Environmental factors, leaf traits and ozone visible symptoms are interrelated in *Viburnum lantana*

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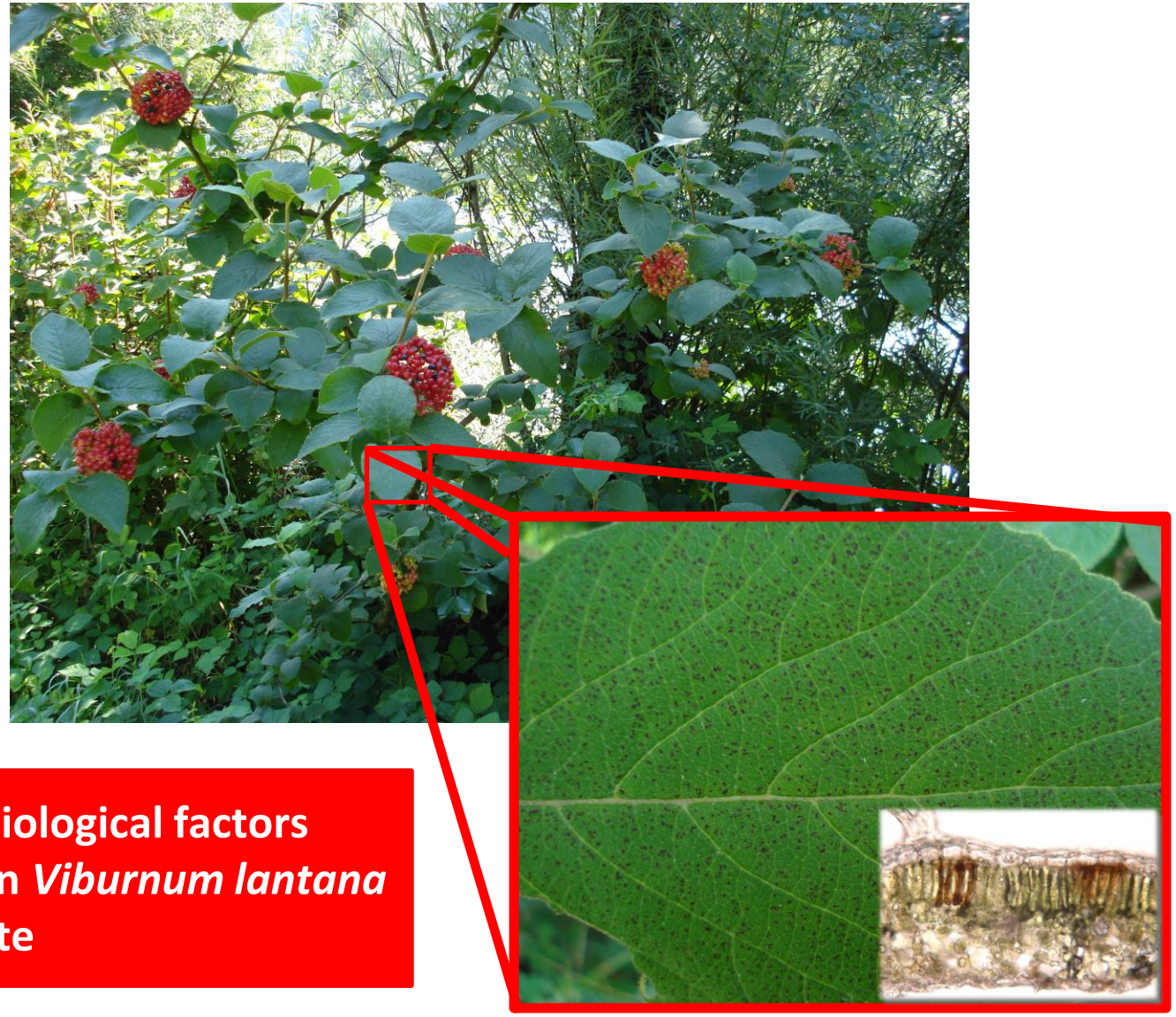
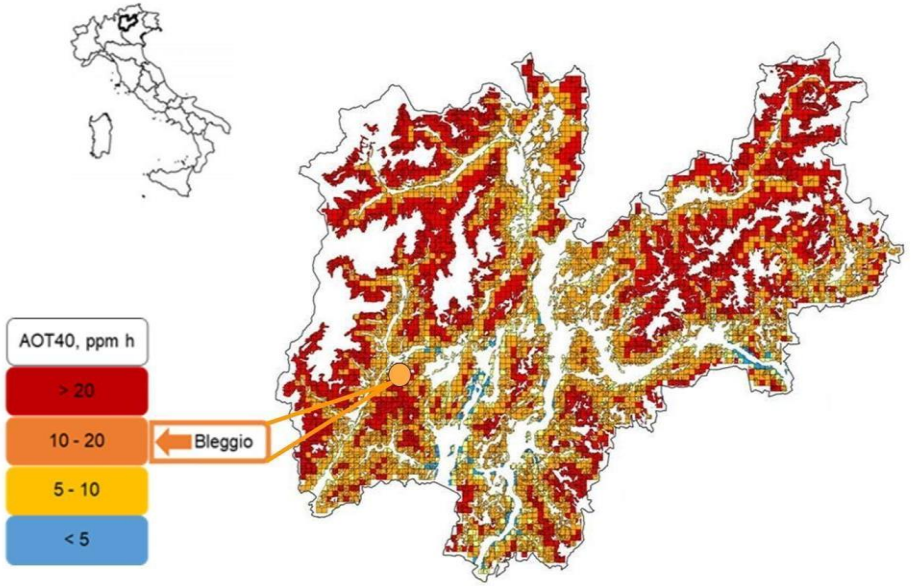
Introduction

