Growth, physiology, and early root development in seedlings of different woody species treated with biostumulants

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Ecosystem services

Green areas provide **ecosystem services = benefits arising from ecological processes which directly or indirectly increase human well-being**

CICES theme	*CICES class*	*TEEB categories*
Provisioning	• Nutrition	• Food, water
	• Materials	• Raw materials, genetic resources, medicinal resources, ornamental resources
	• Energy	
Regulating and maintenance	• Regulation of waste	• Air purification, waste treatment
	• Flow regulation	• Disturbance prevention, regulation of water flows, erosion prevention
	• Regulation of physical environment	• Climate regulation, maintaining soil fertility
	• Regulation of biotic environment	• Gene pool protection, lifecycle maintenance, pollination, biological control
Cultural	• Symbolic	• Information of cognitive development
	• Intellectual and experiential	• Aesthetic, inspiration for culture, art and design, spiritual experience, recreation and tourism

Cices, 2018

Provisioning of
Food, water, energy

Regulation of the environmental processes and cycles (climate regulation, disturbance prevention)

Cultural

Tree planting programs

milliontreesNYC

A PLANYC INITIATIVE WITH NYC PARKS AND NEW YORK RESTORATION PROJECT

1 000 000

TREES PLANTED

10MILA ALBERI PER PADOVA

Forestami

The benefits of one Million Trees in LA

New EU Forest Strategy : planting three billion additional trees across Europe by 2030

ISPRA

As a flagship initiative of the **European Green Deal**, the European Commission has recently adopted the **New EU Forest Strategy for 2030.**

Tree planting programs: planting material

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Tree mortality after transplant can range between 7 and 34% within 5 years from planting (Koeser et al., 2014; Roman et al., 2014).

Was planning carefully done?

- **Are there suitable locations to accommodate new plantings and resources for site amelioration?**
- Are selected species and cv. available in nurseries in adequate quantity and quality?
- Are there resources available for post-planting care?

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Nursery pre-conditioning

Includes a series of cultural techniques applied during nursery production with the aim of producing sturdy plants, with superior tools to recover from transplant shock (Franco et al., 2006):

- Container size and typology
- Nursery substrates
- Deficit irrigation
- Fertilization management
- Root pruning
- Inoculation with mycorrhizal fungi
- Application of biostimulants and plant-growth regulators

HortScience 45(12):1824–1829. 2010.

Effect of Container Design on Plant Growth and Root Deformation of Littleleaf Linden and Field Elm

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Effect of pot type and root structure on the establishment of *Tilia cordata* and *Ulmus minor* plants after transplanting

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Biostimulants

A plant biostimulant is any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrients content.

Diverse nature: single vs. mixed substances and microorganisms

Diverse physiological effects: improve nutrient efficiency and/or abiotic stress tolerance and/or crop quality traits

Humic and fulvic acids (31%; 450 M euro in 2016)

Beneficial fungi and bacteria (9,7%; 141 M euro in 2016)

Inorganic compounds (9,6%; 140 M euro in 2016)

Chitosan and other biopolymers (6%; 87 M euro in 2016)

Seaweed extracts (33,3%; 483 M euro in 2016)

Protein hydrolysates and other N-containing compounds (10,2%; 148 M euro in 2016)

Du Jardin, 2015
Critchley et al., 2021

Seaweed extracts

Algae, especially brown-algae have been applied for agricultural purposes. *Ascophyllum nodosum* is most widely studied (Khan et al., 2009)

About 15 millions metric tons of algae-based products are manufactured annually (FAO, 2006)

Algae-extracts contain several bio-active ingredients:

Laminarin (glucan)

Triggers plant defences

Polyphenols

Trigger plant defences

Mannitol (sugar alchool)

Osmotic adjustment

Organic acids (D-mannuronic; L-guluronic)

Water and nutrient uptake

Fucoidan (sufurated polysaccharide)

Biological activity in mammals

Plant hormones (cytokinins, auxins)

Modulate biosynthetic pathways

Alginic acid

Soil and rooting improvements, mycorrhiza

Arginin

(N source)

Inorganic ions

(Na, Mg, K, Cl e SO4)

Aims of the research

1- Test the hypothesis that a seaweed extract can improve seedling growth, therefore fastening plant production process.

2- Test the hypothesis that a seaweed extract improves root morphological traits and root:shoot ratio, so that transplant success can be improved

3- Identify optimal dose of application

Experimental site

The experiment was carried out at ERSAF, in a regional forest nursery (Curno, BG, Italy), under a Cfb (Koppen-Geiger classification) climate.

Average temperature = 11.5 °C

Average rainfall = 1420 mm/year


Plant material

Seeds of 5 species known to have poor rooting performances in forest nurseries were selected

Amelanchier ovalis

Carpinus betulus

Crateagus monogyna

Fagus sylvatica

Ligustrum vulgare

Plant material and growing conditions

- Seeds of the five species were obtained from local certified seed forests.
- Seeds were cleaned using sodium hypochlorite and stratified in moist sand at 4 °C to overcome dormancy
- In Feb. 2021, seeds were seeded in 0.4 L plastic trays, according to a randomized block design with 10 blocks (19200 seeds in total which yielded 6400 seedlings)
- A commercial substrate specifically developed for seedlings (Hochmoor, Teflor, Italy). The substrate contains with 1 kg/m3 of soluble fertilizer and 1 kg/m3 a controlled release fertilizer, was used.

Substrate component or trait	Amount (v/v) or value
Brown peat + montmorillonite	30%
Blond peat (10-20 mm)	50%
Coconut fiber	20%
pH	6.0
EC	0.3 dS/m
Density	100 kg/m^3
Porosity	95%

The seaweed extract tested

Ascophyllum nodosum is a brown-algae which grows in coasts of Northern Europe, Iceland, Eastern Canada and Greenland with possible applications for horticulture

We used a pure extract (Agrofertil, France) made by grinding the algae at low temperatures. The extract was a powder

The extract was mixed with the nursery substrate at different rates

D0: no extract (control)

D1: 1 kg/m^3 (label dose)

D2: 2 kg/m^3 (2x label dose)

D3: 3 kg/m^3 (3x label dose)

COMPOSIZIONE MEDIA			
Umidità	12-15%	Carboidrati	45-60%
Proteine grezze	5-8%	Acido alginico	20-26%
Grassi grezzi	2-4%	Mannitolo	5-8%
Fibre grezze	>8%	Laminarina	2-6%
Materia minerale	40-55%	Fucoidano	8-10%
Sabbia	>0,5%		
MACROELEMENTI		**MICROELEMENTI**	
Azoto (N)	0,8-1,3%	Iodio (I)	500-1200 ppm
Fosforo (P)	0,05-0,15%	Rame (Cu)	1-10 ppm
Potassio (K)	1-3%	Ferro (Fe)	150-1000 ppm
Calcio (Ca)	1-3%	Manganese (Mn)	0-50 ppm
Zolfo (S)	2-5%	Boro (B)	20-100 ppm
Magnesio (Mg)	0,5-0,9%	Molibdeno (Mo)	1-5 ppm
Cloro (Cl)	2-5%	Zinco (Zn)	40-200 ppm
Sodio (Na)	2-4%	Arsenico (As)	20-45 ppm
VITAMINE presenti in quantità approssimative			
Carotene (A)	20-60 ppm	Tocoferolo (E)	50-200 ppm
Acido ascorbico (C)	2000-1000 ppm	Niacina (B3)	10-30 ppm
Riboflavina (B2)	5-10 ppm	B12 ca. (M)	0,004 ppm
Tiamina (B1)	1-5 ppm	Acido folico (B9)	0,2 ppm
Biotina (B)	0,1-0,4 ppm	Acido folinico	0,2 ppm

Tabella 4 – Composizione media del biostimolante a base di Ascophyllum nodosum usato nella sperimentazione (agrofertil.fr).

Plant material and growing conditions

In Jan. 2022, plants were re-potted into 1.7 L containers and grown under a tunnel under full sunlight for one additional growing season.

<u>Algae extracts were not applied at repotting</u>

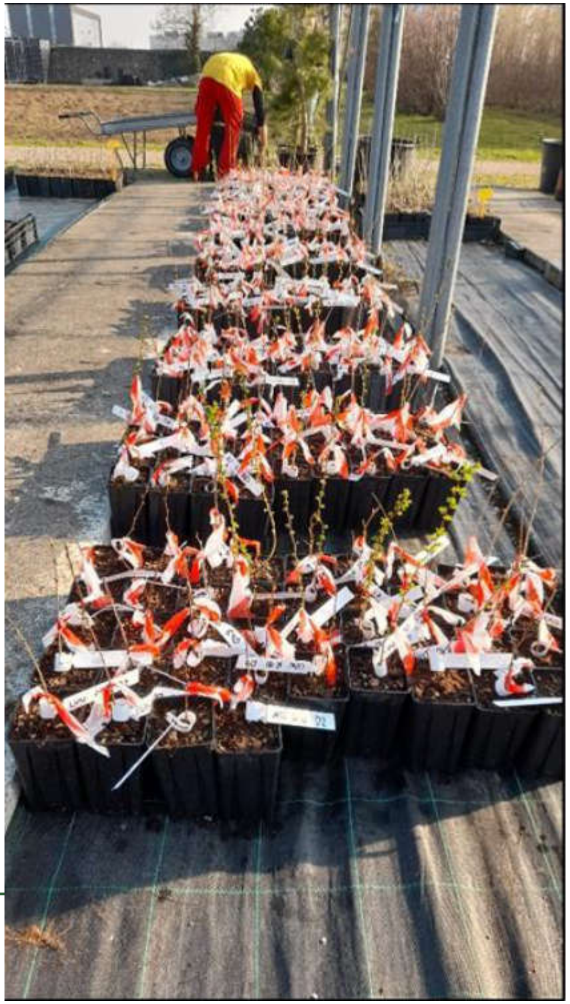
After repotting, plants were arranged according to a randomized block design with 10 blocks (2 plants per treatment and species in each block; 240 plants in total)

