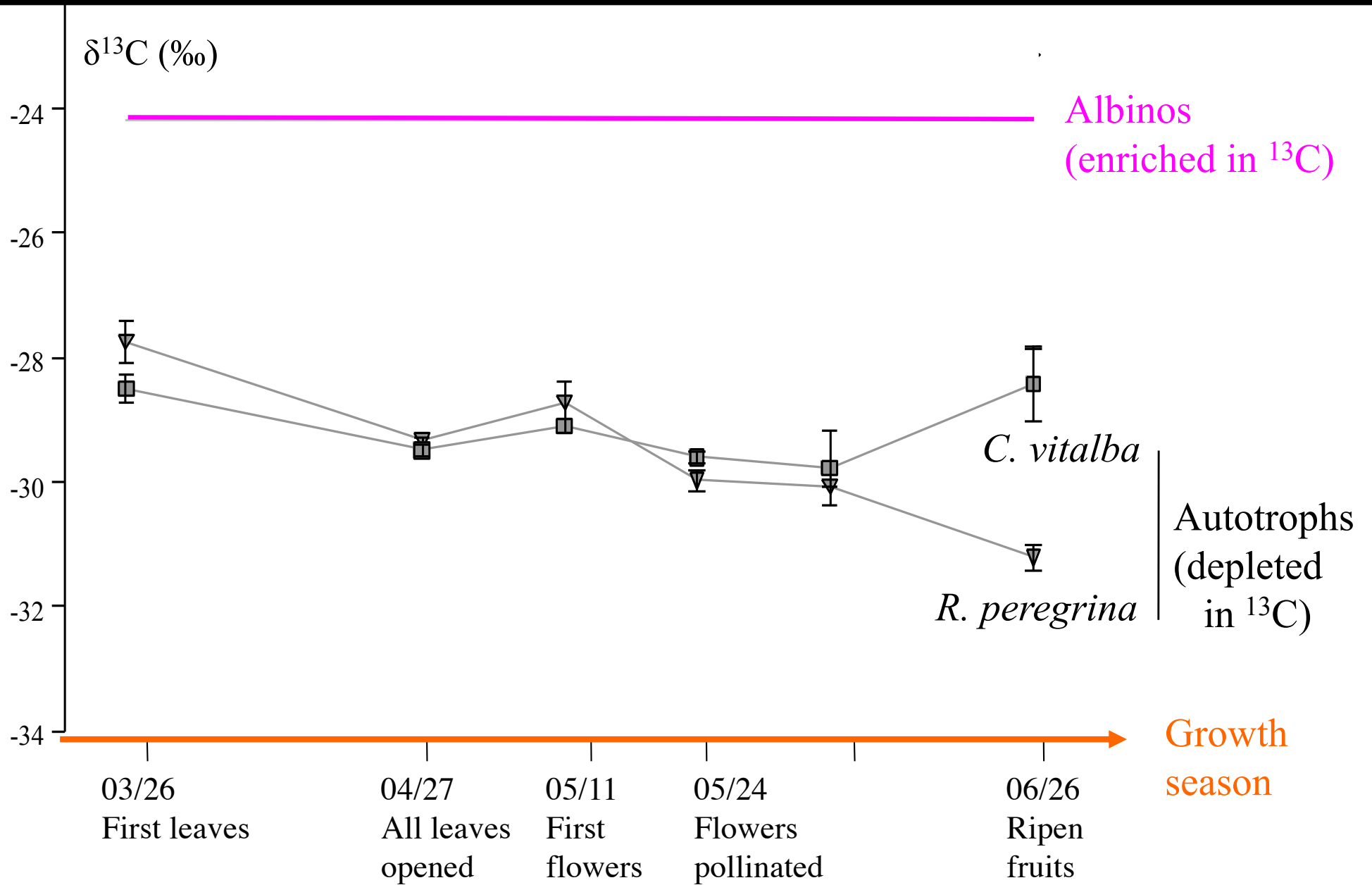


Allocation of fungal *versus* photosynthetic C

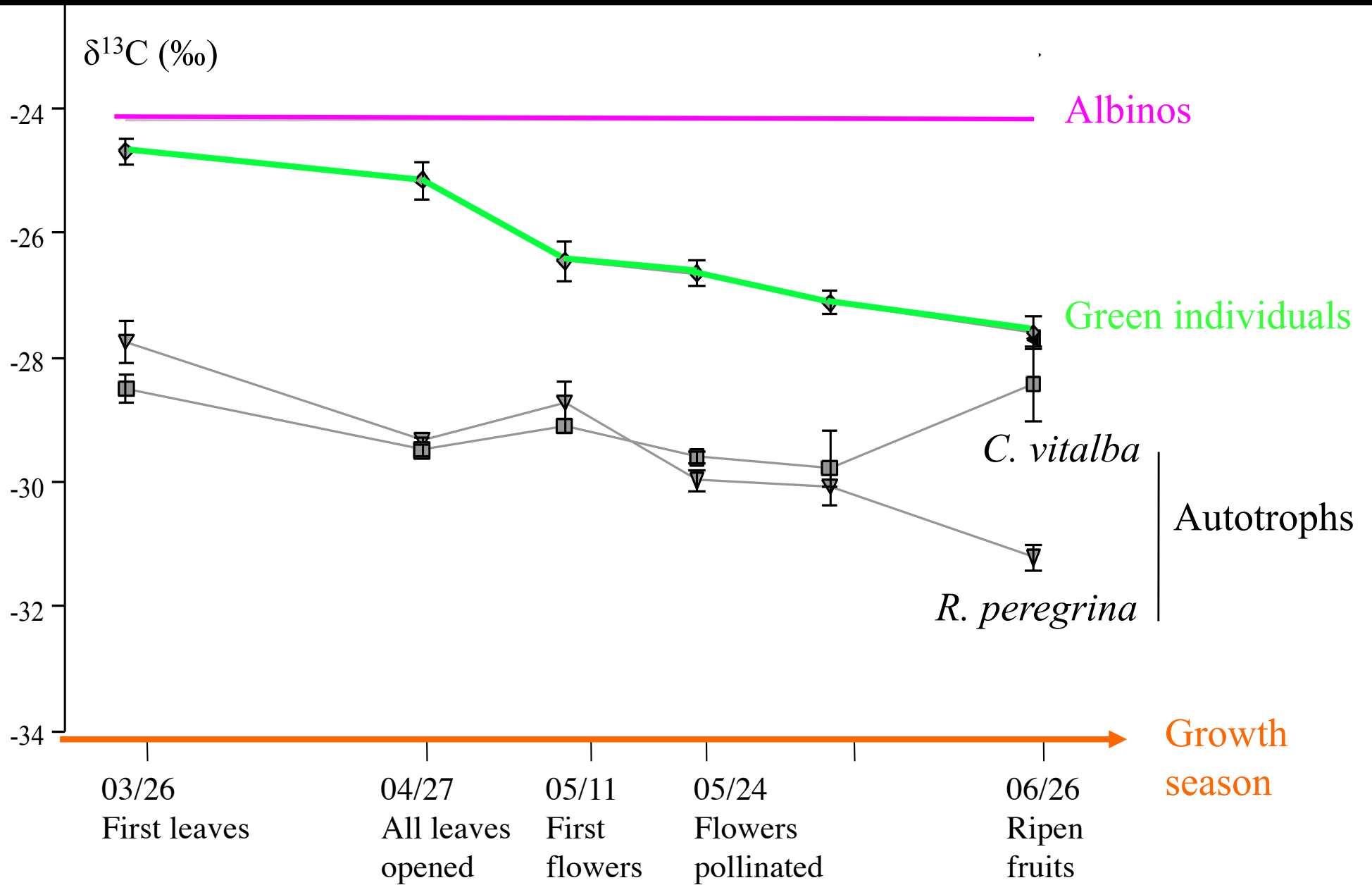
In shoots



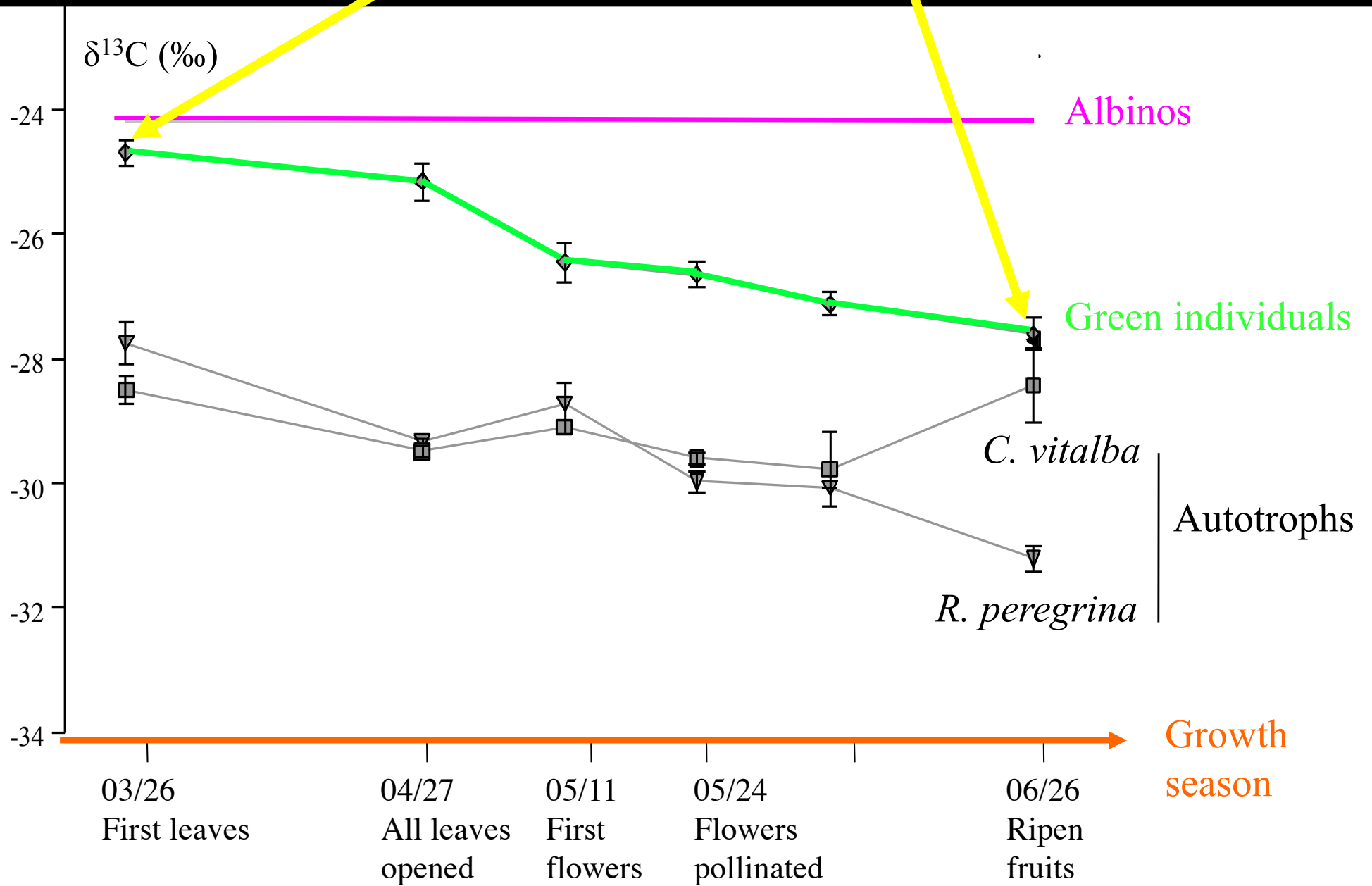
Shoot C nutrition over the growth season in *C. damasonium*