- **Tropospheric ozone ([O_3]) has detrimental effects** on plants
- *Viburnum lantana*, an excellent model plant for **assessing Visible Foliar Symptoms (VFS)** caused by tropospheric ozone
- Occurrence of **VFS differs among individuals at the same site, and within leaves of the same individual.**
- Very few attempts exist to investigate which **factors are associated with such a difference**



Identify environmental, morphological and physiological factors associated with the occurrence and severity of VFS in *Viburnum lantana* and at the scale of an individual site

Methods

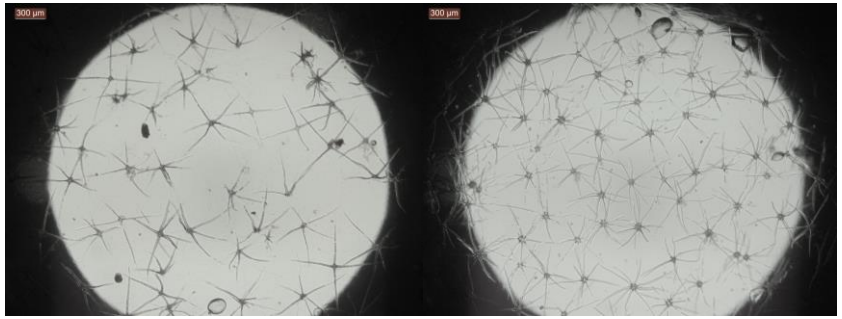


- Study performed in **the Trento province (North Italy) at Bleggio (~3-4 ha)**, one of the n=10 sites belonging to the ViburNeT network (Viburnum lantana ozone biological response Network in Trentino), at 824 m a.s.l.
- Sites yearly assessed since 2010 for the presence of ozone-induced VFS on *Viburnum lantana* (Gottardini et al. 2017)
- **30 *Viburnum lantana*** plants surveyed in 2020

- Selected for: i) **aspect of the light exposed part of the plant** ; ii) **plant shading** iii) **plant height**; iv) **VFS**
- Selected **six symptomatic plants**, three for the small and three for the large plant height classes (same for non-symptomatic)
- Random stem for each plant (n=12) was collected, and **leaves were sampled at each stem portion**