Irrigation was carried out daily (10-15 minutes during nighttime)

No additional fertilization other than that included in the substrate was done

Weeding was conducted manually, twice per month

No pesticide was applied



Measurements

Growth traits (measured on 2, 9, 15, 30, 58 and 80 weeks after emergence):

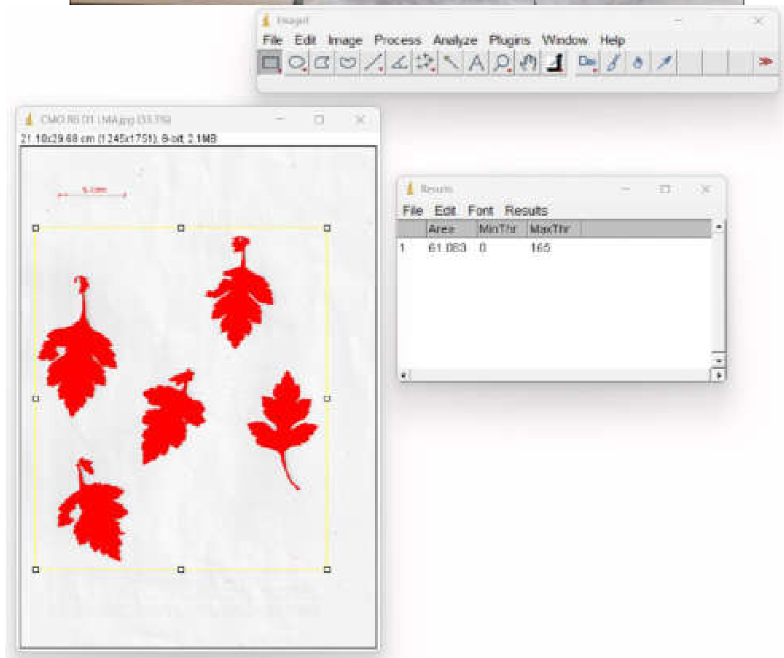
1- **leaf, stem, and root dry weights** (g):
measured after cutting the stem at the flare, separating the leaves, cleaning the roots with a flush of air, and oven-drying leaves, stems, and roots separately at 70°C until constant weight

2- **Average and Total leaf area** (cm^2):
measured scanning all plant leaves with an A3-scanner

3- **Relative Growth Rate of the plant and of roots**: calculated as (lnDWt1 - lnDWt0)/(t1-t0)

4- **Root:shoot**: calculated as DWroots/(DWstem + DWleaves)

5- **Total leaf area to total root length ratio (cm2 leaf/cm root)**



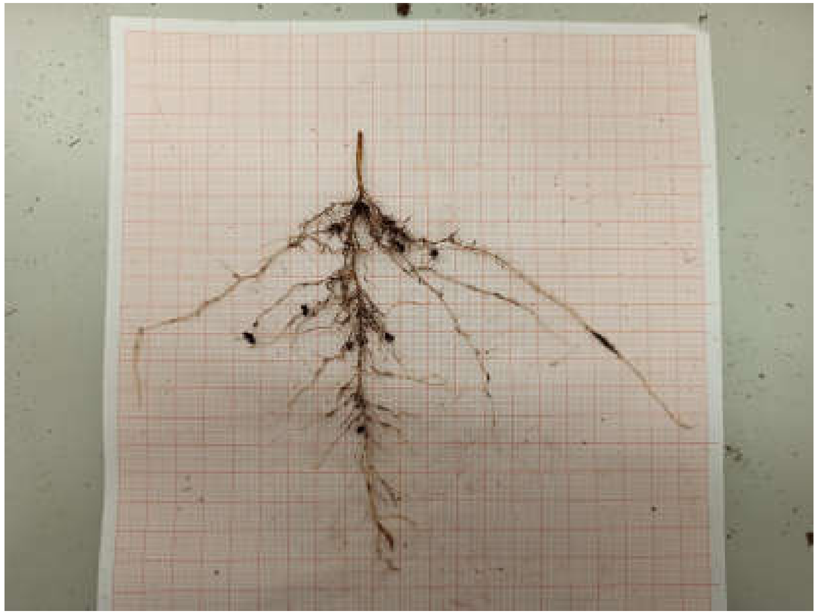
Measurements

Root traits (measured on 8, 15, 30, 58, and 80 weeks after emergence):

1- **Total root length** (cm): measured using the root-line intersect method (Tennant, 1975)

2- **Root density (cm/dm3):** directly root length per unit container volume

3- **Specific root length** (cm/g): root length per unit weight.



Physiology (measured on 2, 6, 12, 24, and 72 weeks after emergence on 3 species):

1- **Net CO_2 assimilation** (μmol m^{-2} s^{-1})

2- **Stomatal conductance** (mmol m^{-2} s^{-1})

3- **Sub-stomatal and chloroplastic CO2 concentration** (ppm)

4- **Water use efficiency**: calculated as A/E

5- **Leaf greenness index**: measured using a SPAD-meter

Results

All data were analysed using mixed models tool in SPSS. Homogeneous subsets were separated using Sidak test

Root systems as affected by species and seaweed extract: P-values from statistics

	Root DW	Root:Shoot	Specific root length	Total root length	Root density
$P_{species}$	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
P_{dose}	0.092	0.061	0.151	0.850	0.440
P_{time}	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
$P_{speciesXdose}$	0.322	0.484	0.370	0.405	0.332
$P_{speciesXtime}$	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
$P_{doseXtime}$	0.550	0.681	**0.028**	1.000	0.911
$P_{spXdoseXtime}$	0.512	0.407	0.422	0.516	0.544

Species and time of sampling significantly affected root traits and a significant species X time interaction was found for all parameters

Species differed in root traits and differences changed overtime

P-values lower than 0.05 indicate significant differences among treatments
P-values lower than 0.01 indicate highly significant differences among treatments

Did seaweed extract affect root traits? P–values from statistics

	Root DW	Root:Shoot	Specific root length	Total root length	Root density
$P_{species}$	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
P_{dose}	0.092	0.061	0.151	0.850	0.440
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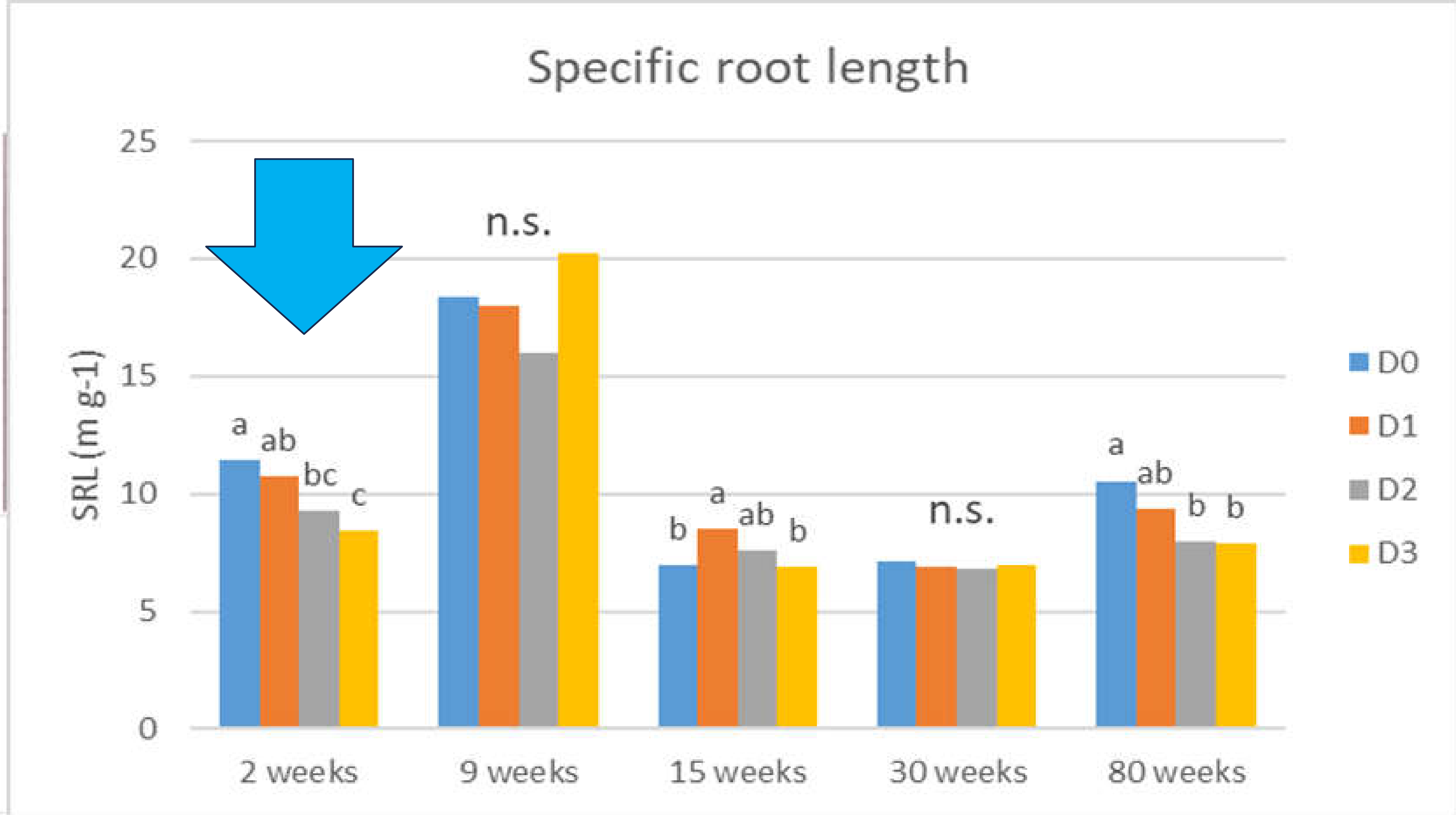
$P_{spXdoseXtime}$	0.512	0.407	0.422	0.516	0.544