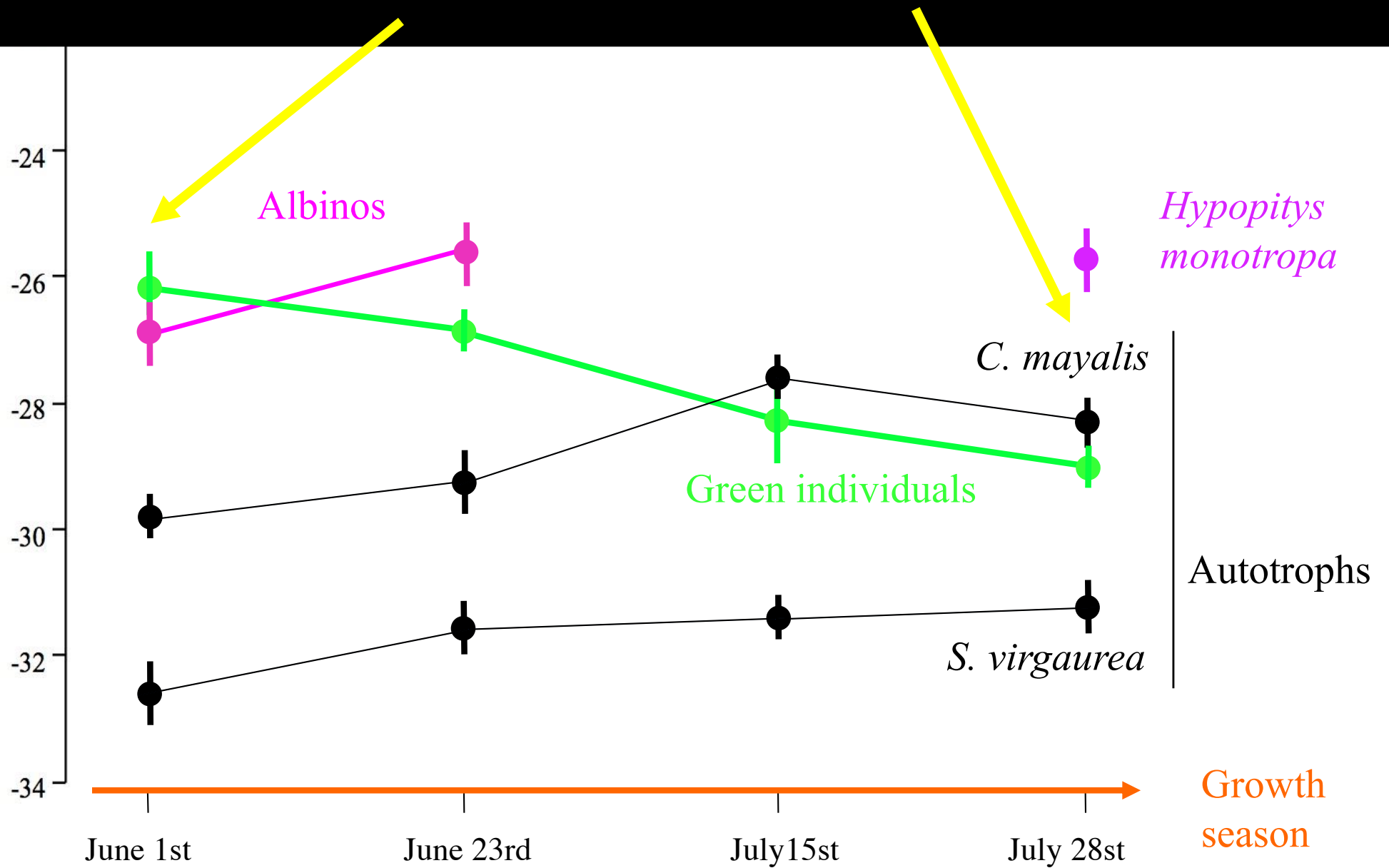
Shoot C nutrition over the growth season in *C. damasonium*



A shift from 80% heterotrophic to 20% at fruiting!



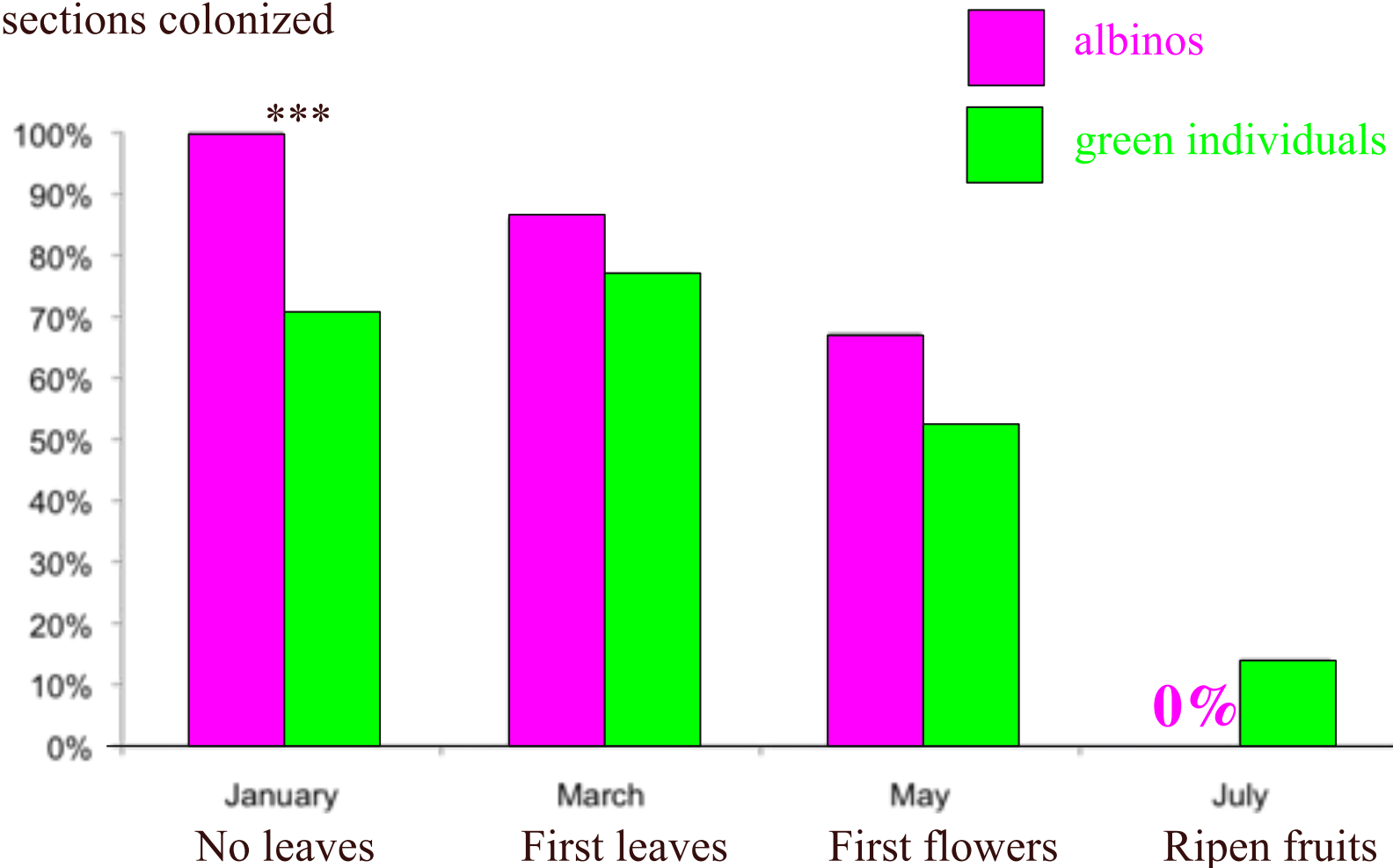
A shift from 100% heterotrophic to 20% in *E. helleborine*