Leaf traits assessment

- **Leaf SPAD** values as a proxy of chlorophyll content (Chl_{SPAD}).
- RGB imaging was used for **Leaf Area (LA) estimation** with ImageJ.
- Visual assessment of **VFS** (0:absent; 1:1-5%; 2:6-50%; 3: >50)
- Analysis of non-glandular **trichomes density** via light microscope on both adaxial and abaxial surface
- Dry weight (DW, g) was obtained for each leaf and **Specific Leaf Area** (SLA=LA/DW) calculated.



Results, environmental effect on leaf traits

	Aspect			Shading				Size			Portion			
	ES	WN	p	High	Mid	No	p	L	S	p	Basal	Mid	Apical	p
SLA, $cm^2\ g^{-1}$	146.0	172.0	p<0.05	178.3	144.3	137.9	p<0.05	137.2	170.7	p<0.01	236.2	173	123.3	p<0.001
Chl_{SPAD}, a.u.	42.6	38.5	p<0.01	39.1	42.7	38.8	p<0.05	43.8	38.9	p<0.001	34.2	41.2	42.7	p<0.001
TrAd, mm^{-2}	1.70	1.70	ns	1.6	1.7	2.0	ns	1.9	1.6	p<0.05	1.6	1.5	1.9	p<0.01
TrAb, mm^{-2}	13.0	10.0	p<0.01	9.9	12.9	13.7	p<0.01	14.9	9.5	p<0.001	10.0	11.2	13.0	ns
TrAd/TrAb	0.25	0.35	p<0.01	0.31	0.27	0.30	ns	0.26	0.31	ns	0.31	0.22	0.32	p<0.05

- **SLA reduced** in plant exposed to ES, subjected to high irradiance and from bottom to top stem portion
- **Trichomes density increased** in plant exposed to ES and not subjected to shading

Environmental effect is high and should be taken into consideration for sampling procedures and **characterization of leaf traits** under natural environmental conditions.

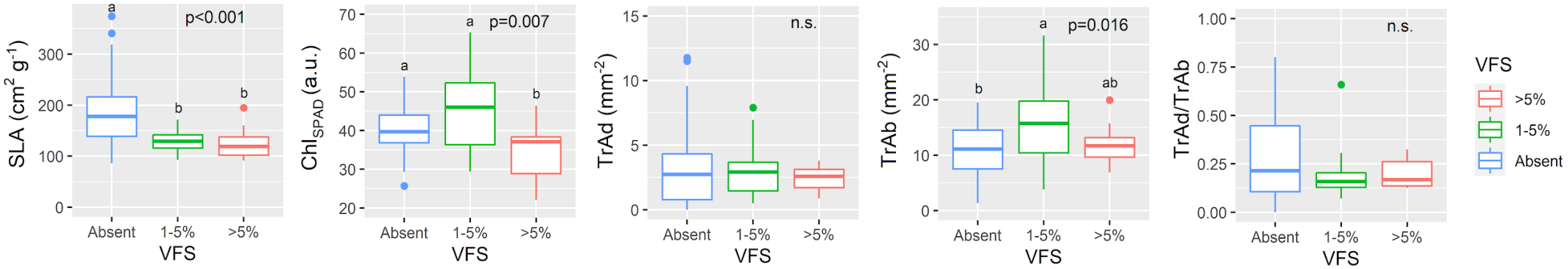
Results, leaf symptoms' association with leaf traits

Table, functional leaf traits calculated for the leaves of non-symptomatic plants, and for both non-symptomatic and symptomatic leaves of the symptomatic plants separately