Seaweed extracts did not affect root DW, root:shoot; total root length, and root density at any dose of application

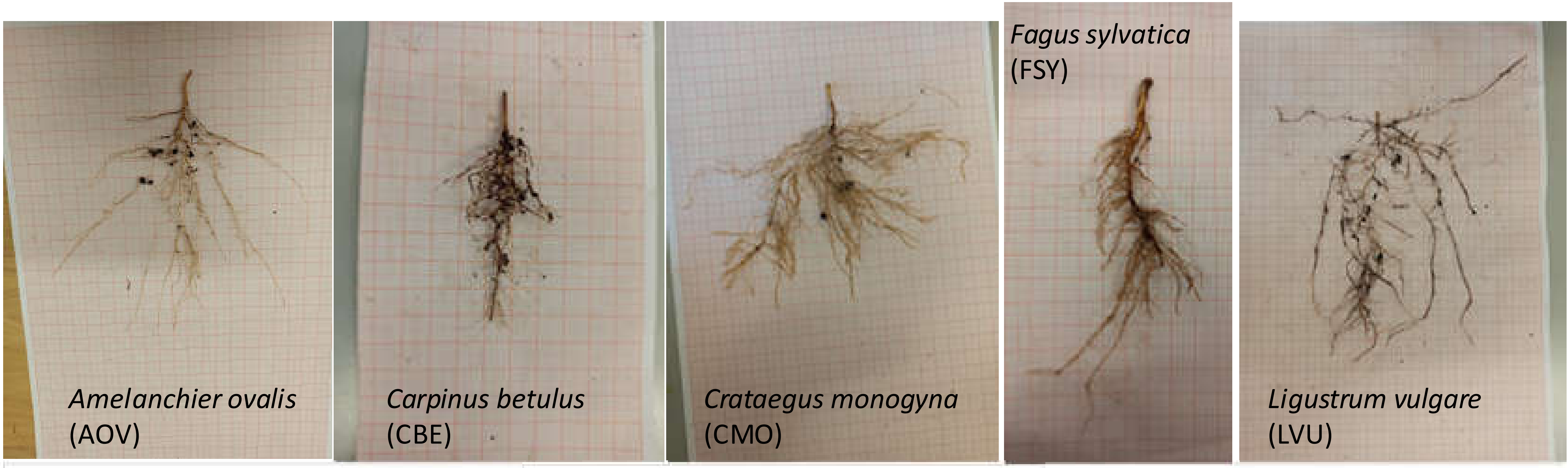
A dose X time of sampling interaction was found for SRL

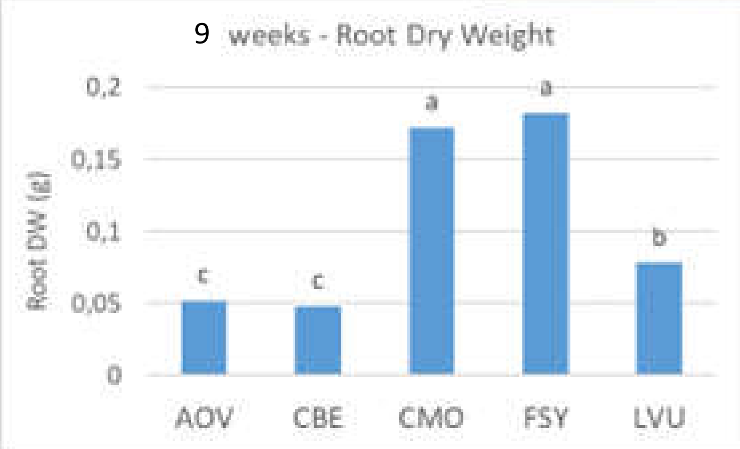
P-values lower than 0.05 indicate significant differences among treatments
P-values lower than 0.01 indicate highly significant differences among treatments

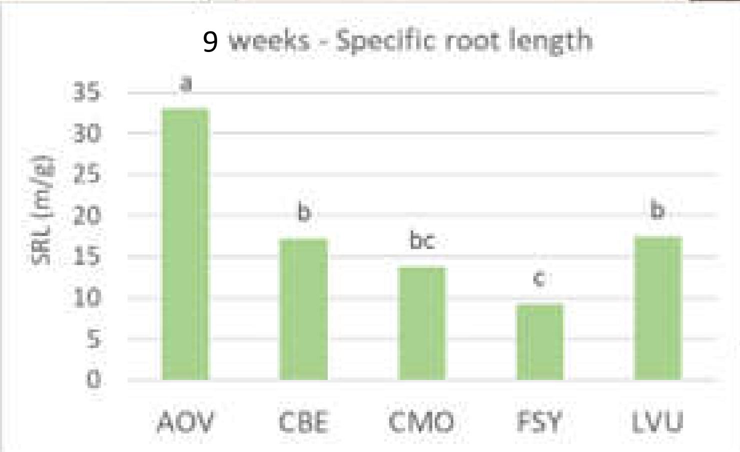


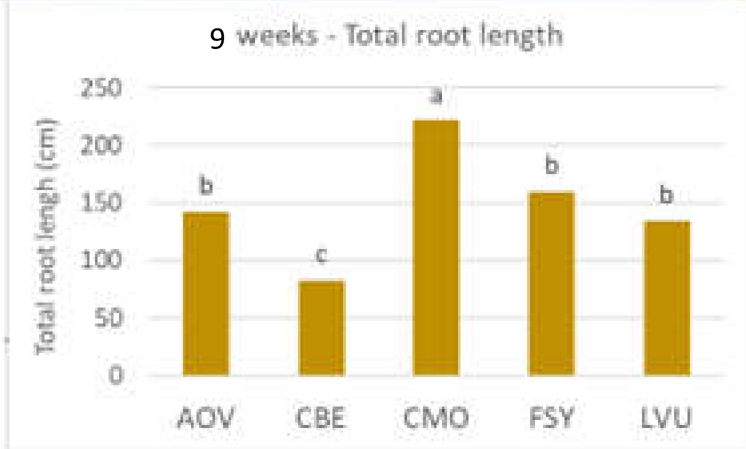
Specific root length
25
20
15
10
5
0
SRL (m g-1)
n.s.
n.s.
a
ab
bc
c
b
a
ab
b
a
ab
b
b
2 weeks
9 weeks
15 weeks
30 weeks
80 weeks
D0
D1
D2
D3

Species differences in root traits (9 weeks after emergence – early June)









Species differences in root traits (15 weeks after emergence – mid July)

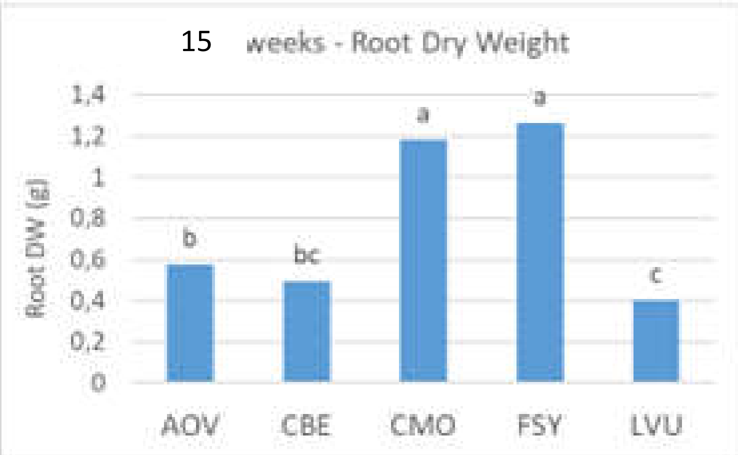
Amelanchier ovalis (AOV)

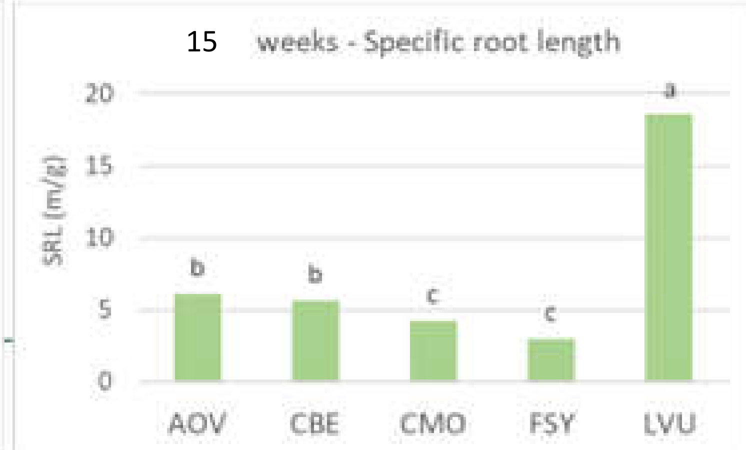
Carpinus betulus (CBE)

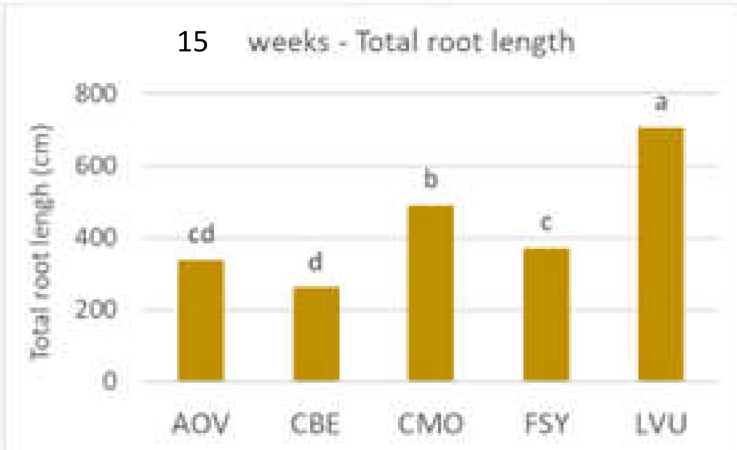
Crataegus monogyna (CMO) (3 of these)