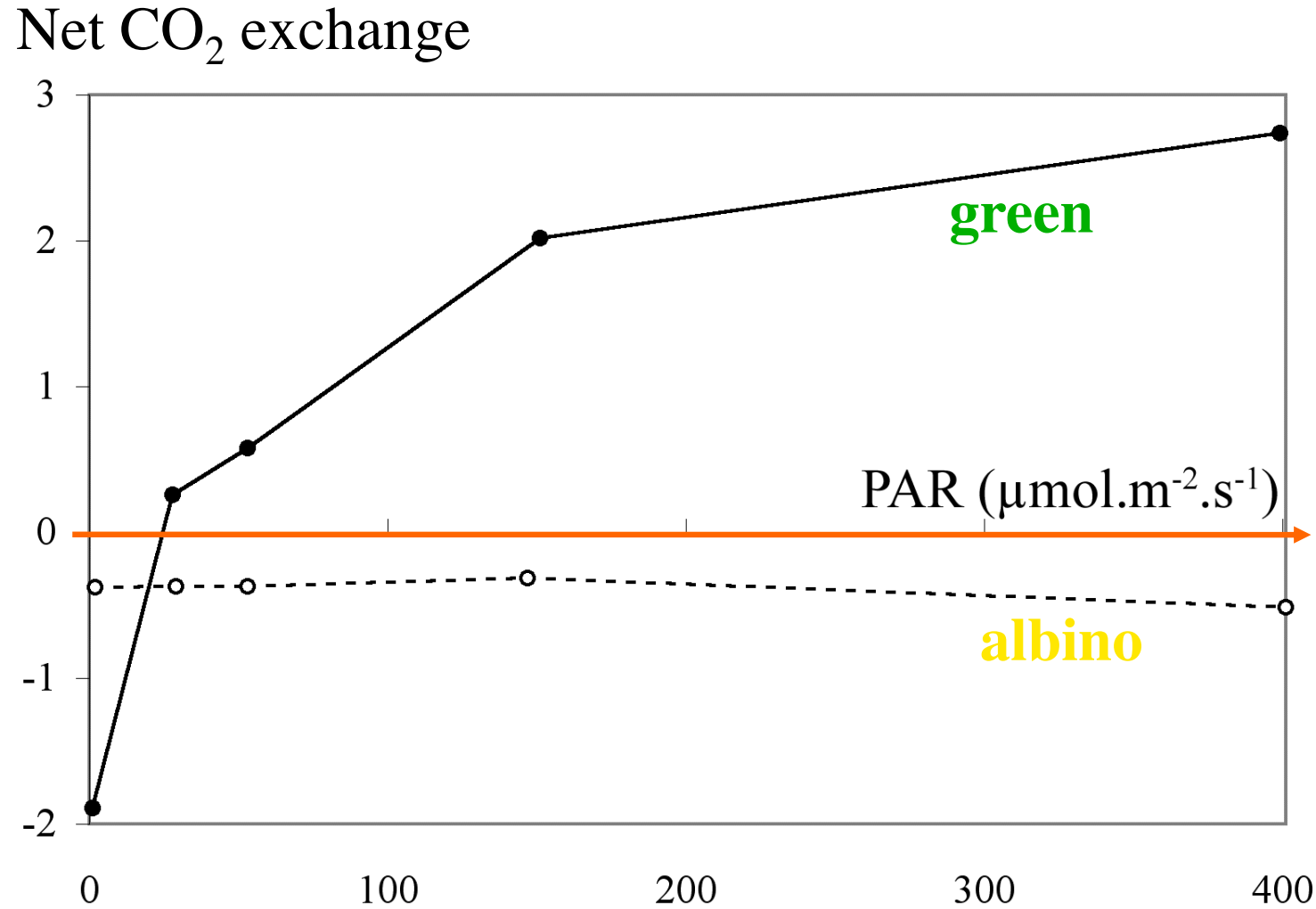
Gonneau *et al.* 2014, *J. Ecol.*, in press

A decrease of mycorrhizal colonization at fruiting in *C. damasonium*

Percentage of root sections colonized

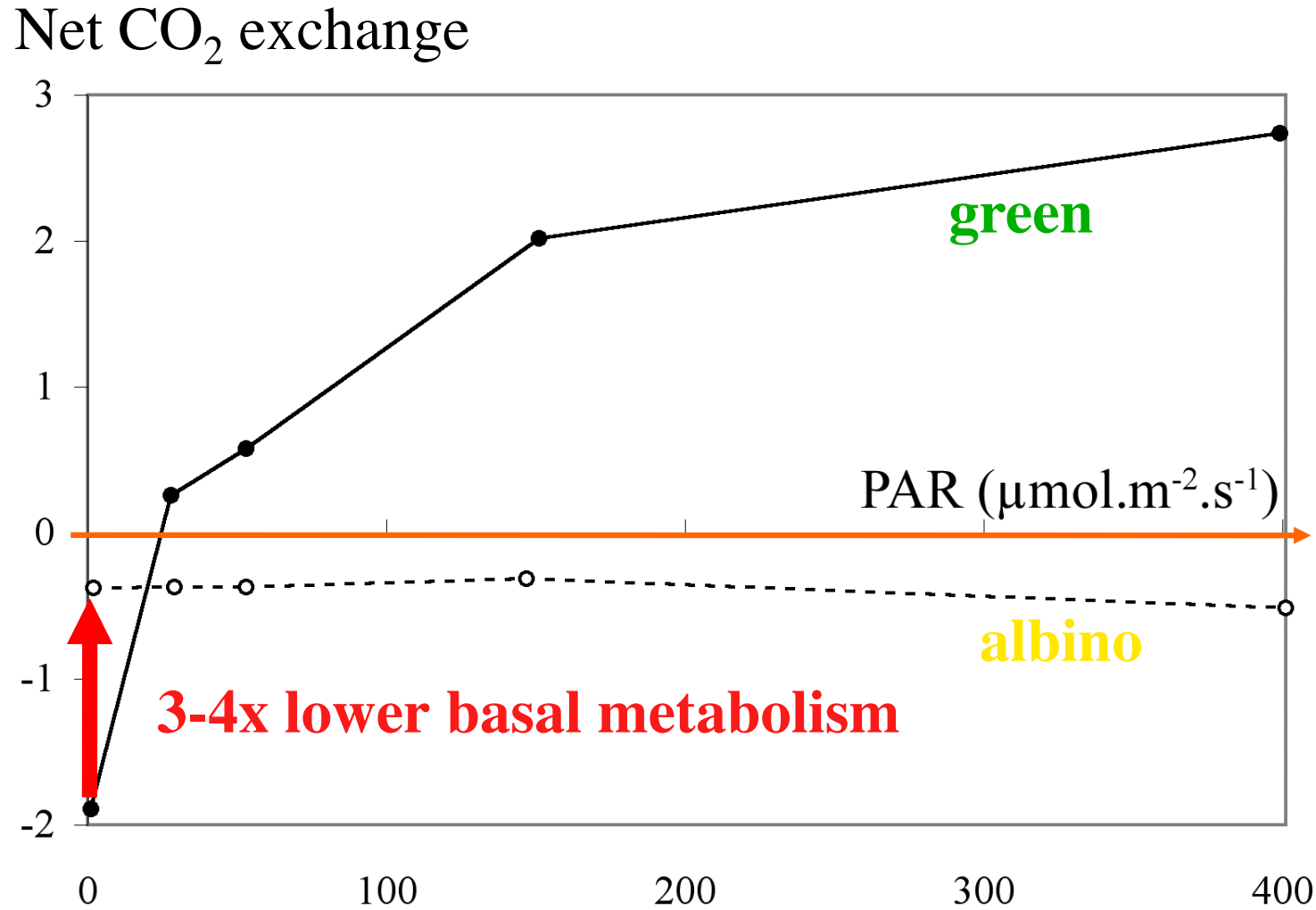


Gaz exchange in *C. damasonium*



Julou *et al.*, 2005, *New Phytol.*

Gaz exchange in *C. damasonium*



... a carbon limitation ?

Julou *et al.*, 2005, *New Phytol.*



CONCLUSIONS

- Shoot autotrophy increases over the growth season.
- Albinos have lower basal metabolism, and especially lack photosynthetic and fungal C at fruiting.