Leaf trait	Non-symptomatic plants, n=6	Symptomatic plants, n=6		F	P value
	Non-symptomatic leaves, n=51	Non-symptomatic leaves, n=43	Symptomatic leaves, n=25		
SLA, $cm^2\ g^{-1}$	140.4a	189.0b	127.2a	13.069	<0.001***
Chl_{SPAD}, a.u.	42.0	40.3	40.8	0.619	0.540
TrAd, n mm^{-2}	3.8	3.3	2.8	1.810	0.168
TrAb, n mm^{-2}	11.4a	11.2a	14.5b	3.535	0.032*
TrAd/TrAb	0.33a	0.28ab	0.20b	4.828	0.010**

- **SLA resulted significantly (p<0.001) lower for symptomatic leaves**
- **Abaxial trichome density was significantly higher for symptomatic leaves**
- When all asymptomatic leaves were grouped **the significant differences for SLA and abaxial trichomes were confirmed**

Results, VFS intensity and association with leaf traits (symptomatic plants)



- **SLA decreases with the increase of VFS intensity (p<0.001)**

- **Lower Chl_{SPAD} values were observed in leaves with >5% VFS (p=0.007)** when compared to asymptomatic or slightly symptomatic leaves

- **An increase in abaxial trichomes density was observed with the increase of VFS intensity** in leaves with VFS >5% (p=0.016)

Reduced SLA as a defensive strategy against O_3

OPINION

WILEY Global Change Biology

A unifying explanation for variation in ozone sensitivity among woody plants

Zhaozhong Feng[1,2] | Patrick Büker[3] | Håkan Pleijel[2]
Erik Karlsson[4] | Johan Uddling[2]

Global Change Biology (2008) **14**, 2727–2739, doi: 10.1111/j.1365-2486.2008.01677.x

Functional leaf traits, plant communities and acclimation processes in relation to oxidative stress in trees: a critical overview

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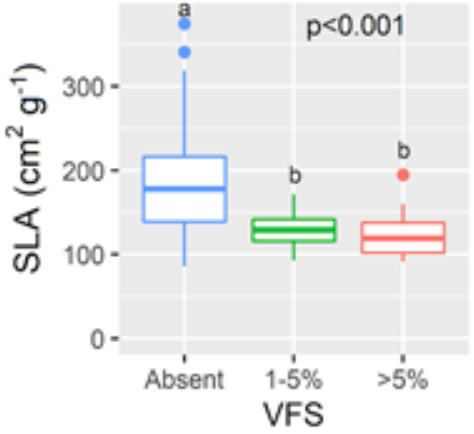
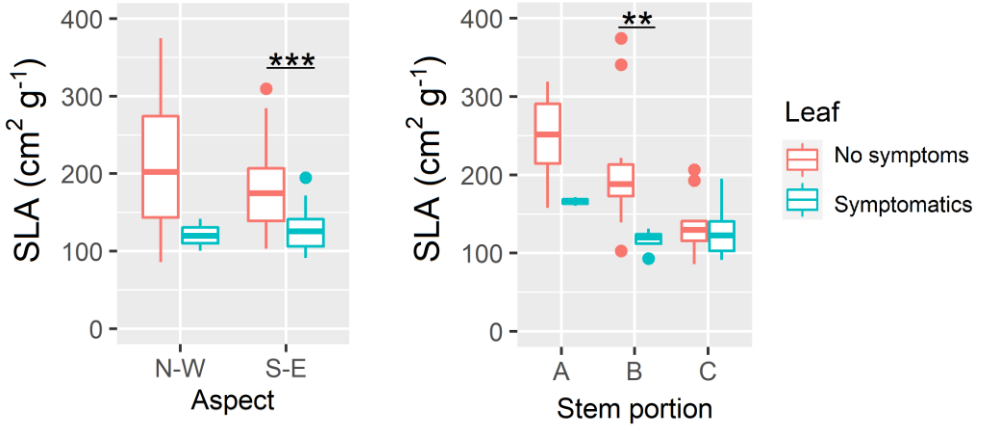
- In *V. lantana* symptomatic plants, **VFS was clearly associated with reduced SLA**
- **SLA plasticity is a defensive strategy** implemented to reduce stress damages