Fagus sylvatica (FSY)

Ligustrum vulgare (LVU) (6 of these)







Species differences in root traits (30 weeks after emergence – October)

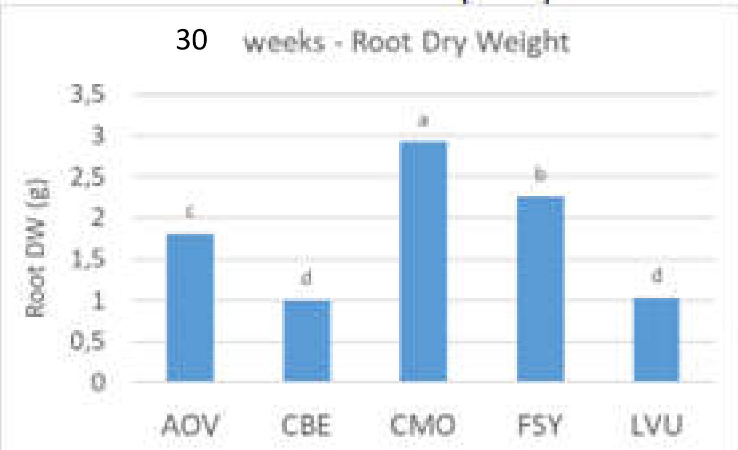
Amelanchier ovalis (AOV)

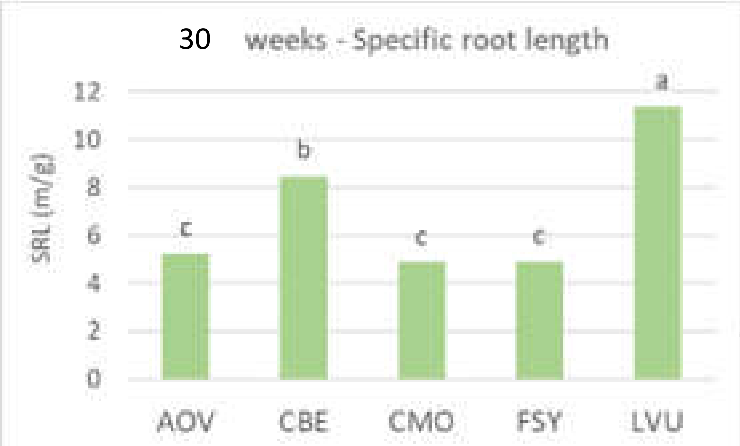
Carpinus betulus (CBE)

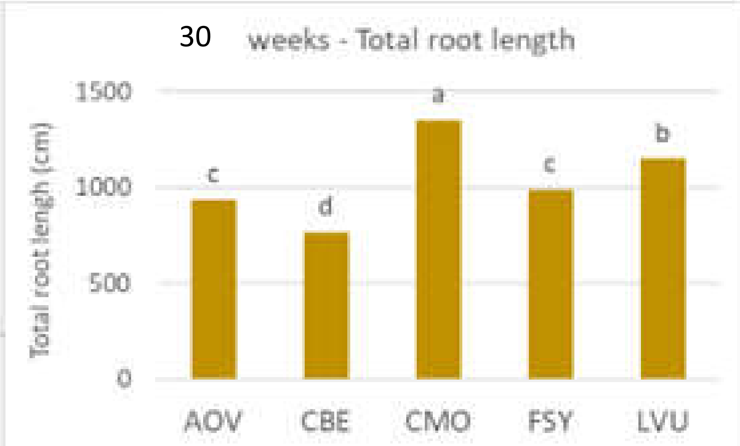
Crataegus monogyna (CMO)

Fagus sylvatica (FSY)

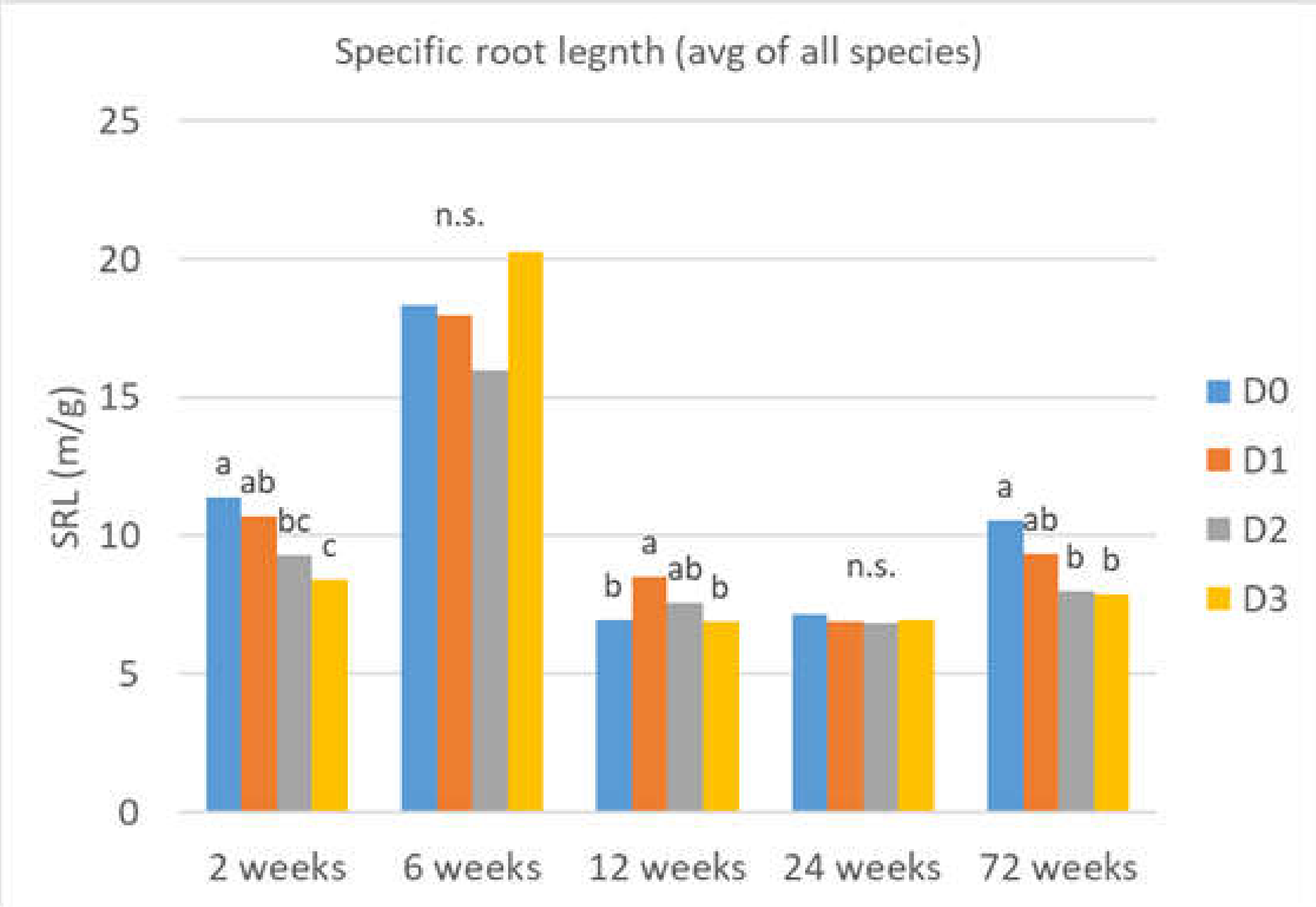
Ligustrum vulgare (LVU)







Specific root length (dose x time)



Overall, the application of biostimulants, decreased SRL.

Lower SRL has been associated to higher soil nutrient availability (Ostonen et al., 2007)

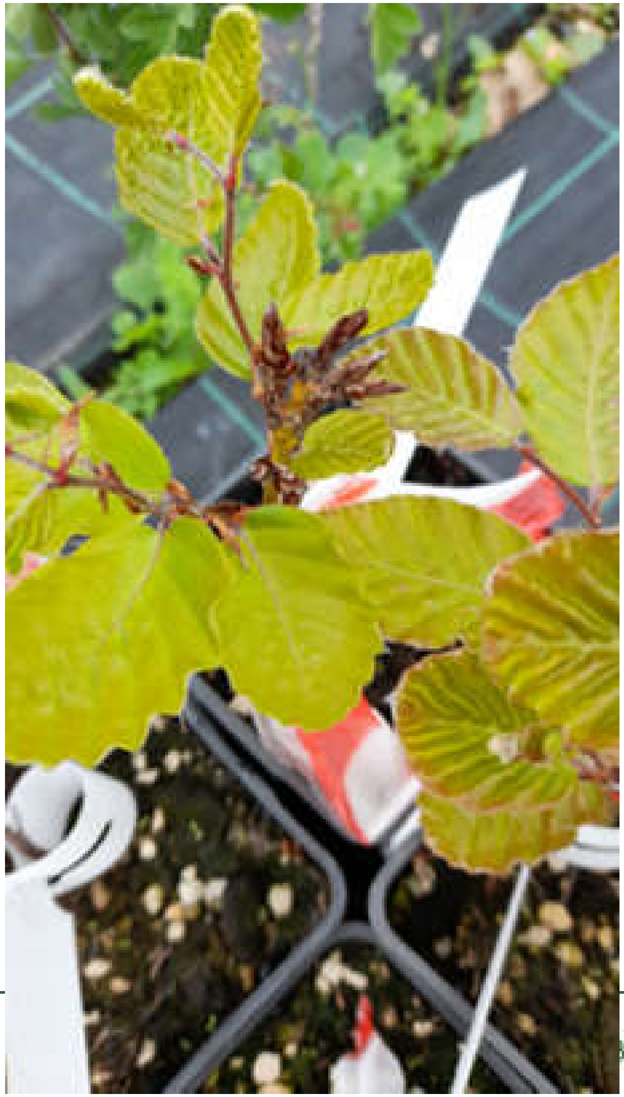
Lower SRL has been related to lower root metabolic activity and respiration, lower capacity to explore the soil for water but higher root longevity (Poorter et al., 2015)