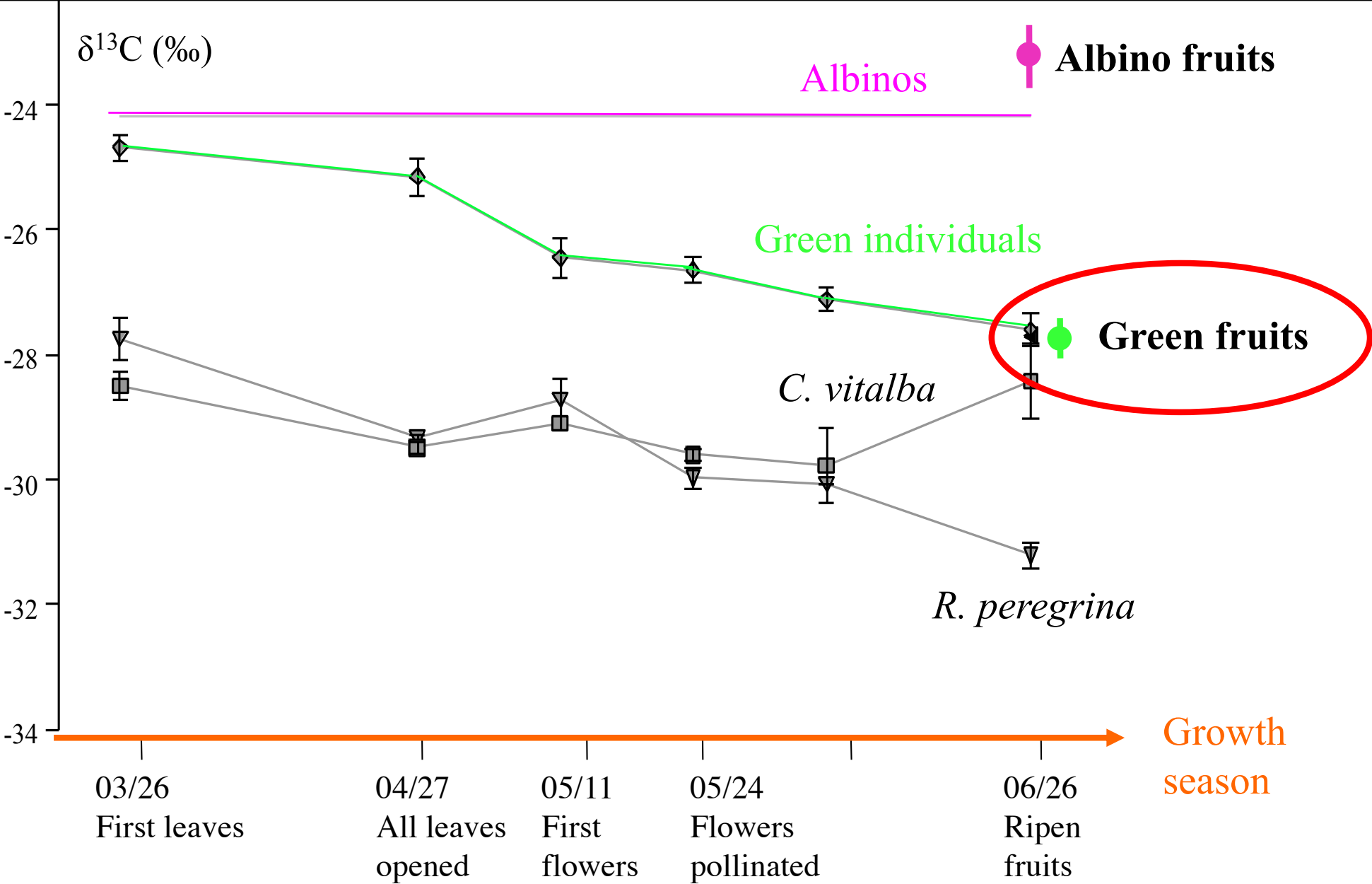
Allocation of fungal versus photosynthetic C

In shoots

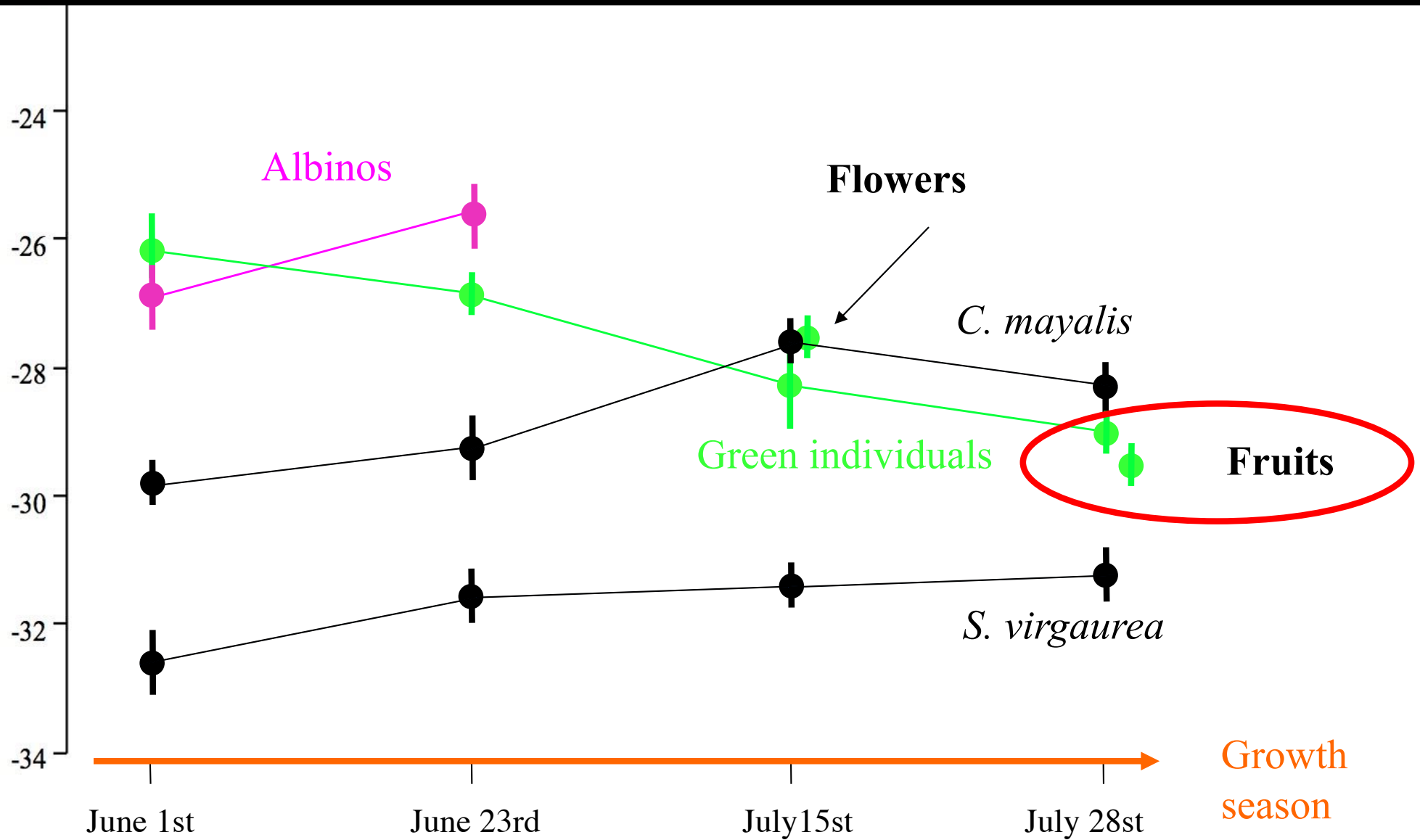
In fruits



Fruit C nutrition in *Cephalanthera damasonium*



Fruit C nutrition in *Epipactis helleborine*



Gonneau *et al.* 2014, *J. Ecol.*, in press

TESTING THE ROLE OF FUNGAL C

Limodorum abortivum, control vs. treated with iprodione

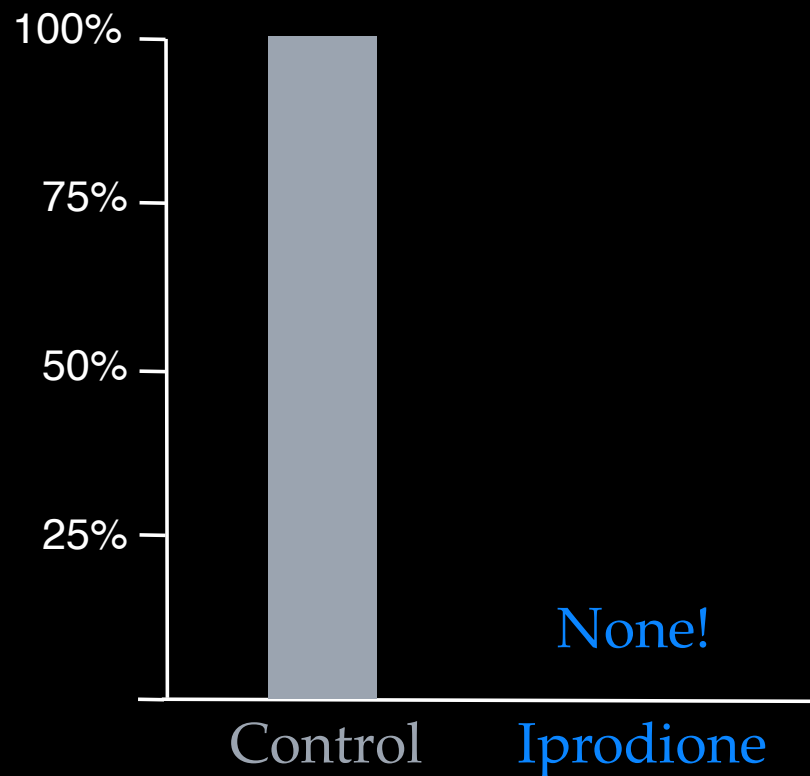


Bellino *et al.*, 2014
Oecologia **175**: 875-885

TESTING THE ROLE OF FUNGAL C

Limodorum abortivum, control vs. treated with iprodione twice, during and after anthesis

In situ root of *L. abortivum*
with fungus at fruiting



TESTING THE ROLE OF FUNGAL C

Limodorum abortivum, control vs. treated



	Control	Iprodione	
Fruit length (mm)	25.7 ± 2.3 <i>a</i>	26.7 ± 3.4 <i>a</i>	(<i>n</i> =42)
Fruit diam. (mm)	8.4 ± 0.3 <i>a</i>	9.0 ± 0.5 <i>a</i>	(<i>n</i> =42)
Seed (mm ²)	0.26 ± 0.06 <i>a</i>	0.28 ± 0.07 <i>a</i>	(<i>n</i> =713)
Fruit δ ¹³ C (‰)	-25.06 ± 0.08 <i>a</i>	-25.50 ± 0.17 <i>b</i>	(<i>n</i> =21)

No impact on seed set, full compensation by photosynthesis

CONCLUSIONS

- Shoot autotrophy increases over the growth season.
- Albinos have lower basal metabolism, and especially lack photosynthetic and fungal C at fruiting.
- Fruits use photosynthates, even if fungal C may help (a bit).