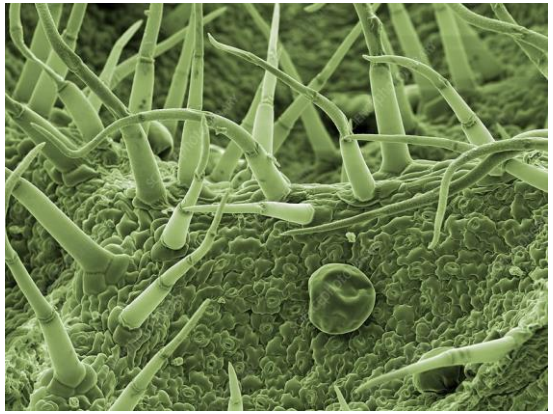
- SLA response was different at e.g. **different stem portion (A basal, C apical) and for plants with different exposure**
- **Intra-site and intra-plant environmental conditions** (e.g. light, RH) affect stomatal physiology (i.e. *gs*) **leading to different stress intensity and O_3 uptake capacity**

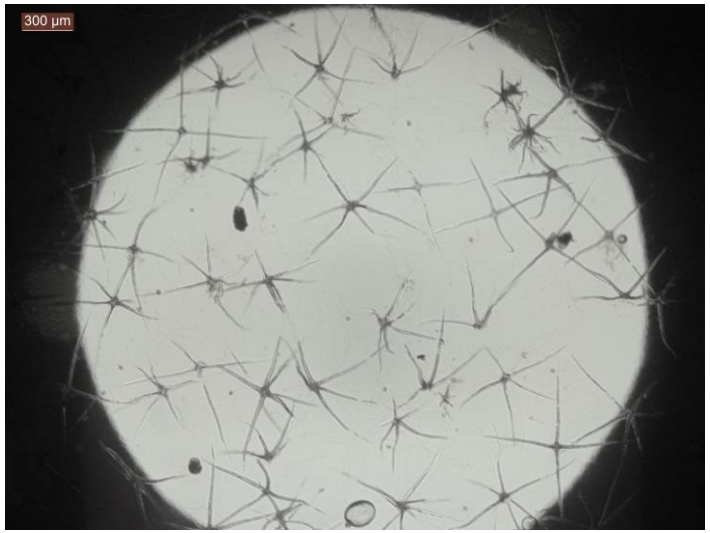


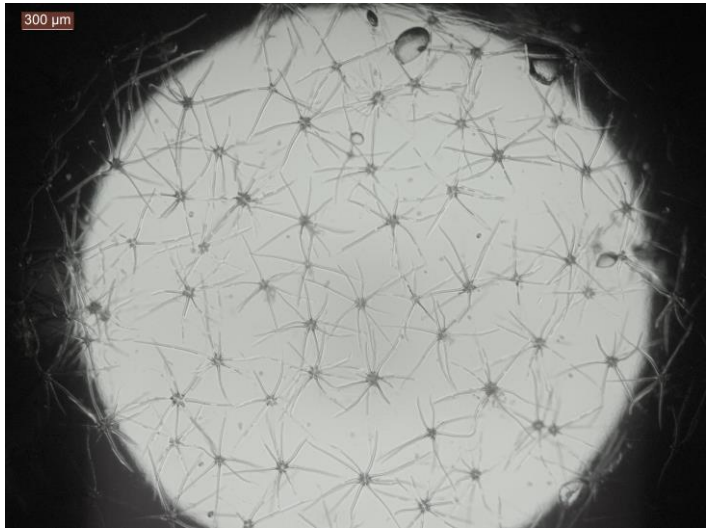
Evidence of a leaf-specific response to ozone via SLA plasticity in *Viburnum lantana* strongly influenced by environment

Non-glandular trichomes may not be involved in protection against O_3

- **Glandular trichromes involved in protection against [O_3]**: **able to extinguish O_3 before it enters the leaf** (Karabourniotis et al., 2020).
- Non-glandular trichromes: **not involved in any defensive role under elevated [O_3]**