Only at 12 weeks after emengence, when exracts were applied at label dose, SRL increased compared to control

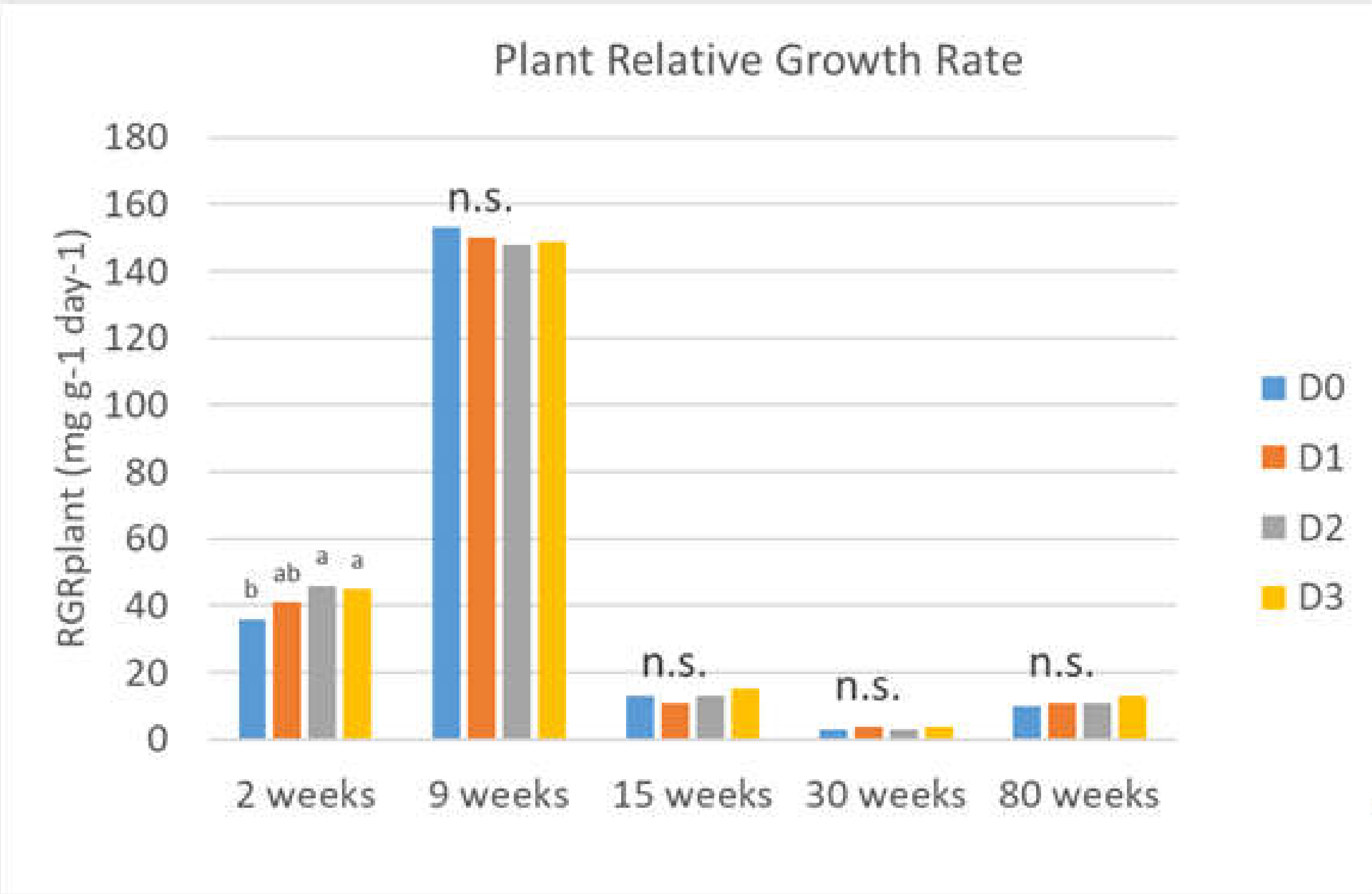
Effect of seaweed extract on plant growth and leaf area

	RGR plant	Plant DW	Total leaf area	TLA/TRL
$P_{species}$	**0.000**	**0.000**	**0.000**	**0.000**
P_{dose}	0.169	**0.000**	0.433	0.067
P_{time}	**0.000**	**0.000**	**0.000**	**0.000**
$P_{speciesXdose}$	0.992	0.069	0.566	0.219
$P_{speciesXtime}$	**0.000**	**0.000**	**0.000**	**0.000**
$P_{doseXtime}$	**0.001**	**0.004**	**0.001**	0.717
$P_{spXdoseXtime}$	0.701	0.079	0.854	0.937

- Specie differed for all investigate traits
- The effects of biostimulant application were similar in all species
- A dose x time interaction was found for RGRplant, plant DW, and TLA indicating that biostimulant application had an effect which changed over time