Allocation of fungal *versus* photosynthetic C

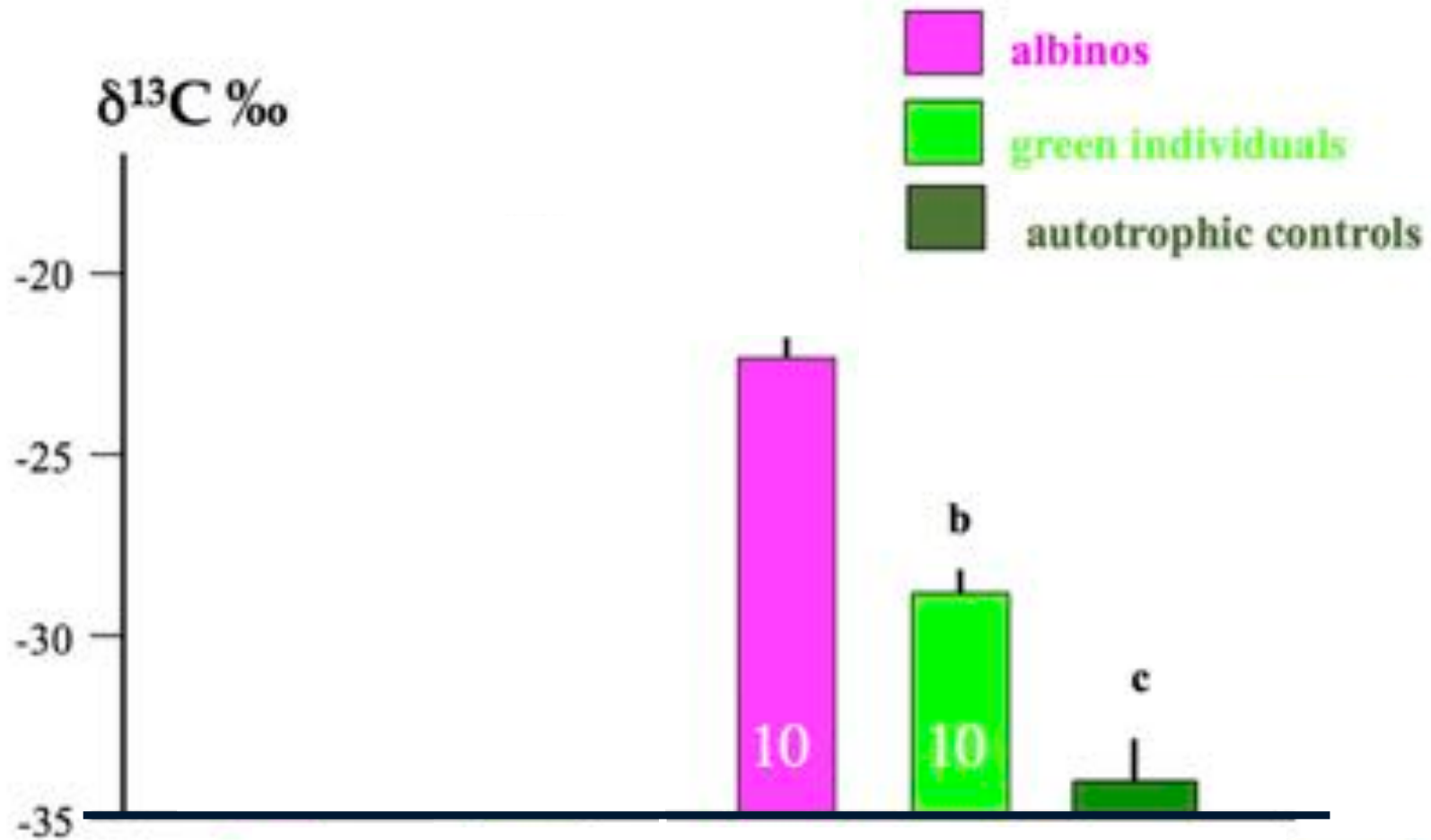
In shoots

In fruits

In underground parts
and emerging shoots

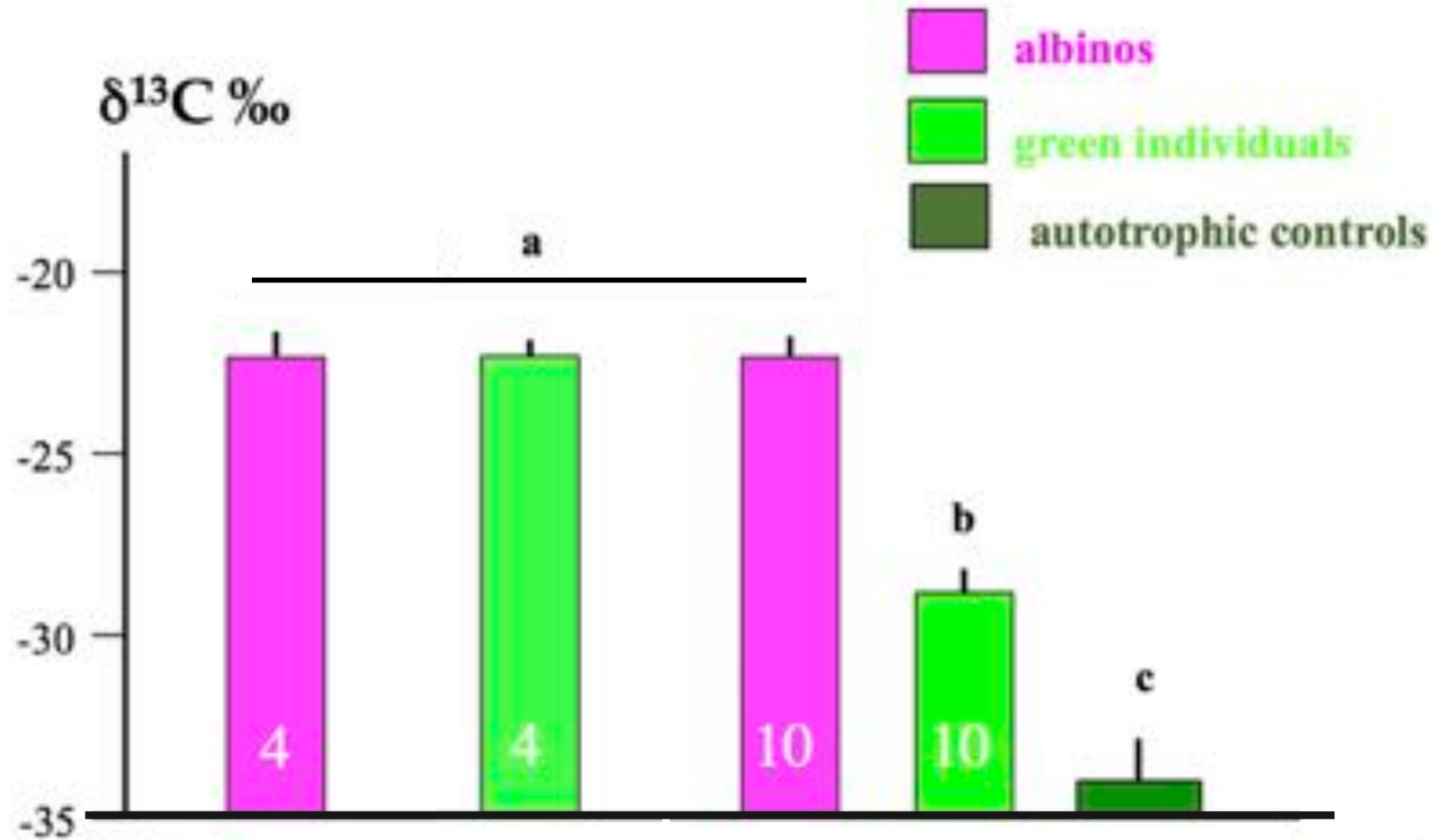


RHIZOME FEED ON FUNGAL C



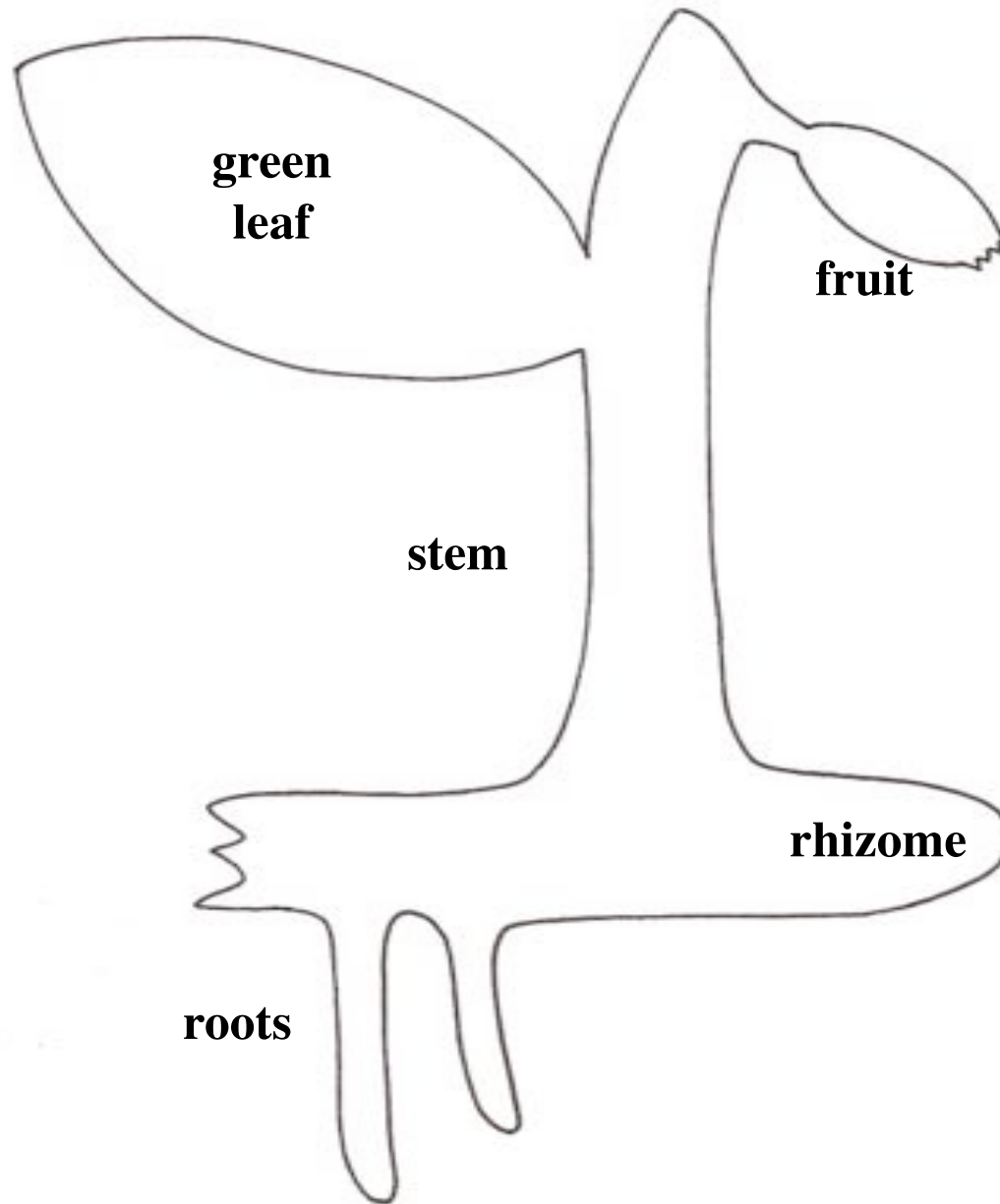
Gonneau *et al.* 2015

RHIZOME FEED ON FUNGAL C



