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UV rays Decrease Foliar pH in Cress (*Lepidium Sativum*) and Modify NIR Spectrum

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Abstract

An experiment on watercress (*Lepidium sativum*) 42 d days after sowing was set up to evaluate the effect of UVAB radiation on foliar pH and on NIR tomoscopy. The effect of 8 h of UV exposure (about 500 µWcm^{-2}) was clear, causing a reduction in the foliar pH of 6.0%, compared to the contemporary control group (5.22 *vs.* 5.56). When the treated leaves were returned to white light, their pH rose by 1.7% (5.31), reaching the same level as leaves grown outdoors (5.33). On the basis of the NIR spectra, the leaves of the plants grown outdoors were recognized 100%, that is, more than the control leaves (87%) and the plants treated with UV on the day of measurement (81%). However, the effect of the UV marking on the leaves is not permanent as, after the UV treatment, the leaves returned to white light were classified at 61%, which is a greater value than the 37% of overlapping between the two groups. The foliar pH was highly correlated with the NIR spectra, with R^2 0.43. These results corroborate the hypothesis of the influence of the solar cycle on the variation of the pH of leaves in plants, which we believe may have played a major role in past agricultural pandemics. These results call for the introduction of the pH test for the evaluation of resistance to pathogens. From the environmental point of view, the foliar pH could be enlisted as an *in vivo* long-time indicator of the response of plants to climate changes.

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Introduction

A progressive decrease in foliar pH, which is correlated with the number of sunspots during the second (descending) phase of the 24th cycle, has been highlighted in a multi-year study on vine [1]. It has been hypothesized, in studies on the cycles of forest moth pest species [2], that such a decrease was due to an excess of UVB radiation, which is counter-correlated with the number of sunspots and is measured through the relationship with Ozone, as detected by means of TOMS [3]. However, there is no experimental evidence that clearly identifies the dependence of foliar pH on the effective irradiation that comes from light containing UV rays. The pH of leaves is a physiological parameter that has so far been neglected in both research and practice, and is completely absent in publications due to its relationship with UV. However, the pH of leaves seems to affect the defense of plants against fungi and viruses. In fact, on the basis of a critical review of the phytopathological pandemics historically recorded during the 24th solar cycles [1], it appears that, in 22 out of 25 cases, the sunspots were in a position below the average position of the cycle for the year in which the pandemic event exploded. Considering the result observed on vine, and extrapolating it to the past, this finding suggests that the pH of the plants is tending toward an acid phase, enriched with energy and protons and - in a certain as yet not defined way - favorable for the spread of parasites, which could result in their prevalence.

The aim of the present research has been to verify whether an increase in UVAB radiation can induce a response in plants that result in a reduction of the foliar pH and, in parallel, produce modifications of the electromagnetic spectrum NIR (740-1070 nm) of the leaf blade.

Materials and Methods

Plants and Lights

Watercress plants (*Lepidium sativum*), obtained from seed (cv. Sais 1143) planted in a peaty soil substrate, were grown in an underground cell at 18.5° C and illuminated 24 hours a day with a white LED light. At 42 days after seeding (October 30, 2020), 45 leaves were sampled to monitor the foliar pH and NIR spectrum. A 12 h time lapse of darkness was then introduced, after which half of the vessels were left in the white light cell (group 1_White), while the other half of the vessels were transferred, 1 m away, to an area illuminated with the same white LED light integrated with 2 UVA filament lamps and a UVAB neon lamp (Table 1, Figure 1) (group 2_UV). After 12 hours of light, the lighting was interrupted for 12 hours, and a crossover was made between the plants and the light devices, so that the 2_UV group became 3_UV + White, while the 1_White group became 2_UV. Leaf measurements were carried out on the treatment days, starting after 8 hours of light, and on another group of contemporary plants grown outdoors (group 4_Outdoor). In total the UV irradiance was about 500 μWcm^{-2}.

Foliar pH and NIRS Measurements.

The foliar pH was measured with a Hamilton double pore PF electrode (Figure 2), the only one that proved suitable for the purpose, according to the positive results of previous studies conducted by our group [4-7].

