



Coordination of morpho-physiological and metabolic traits of *C. incanus* L. to overcome heatwave-associated summer drought: a two-year on-site field study



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## Background

*Cistus* x *incanus* L. (pink rock-rose, syn. *C. incanus*) is a semi-deciduous shrub well-adapted to Mediterranean summer drought thanks to its high plasticity in morpho-anatomical and physiological traits <sup>1</sup>. However, increasing frequencies of heat waves are threatening *C. incanus* distribution and survival in coastal dune areas. We took advantage of the contrasting climatic conditions of summers 2014 and 2015 to study morpho-physiological and metabolic changes in *C. incanus* experiencing drought and heatwave in its natural environment and to test the resilience of this shrubs against environmental pressures associated to climate change.





## **Materials and Methods**

Eight homogenous healthy individuals of *C. incanus* were selected on the coastal sand dunes located in Castiglione della Pescaia (GR, Tuscany, Italy, 42 460 N, 10 530 E) and sampled over two consecutive years on cloudless days in summer 2014(8–9 July) and 2015 (14-15 July), which was one of the driest and hottest in Europe in the last 70 years <sup>2</sup>.

Photosynthesis ( $P_n$ ). and stomatal conductance ( $g_s$ ) were measured at midday and the ratio between Electron transport rate ( $J_F$ ) and ( $P_n$ ) was calculated on a daily basis.

Morpho-anatomical traits were monitored: upper epidermis (UE); palisade mesophyll (PM); spongy mesophyll (SM); lower Epidermis (LE); palisade/spongy mesophyll ratio (PM/SM); leaf thickness (LT) and leaf mass per area (LMA).

At midday, total Biogenic Volatile Organic Compounds ( $BVOC_{Tot}$ ) were sampled by partially diverting the outlet of the cuvette of the Li-Cor system into a silico-steel cartridge which was then analyzed by GC-MS, In addition, two leaves per plant were collected to detect tannin content by HPLC-DAD analysis.

## **Results and discussion**

Leaf thickness (+21.6%) and LMA (+34.5%) strongly increased from summer 2014 to 2015. The palisade parenchyma was the most contributing tissue to the total increment in leaf thickness ( $R^2 = 0.66$ ). The higher  $J_f/P_n$  in 2015 indicated an excess of reducing power along the electron transport chain that could have been diverted toward alternative electron sinks such as the production of secondary metabolites<sup>3,4</sup>. Among these, BVOCs emission, in particular monoterpenes, strongly increased in 2015 compared to 2014 (+81.7%), contributing to the thermal protection of photosystem II<sup>3</sup>. In addition, the higher accumulation of leaf condensed tannins (+27.1%) in 2015 compared to 2014 may have played a structural role besides a photo-protective function<sup>5</sup>.

## Conclusion

This study provides new evidence on the substantial contribution of secondary metabolites, both condensed tannins and monoterpenes, in conferring high plasticity to *C. incanus* to cope with extreme drought and heatwaves. Therefore, our results suggest that *C. incanus* might have greater potential to acclimate to climate change in its natural environment.



 $g_{s}$ 

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<sup>1</sup>Puglielli, G., Varone, L., and Gratani, L. (2019). Diachronic adjustments of functional traits scaling relationships to track environmental changes: revisiting *Cistus* species leaf cohort classification. *Flora* 254, 173-180.

<sup>2</sup>Russo, S., Sillmann, J., and Fischer, E. M. (2015). Top ten European heatwaves since 1950 and their occurrence in the coming decades. *Environ. Res. Lett.* 10:124003.

<sup>3</sup>Harrison, S. P., Morfopoulos, C., Dani, K. S., Prentice, I. C., Arneth, A., Atwell, B. J., et al. (2013). Volatile isoprenoid emissions from plastid to planet. *New Phytol*. 197, 49-57.

<sup>4</sup>Gori, A., Tattini, M., Centritto, M., Ferrini, F., Marino, G., Mori, J., et al. (2019). Seasonal and daily variations in primary and secondary metabolism of three maquis shrubs unveil different adaptive responses to Mediterranean climate. *Conserv. Physiol*.7:coz070

<sup>5</sup>Bussotti, F. (2008). Functional leaf traits, plant communities and acclimation processes in relation to oxidative stress in trees: a critical overview. *Glob Chang Biol*. 14, 2727-2739