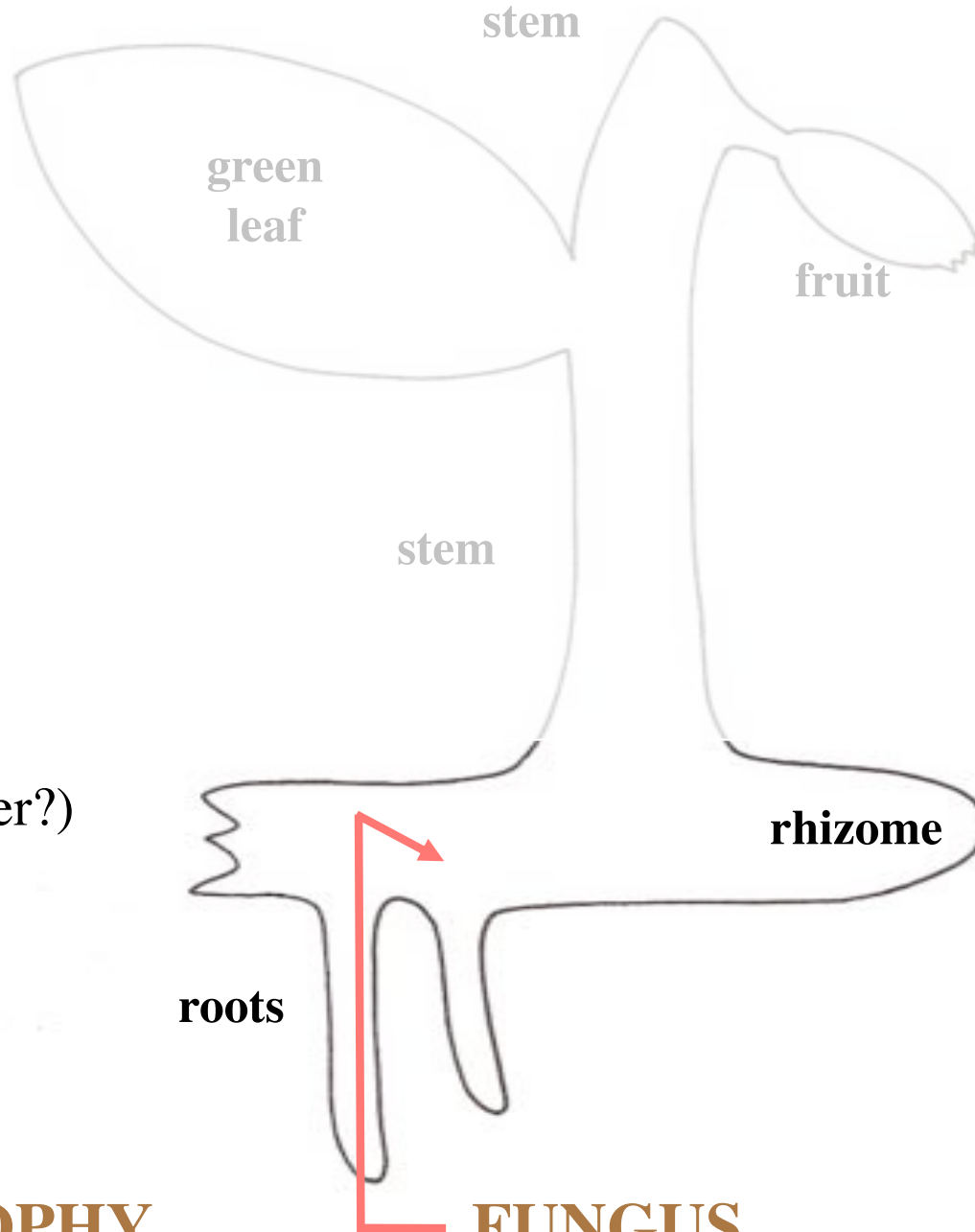
Gonneau *et al.* 2015

PHOTOSYNTHESIS



MYCOHETEROTROPHY

PHOTOSYNTHESIS



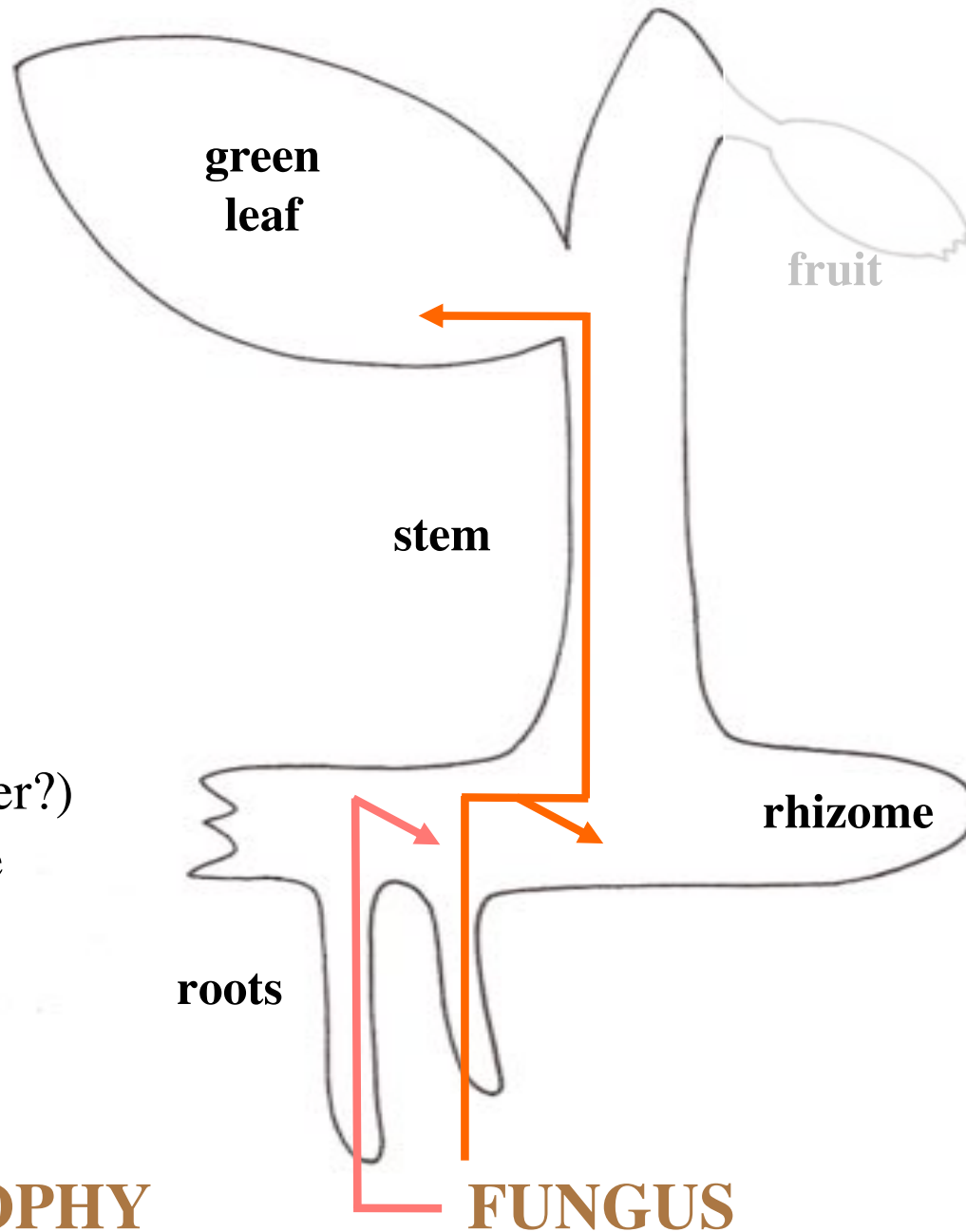
Fungal C

— dormancy (& winter?)

MYCOHETEROTROPHY

FUNGUS

PHOTOSYNTHESIS



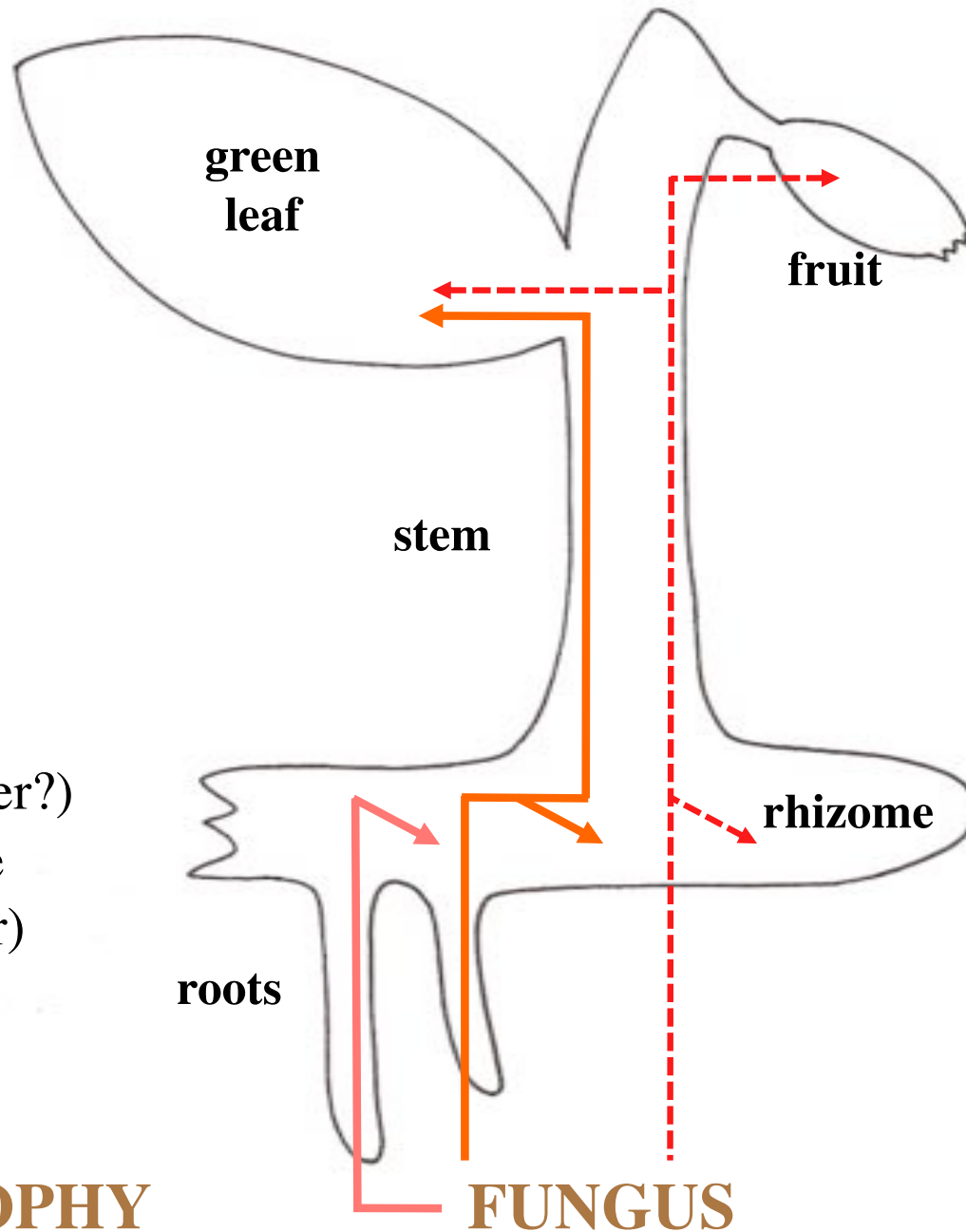
Fungal C