After the pH measurement, the leaf fragments were placed in a reverberant holder for the NIR scan, which was performed using the SCiO™ v.2 device, a low-cost spectrometry system for end-user food analysis [8] (Figure 5) which is systematically employed in the research conducted by our group [9-12].

Statistical Processing.

The data obtained from the pH measurement were processed with SAS software (vs 9.0), via PROC GLM, with a single-factor model (group) with four levels, using the PDIFF command to determine the significance of the differences.

The spectral reflectance data were processed after Log mathematical transformation and second derivation by setting a four-element classification matrix, resolved by The LabSCiO™ proprietary software, in which the Random Forest algorithm is used.

The degree of association between the spectral data and the foliar pH was ascertained by means of a Partial Least Squares (PLS-R) regression calculation, using The LabSCiO™ proprietary software.

Results

A total of 299 packets of leaves, divided into

Table 1. Irradiance of the UV bulbs at 20 cm.

Source	Irradiance UVC 250-280nm µWcm^{-2}	Irradiance UVB 280-315nm µWcm^{-2}	Irradiance UVA 315-400nm µWcm^{-2}	Irradiance UV 250-400nm µWcm^{-2}
Sylvania Reptistar	-	11.7	110	122
Sylvania UVA	-	0.917	204	205

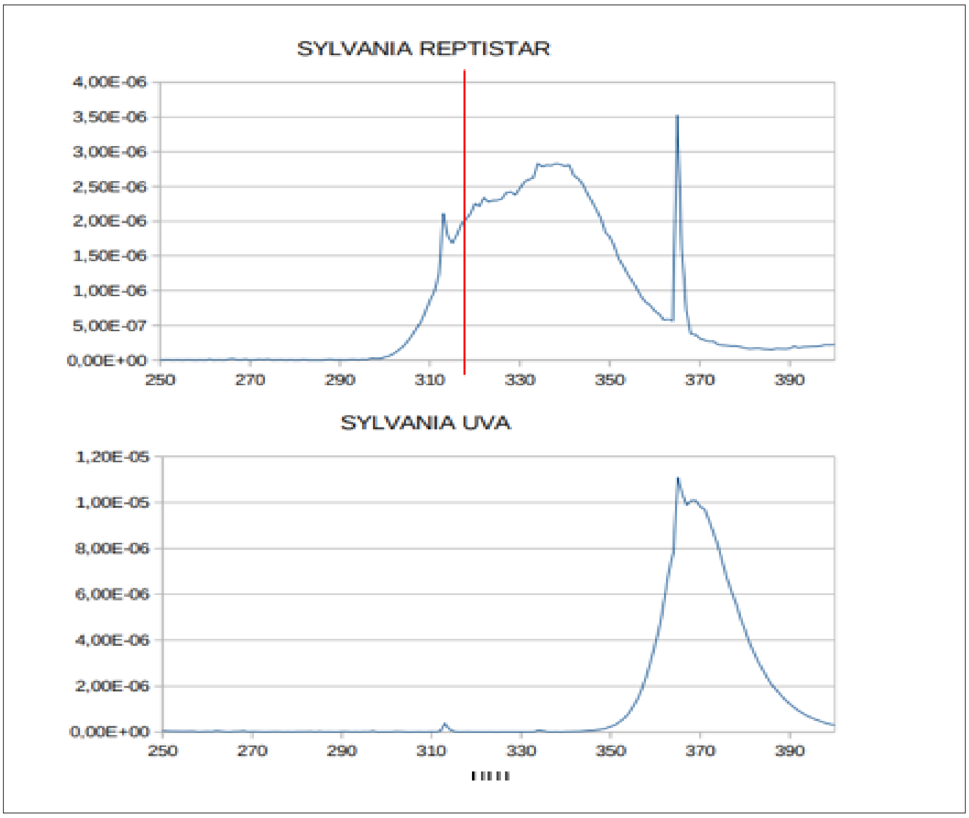

Figure 1. Spectral irradiance, in µWcm^{-2} versus wavelength for the bulbs UVAB (Sylvania Reptistar) and UVA (Sylvania UVA).

four groups, were examined in the experiment. The pH was found to be very stable, and provided a coefficient of variation that was limited to 2.94%. The r-square value of ANOVA was 0.427.