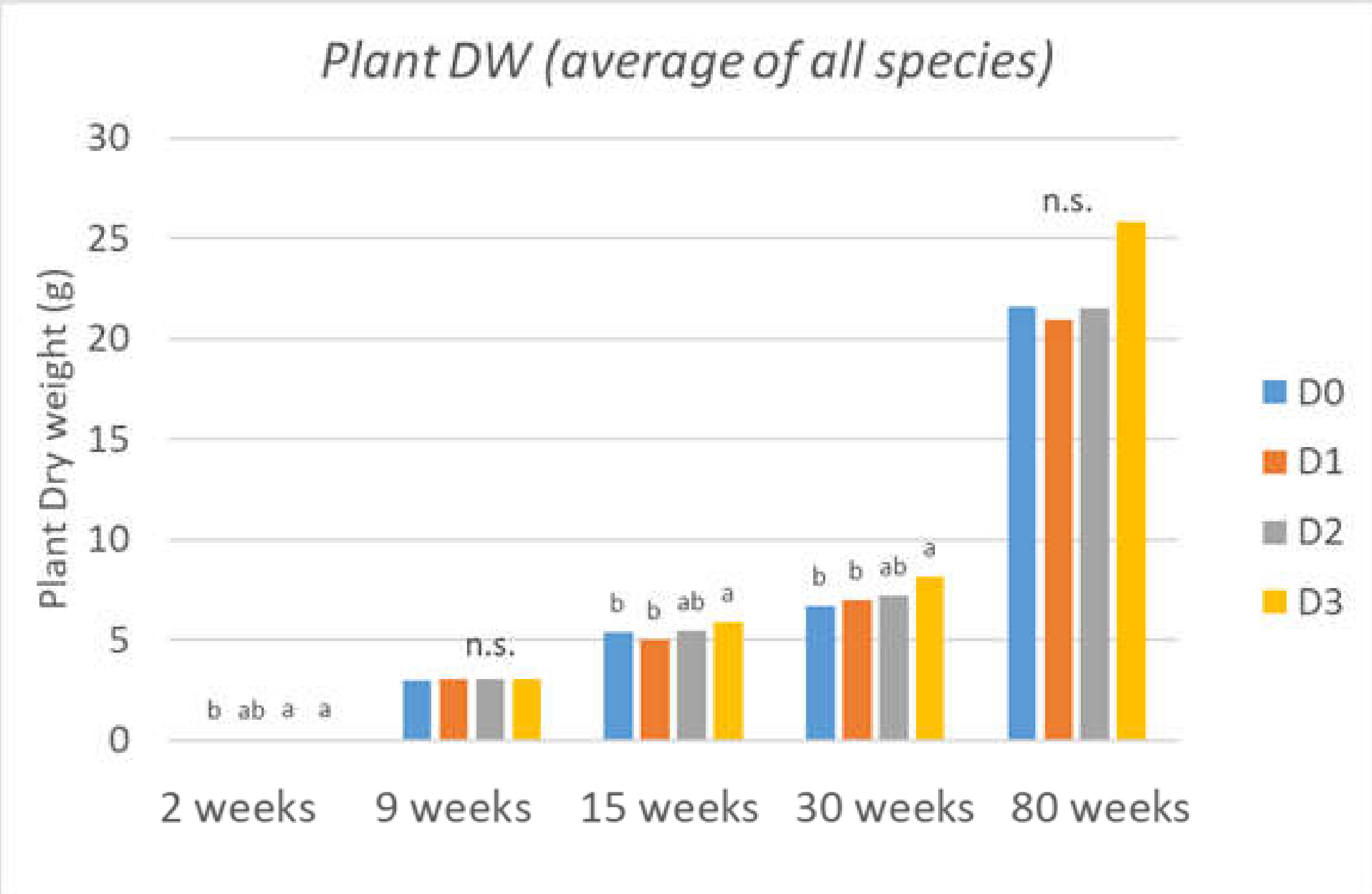
Relative growth rate: whole plant



- It indicates the amount of dry biomass produced per unit existing g DW per day

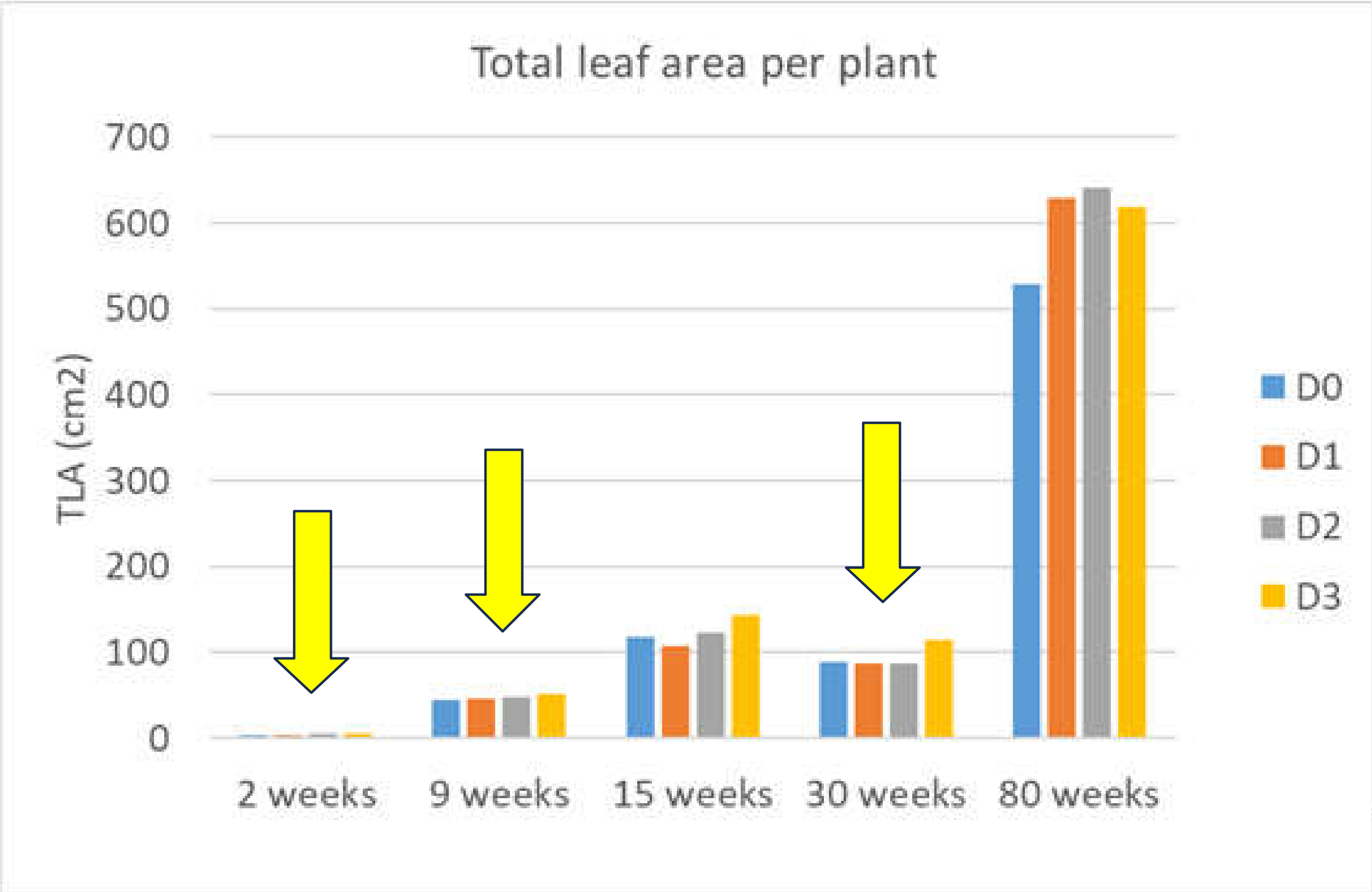
- Higher RGRplant was observed in the 2-6 weeks after emergence period in all species
- RGRplant transiently increased when biostimulants werea applied at 2x or 3x label doses, compared to control

Plant dry weight



- Higher RGR at the earliest stages of plant development resulted in transiently higher plant biomass produced in plants amended with 3x label dose, compared to control
- The effect was significant for 1 growing season.
- After repotting, differences among doses were no longer significant

Total Leaf Area

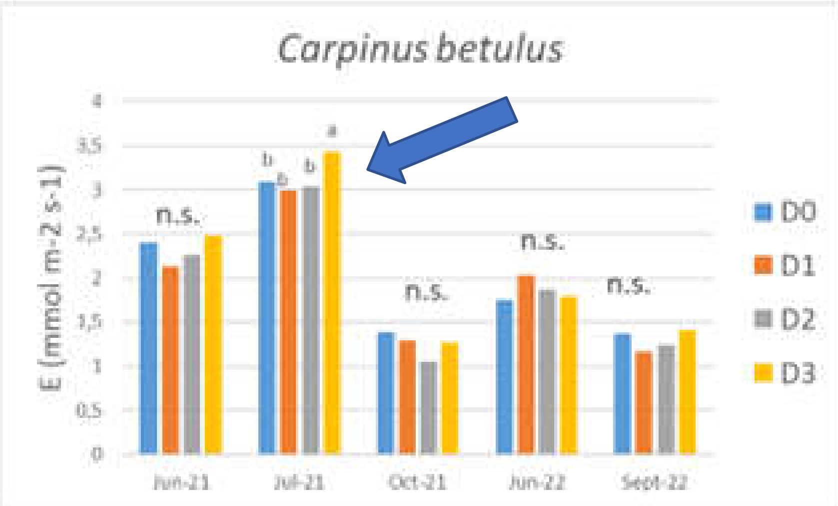


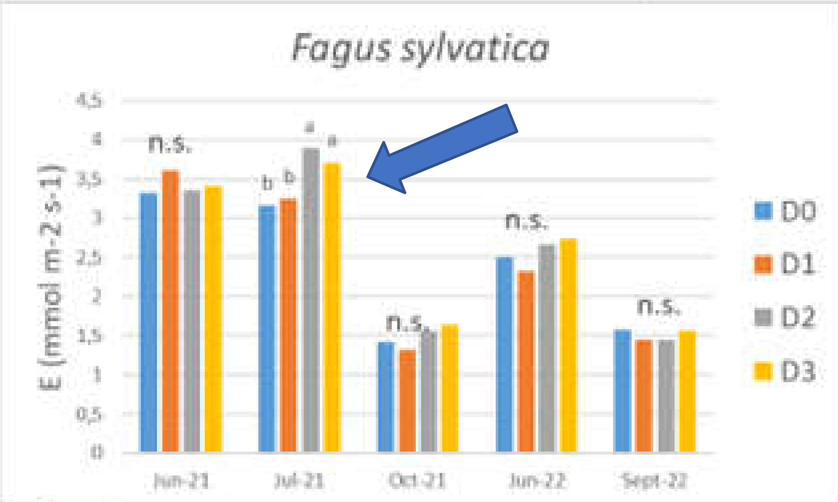
- Biostimulant application temporary increased total leaf area per plant when they were applied a 3x label dose.
- Larger changes were observed during fall (24 weeks).
- Such effect may be due to enhanced tolerance to summer heat or delayed stress-induced leaf senescence (polyphenols?)

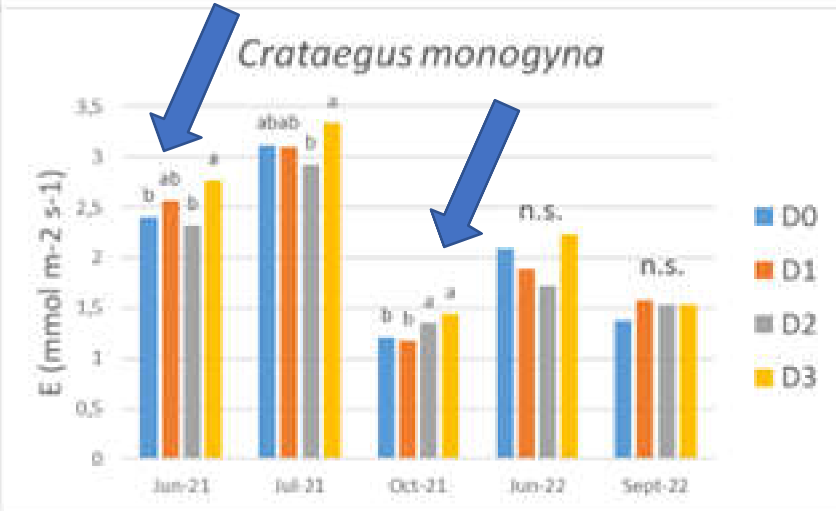
Leaf gas exchange: transpiration

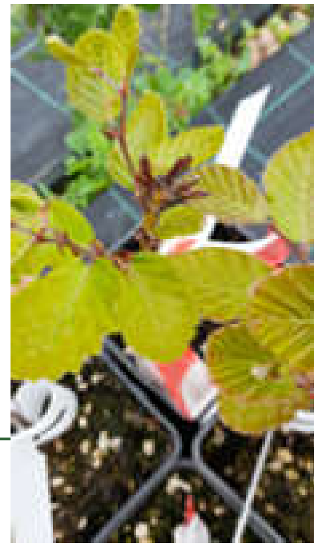
Factor	P-value
Pspecies	**
Pdose	**
Ptime	**
PspeciesXdose	n.s.
PspeciesXtime	**
PdoseXtime	n.s.
PspXdoseXtime	*

- Mannitol in algae-extracts may have improved osmotic adjustment and water uptake.
- Seaweed extracts transiently increased the amount of water transpired per unit leaf area compared to control.
- The effect was significant only in the 1st year and only when algae were applied a 3x label dose