- dormancy (& winter?)
- at shoot emergence

MYCOHETEROTROPHY

FUNGUS

PHOTOSYNTHESIS



Fungal C

- dormancy (& winter?)
- at shoot emergence
- at fruiting (summer)

MYCOHETEROTROPHY

FUNGUS

PHOTOSYNTHESIS

Plant C

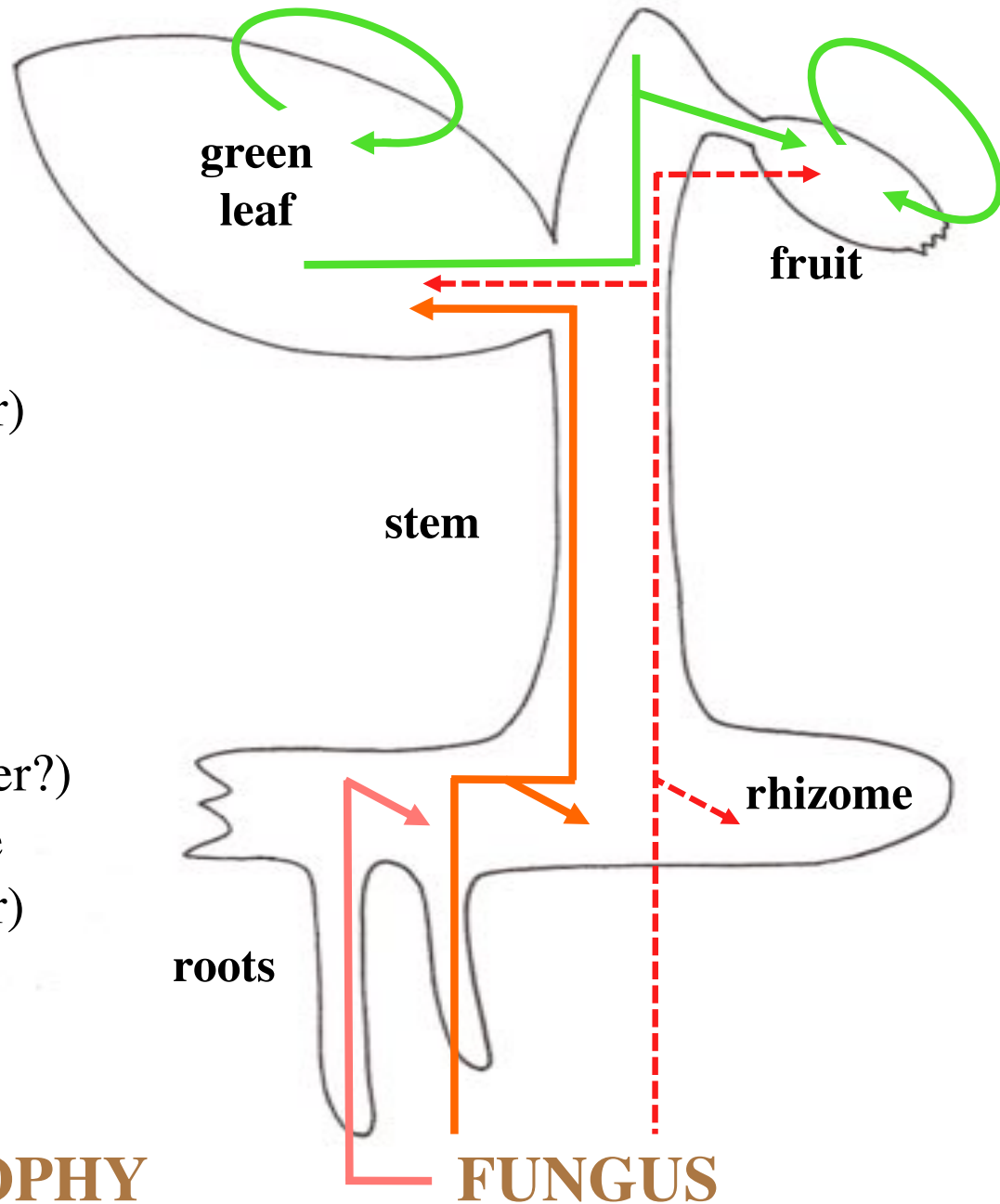
— at fruiting (summer)

Fungal C

— dormancy (& winter?)