The differences between the mean foliar pH of the four groups (Table 2, Figure 4) were all highly significant, except those between the Outdoor group and the 3_UV + White group.

The effect of UV exposure was clear, and caused a reduction in foliar pH of 6.0%, compared to the contemporary control group (5.22 ^{c} *vs.* 5.56 ^{a}). The behavior of the leaves treated with UV is interesting: when these leaves were reintroduced into white light the following day, their pH rose significantly by 1.7% (5.31 ^{b} *vs.* 5.22 ^{c}), reaching the same level as the leaves grown outdoors (5.33 ^{b}).

The NIR spectra were able to characterize the four groups well, thereby allowing a highly significant reclassification to be made (Table 3). The leaves of the plants grown outdoors were recognized 100% . The leaves of the plants grown under white light were much less recognizable (87%), on a par with the leaves of the plants treated with UV on the day of the measurement (81%). However, the UV signature effect on the leaves was not permanent, as the leaves that were returned to a white light after the UV treatment were correctly classified at 61%, which is a greater value than the 37% of overlapping between the two groups; the proportions (61% and 37%) should in fact have at least been reversed in the case of a dominant residual effect.

By observing the average NIR spectra of the four groups (Figure 5), it was noted that the highest reflectance value belonged to the Outdoor group, while the minimum was in Group 3, which included leaves taken from plants that had undergone UV exposure the previous day and then returned to white light before being tested again.

The interrelation between the NIR spectrum and the foliar pH value was significant, with an R^2 coefficient of 0.4317 (Figure 6). The PLSR software procedure that developed the predictive model allowed 3 latent variables to limit overfitting.

Discussion

The correlation between pH and an NIR spectrum that yielded an R^2 of 0.43 is nothing new. An R^2 of 0.52 was observed on tomato[13]. By grouping the results of three of our published works on corn leaves[14] and one unpublished one, for a total of 1627 samples, the cross-validation R^2 value was 0.60, which is similar to the 0.65 value obtained in the research on sorghum[6].

As far as the substances that make up the leaf parenchyma, which can be connected to the pH measurement, are concerned, a survey and processing of the maize dataset was carried out to identify the most interesting correlations. Two positive correlations with pH emerged for the dry matter (r = 0.19) and the non-digestible NDF (0.33). Some negative correlations instead connected the foliar pH to proteins (-0.25), ether extract (-0.14), digestible NDF (-0.19), total digestibility (-0.22), lignin (-0.14) and hemicelluloses (-0.38). Basically, when the pH is lowered than normal, a higher water content may be found, and therefore less non-digestible NDF and more digestible NDF with more hemicelluloses, which indicate a greater digestibility of the dry substance, despite the fact that lignin tends to rise in percentage. Furthermore, an increase in proteins and lipids may be recorded for a drop in pH. This is what emerges from the aforementioned studies, but unfortunately, it was not possible to apply the NIRS equations developed for Sorghum [6], and then transferred to the leaves of other species, to the cress leaves. The spectral difference between leaves that are normally examined at 1 cm with a protective spacer ring and the scanning of a few leaflets at the bottom of a reverberation chamber (Figure 3) is too great. Therefore, what can the spectral signatures of the leaves treated for a short period of 8 hours with UV light refer to? By observing the reflection curves (Figure 5), it was noted that the UV treatment tended to increase the average level of reflection after 900 nm, which is almost like sheltering the leaves from light; on the other hand, after the UV treatment, when the white light was restored, the reflection fell sharply over the entire spectrum, that is, the leaves absorbed the light of the SCiO™ - which is a blue light - with greater intensity, almost as if they wanted to appropriate it more. The outdoor leaves were clearly more reflective, probably because they had received much higher doses of UV radiation and in a less violent way, and probably had a different composition of the

Table 2. Differences in the foliar pH averages for the five groups

Group	No.	LSMeans	SD
1_White	92	5.557 a	0.144
2_UV	94	5.222 c	0.170
3_UV + White	45	5.311 b	0.131
4_Outdoor	68	5.329 b	0.172

a>b>c P<0.05

Table 3. Reclassification of the four groups on the basis of the NIRS spectra, according to the Random Forest algorithm.