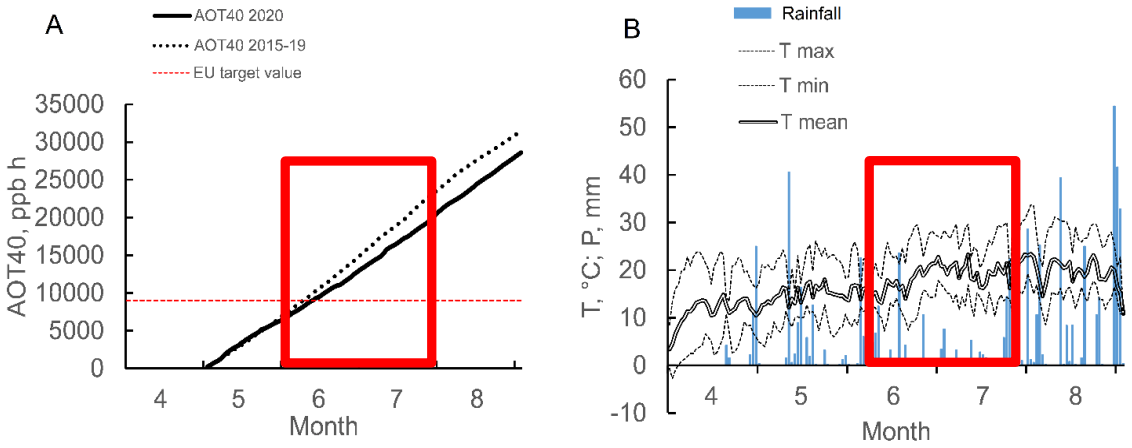




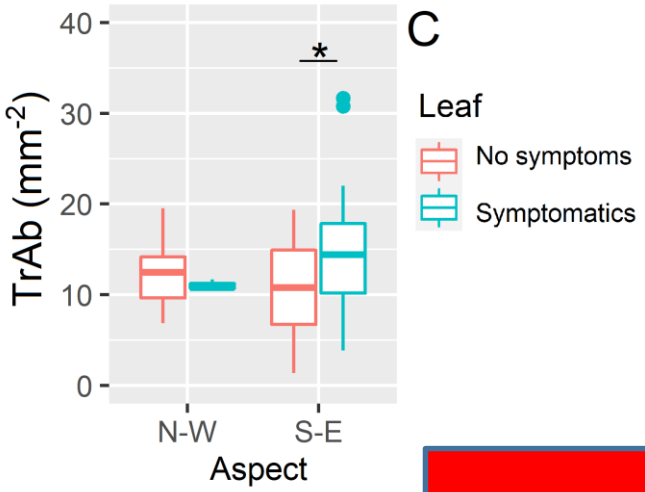


In our experiment only non-glandular trichomes were observed in *Viburnum Lantana* and the density **was higher in symptomatic leaves, with higher VFS frequency, and in plants exposed to higher irradiance level.**