— at shoot emergence

— at fruiting (summer)



MYCOHETEROTROPHY

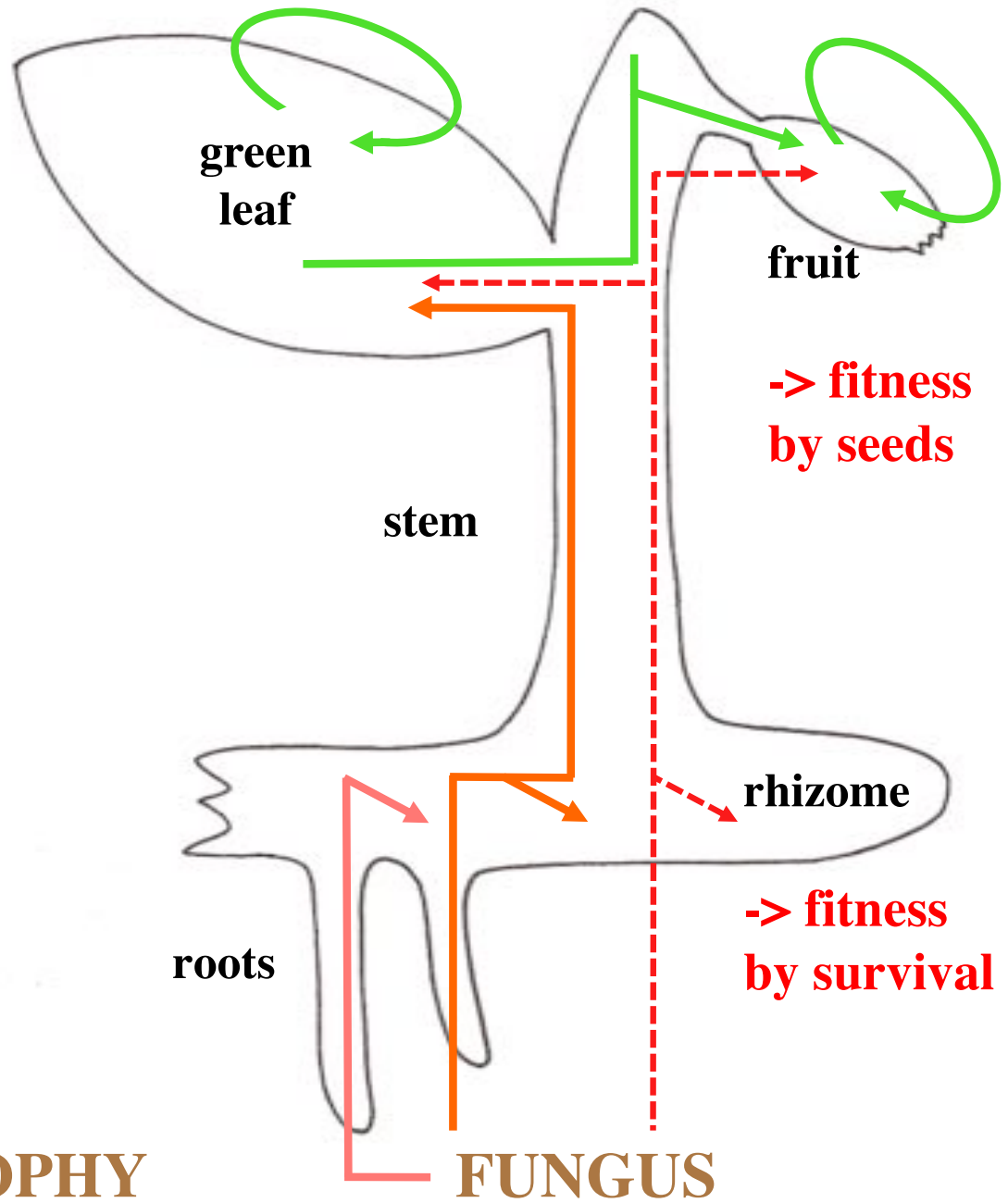
FUNGUS

PHOTOSYNTHESIS

Plant C

Fungal C

MYCOHETEROTROPHY



CONCLUSIONS

- Fruits use mostly photosynthates
- Underground tissues use mostly fungal C
- The resulting optimization of the use of fungal and photosynthetic C is problematic for albino fruiting.

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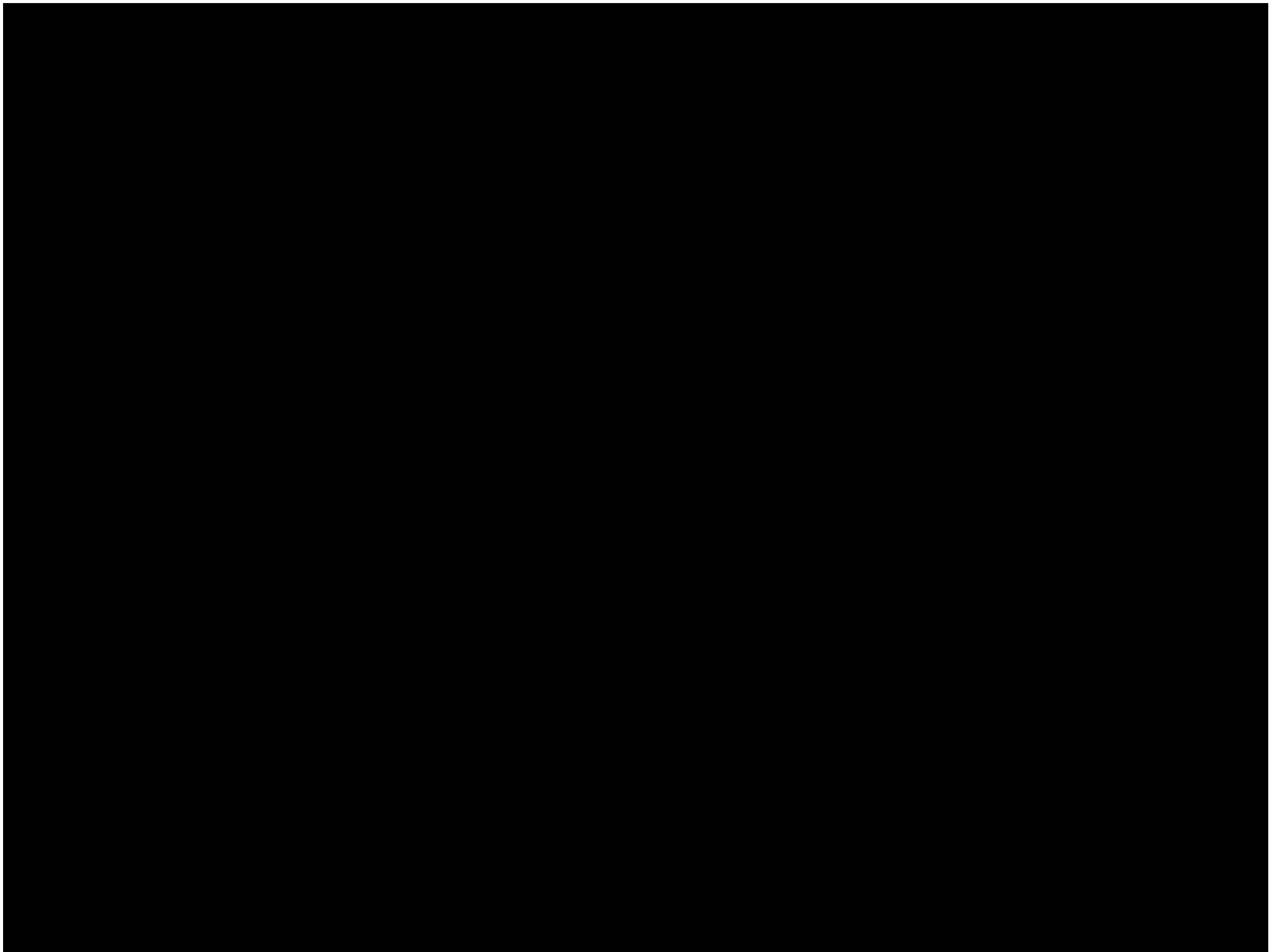
... due to this fatal addiction to light of mixotrophic plants, you cannot lose photosynthesis in one step!

CONCLUSIONS

- Fruits use mostly photosynthates
- Underground tissues use mostly fungal C
- The **resulting optimization** of the use of fungal and photosynthetic C is likely fatal to albino seed production.

The evolutionary emergence of pure C sinks in mycorrhizal networks is a complex process,

... and mixotrophy is metastable in evolution



My partner network...

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... all papers online on ISYEB website

ANR







MARC-ANDRÉ
SELOSSE

JAMAIS SEUL

**Ces microbes qui construisent
les plantes, les animaux
et les civilisations**

postface de Francis Hallé

ACTES SUD