	Known			
Classified	1_White	2_UV	3_UV+White	4_Outdoor
1_White	87% b	6%	2%	0%
2_UV	12%	81% b	37% d	0%
3_UV+White	0%	13%	61% c	0%
4_Outdoor	1%	0%	0%	100% a

a>b>c>d P<0.008

Figure 2. Measuring the foliar pH

Figure 3. Scanning the leaves, after measuring the pH, with the SCiO™ device

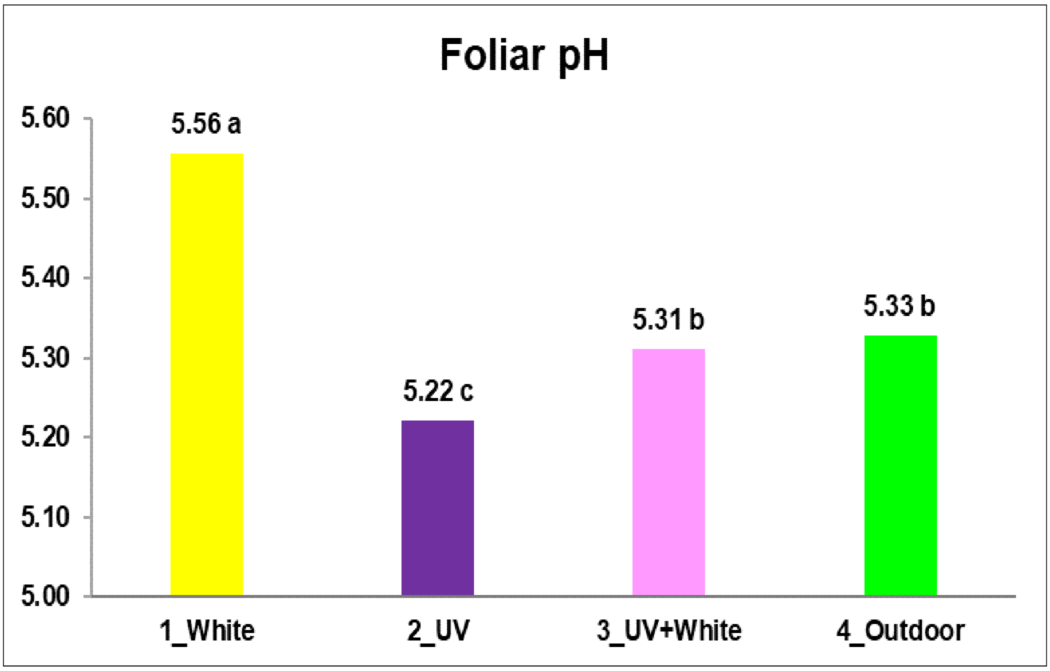

Figure 4. Mean values of the foliar pH for the four groups.

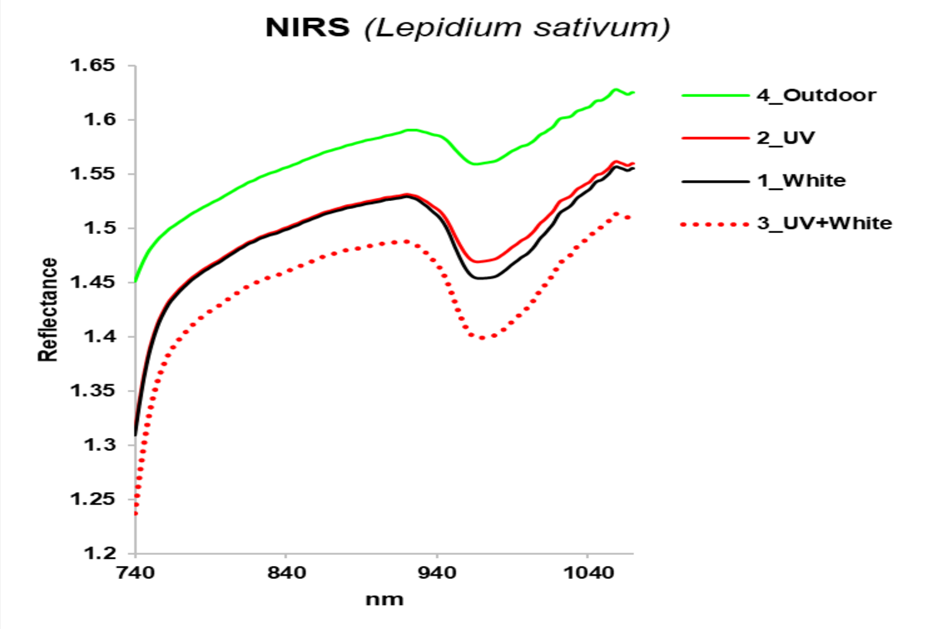

Figure 5. Average reflectance NIR spectra of the four groups of leaves.