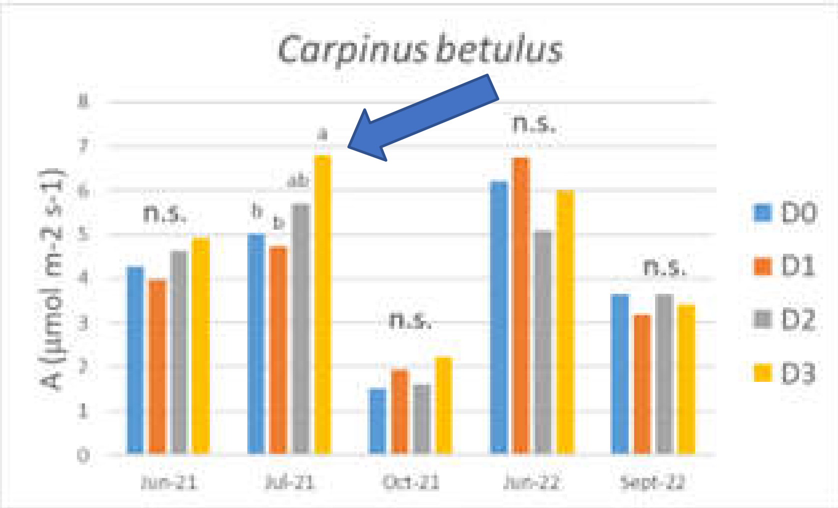




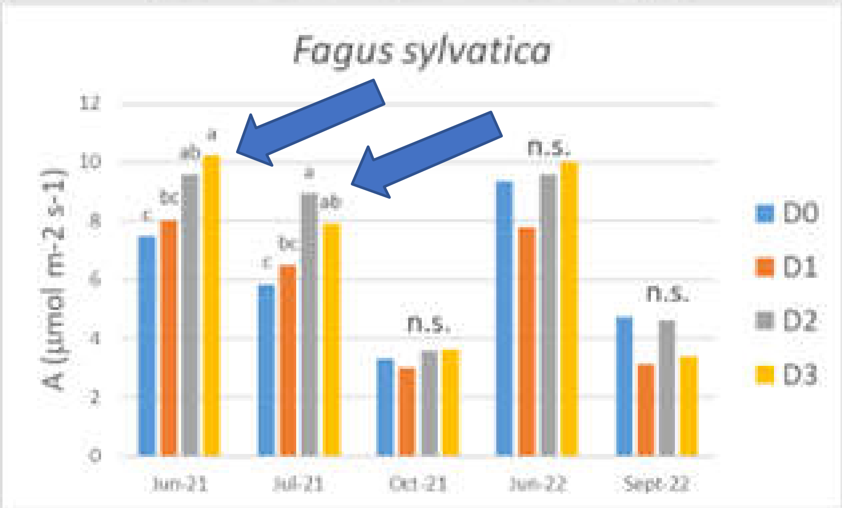


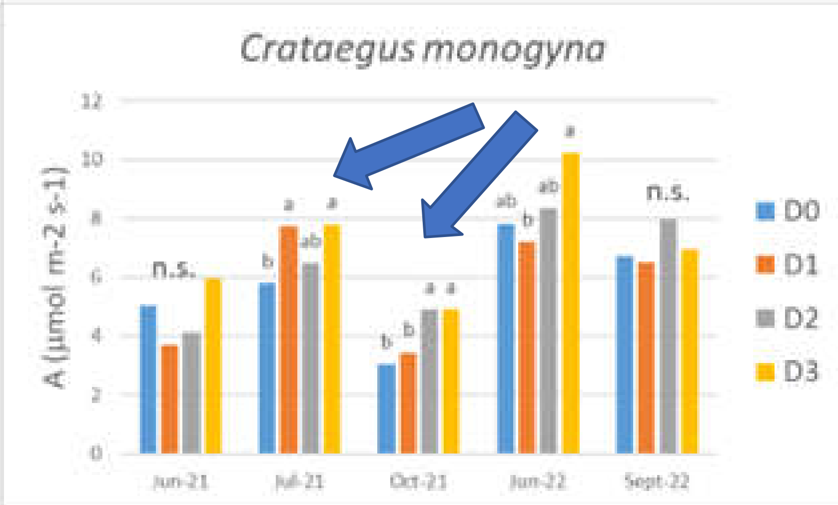


Leaf gas exchange: net CO2 assimilation



Factor	P-value
Pspecies	**
Pdose	**
Ptime	**
PspeciesXdose	*
PspeciesXtime	**
PdoseXtime	*
PspXdoseXtime	n.s.





- The amount of CO2 assimilated by 1 m2 leaf area was increased when seaweed extracts were applied at 3x label dose, compared to control.
- Such effect was observed within 1 year since biostimulant application

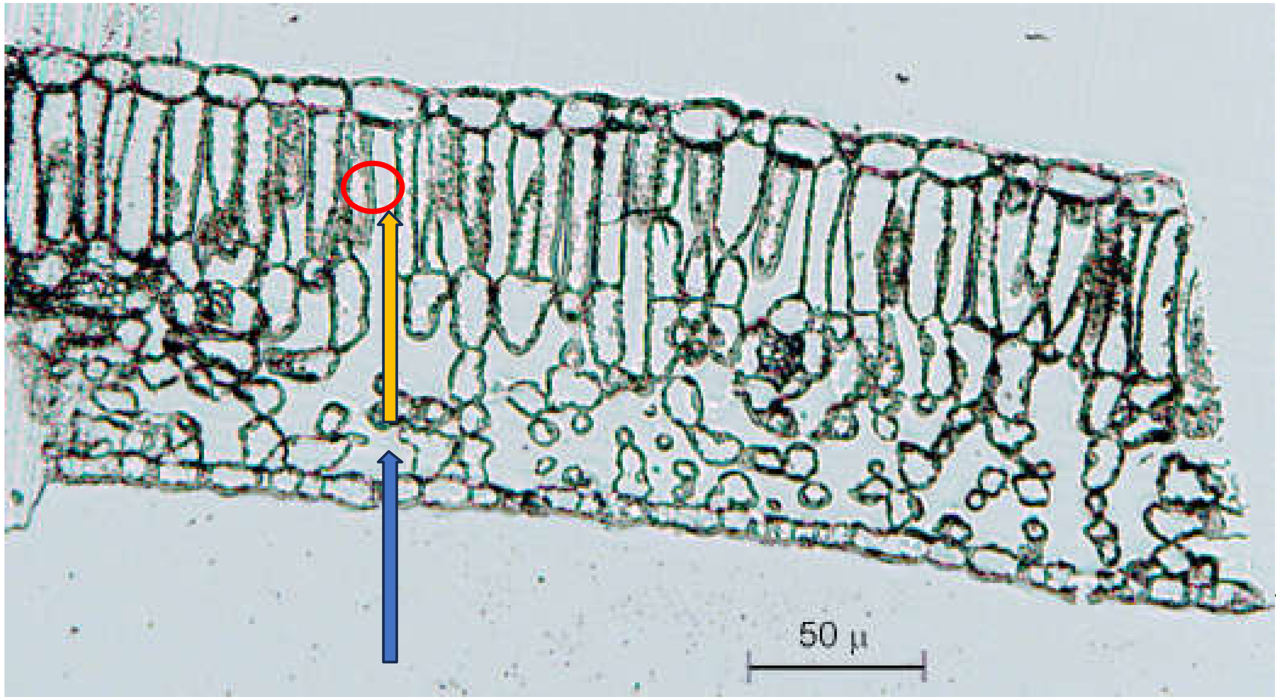
Leaf gas exchange: photosynthetic limitations

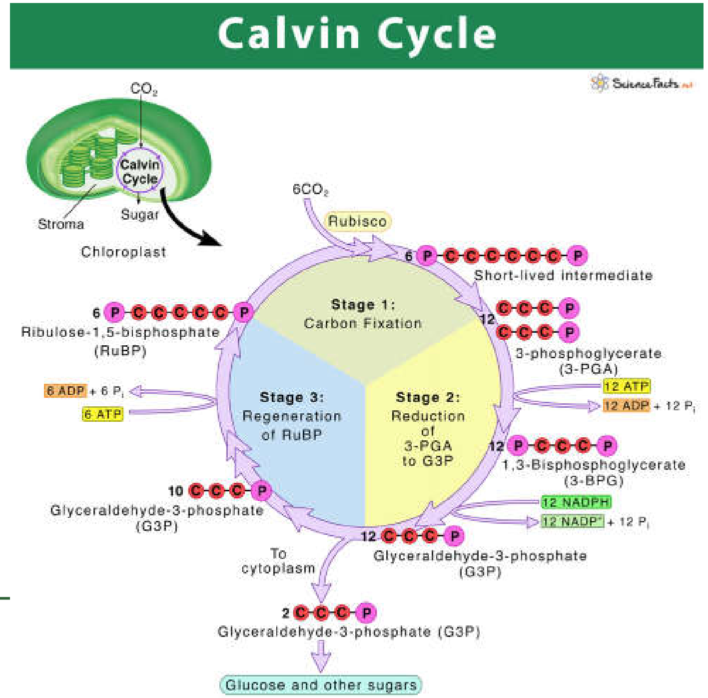
To be converted into carbohydrates, CO_2 must:

1- diffuse from the outer air to the leaf (stomata, blue arrow)

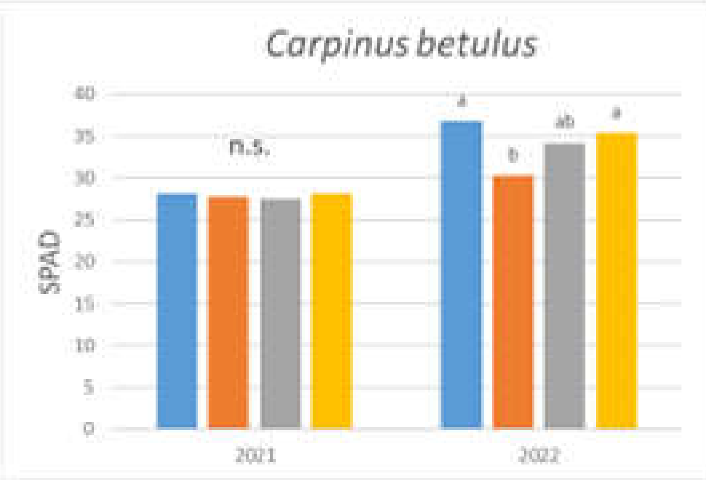
2- diffuse from the substomatal chamber to the chloroplast (mesophyll, orange arrow)