Non-glandular trichomes may not be involved in protection against O_3



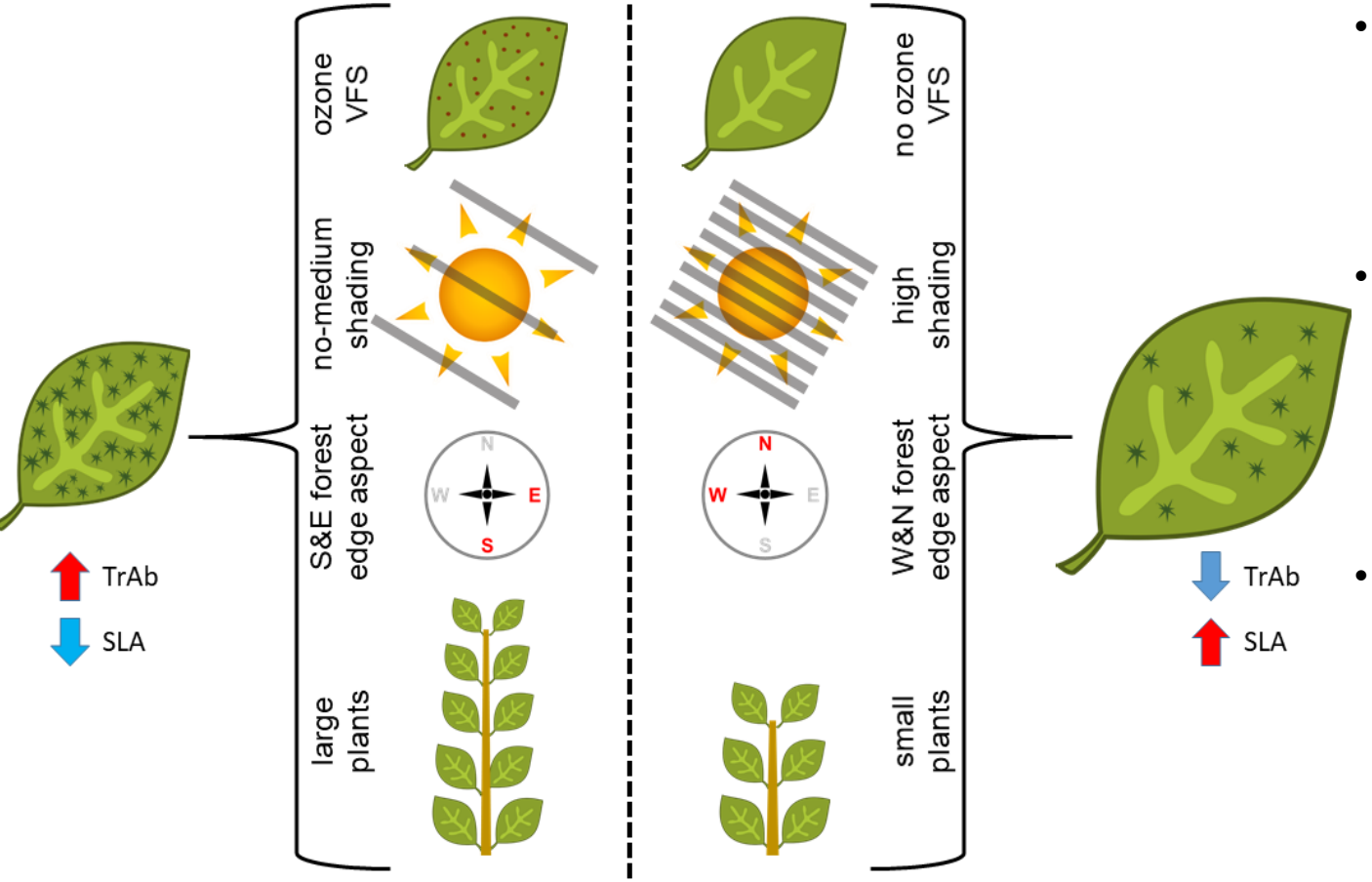
Viburnum lantana plants were subjected to a series of stressors in 2020



- High trichomes as a **strategy to reduce environmental stress damage** *(e.g.* Karabourniotis et al., 2020).
- They allowed **higher O_3 uptake in leaves where non-glandular trichromes were more frequent.**
- Difference between symptomatic and non-symptomatic leaves exposed at S-E, with higher number of trichomes associated with VFS.

Combinations of environmental variables determines the leaf-specific sensitivity to increasing ozone levels

Conclusions



- Significant **environmental effect for several leaf traits irrespective of ozone symptoms in *Viburnum lantana***
- SLA and trichomes showed significant associations with VFS: **strategies adopted for increasing tolerance under multiple stresses may be detrimental and can lead to increasing VFS**
- A complex relationship between ozone symptoms and environmental variables: **take into consideration such complexity into monitoring programs**

Thank you!