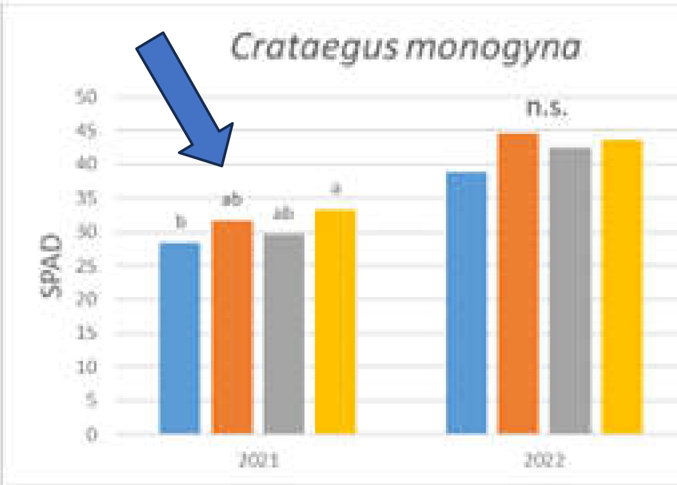
3- be carboxylated (Rubisco and Ribulose, red dot)

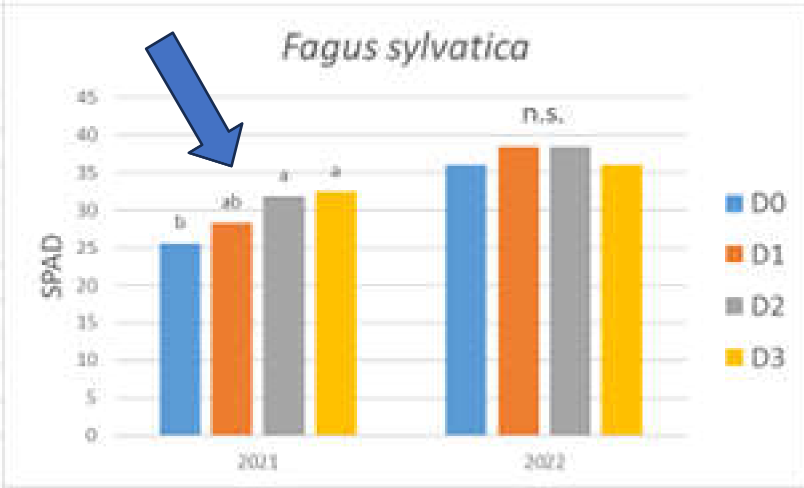




Leaf gas exchange: leaf greenness index







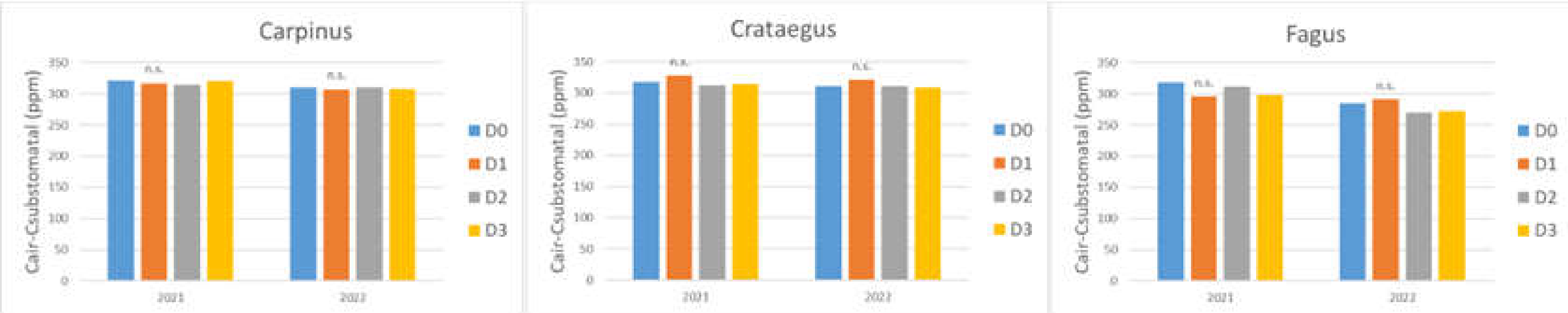
- Biostimulants at 3x label dose increased leaf greenness index in 2 of the 3 species, during the 1st year
- Short-term effect.
- Nitrogen involved? (*A. nodosum* has up to 1.3% N which can yield about 40 g N/m3 substrate when it is applied at 3x label dose; comparable to most organic fertilizers)

Improvements in photosynthetic rate in plants amended with 3x label dose are linked to non-stomatal factors (likely due to better nutrition) rather than to structural improvements that promote stomatal opening

Leaf gas exchange: diffusive limitations

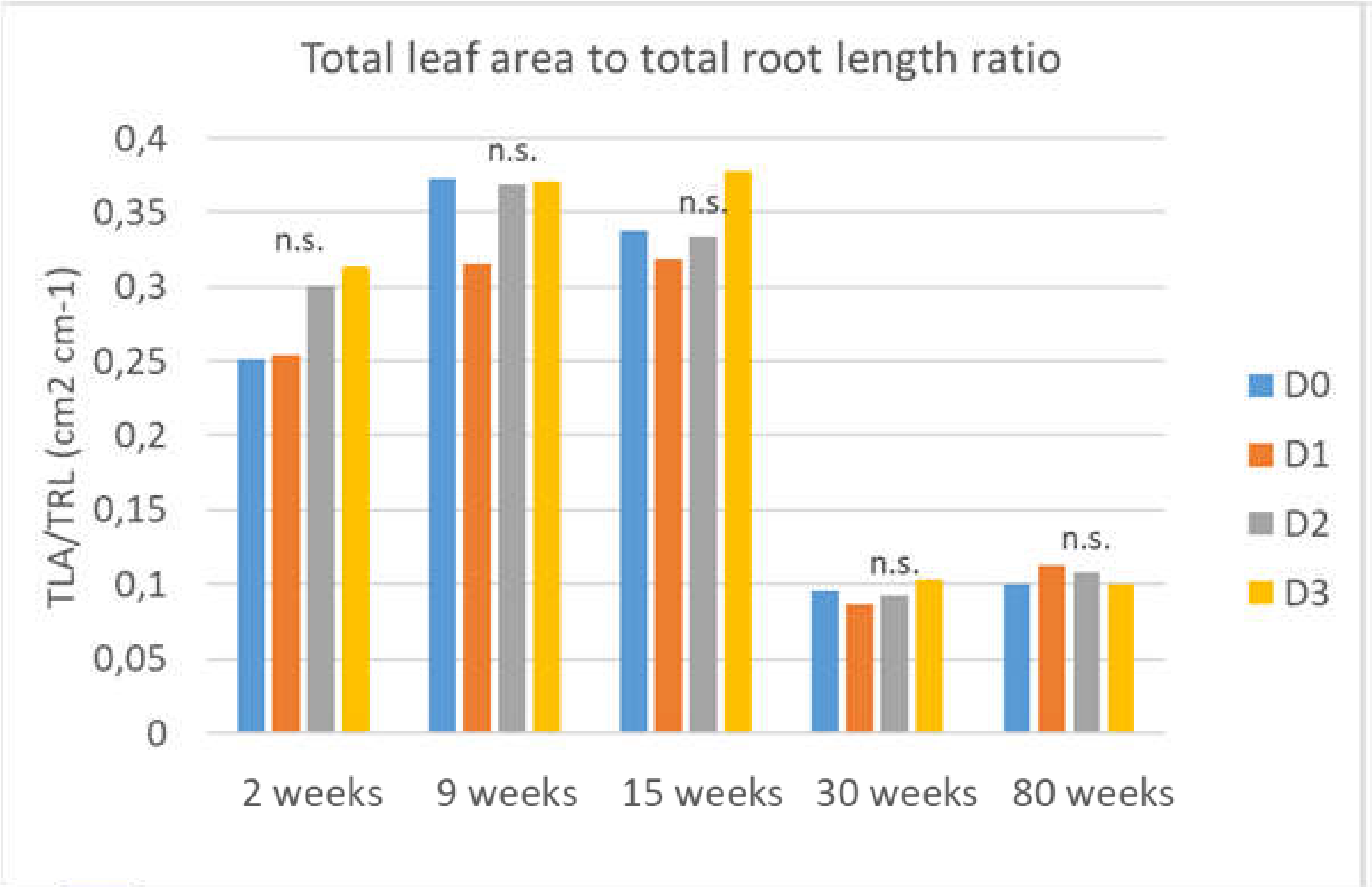
Stomatal and mesophyll factors determine diffusive limitations to photosynthesis, which can be estimated as (Loreto et al., 1994):

- Diffusive drawdown = CO2 air – CO2 chloroplast (the lower it is, the easier the diffusion)



- Diffusive drawdown is higher in *Carpinus* and *Crataegus* than in *Fagus* (lower SRL in water-rich irrigated environment?)
- The seaweed extract did not consistently facilitate CO_2 diffusion from the outer air to the chloroplasts.

Traits which can favor tolerance to transplant



- Biostimulant application did not affect Root to Shoot ratio
- Biostimulant application did not affect the ratio between total leaf area and total root length.
- Biostimulant application did not promote shifts towards higher allocation to roots, compared to shoot.

Take home message

- *A. nodosum* improved growth in the short-run during nursery cultivation. This was partly due to higher chlorophyll content in plants treated with algae at high doses which determined a short-term increase in photosynthetic rate
- Annual applications of *A. nodosum* may fasten plant production process
- The optimal dose of application was 3x label dose = 3 kg/m3
- Water uptake and transpiration increase in the short-run, likely due to osmotic role of some biostimulant components (e.g. mannitol), rather than to improved root architecture
- Little evidences were found that its application may have long-term effects that promote tolerance to transplanting shock.

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Thanks to

… you all for your attention

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