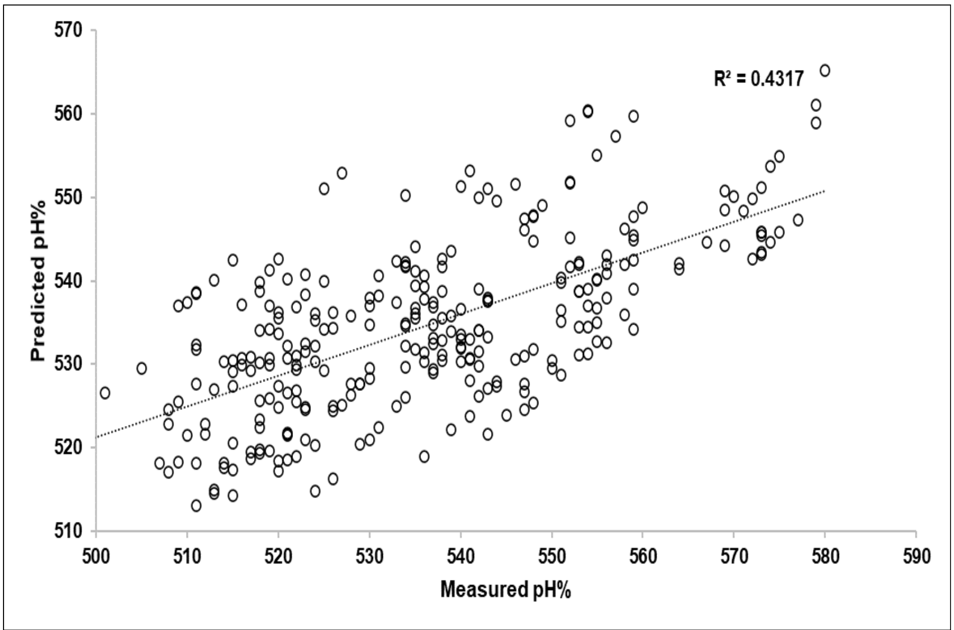

Figure 6. Prediction of the foliar pH from the NIR spectra.

cuticula and parenchyma. The UV treatment, although limited to 8 hours, induced macroscopic changes in the leaves, which manifested residual effects, and thus 37% of the post-treatment leaves were still recognized as being under the UV effect. The bibliography does not report any cases of the NIR spectroscopy of plants irradiated with both spot and permanent UV. Instead, numerous works have highlighted the effects of UVB on substances and cell structures that respond to treatment. In short, rapid increases in ROS and phenolic compounds were observed, after 3 h, in irradiation studies with *Deschampsia antarctica* [15], although these values were then normalized, while the peroxidation membrane increased significantly throughout the exposure (MDA + 50%). The role of peroxidation enzymes has also been highlighted in *Arabidopsis thaliana*[16]. The plasma membrane of plant cells undergoes a number of changes in response to UVB exposure. According Strid et al. [17], these changes include depolarization of the electrical potential of the cell, synthesis of H_2O_2, and oxidation of reduced glutathione to oxidized glutathione, but mainly an efflux of K^+ ions, and this may have been the case for the drop of the foliar pH we observed. Moreover, an abundance of salicylic acid could be implicated in the rise in acidity [18]. Leaf-cell elongation mechanisms were shown to be involved in longer-lasting trials [19], in which the plant growth curbing effect of UVB was clearly revealed. In general, it can be observed that the positive effects of elevated (CO_2) on photosynthesis and growth do not generally overcome the negative effects of UVB radiation, elevated temperatures, or water stress, on the productivity and quality of grain crops [20]. High UVB levels introduce different lesions, and in particular cyclobutane pyrimidine dimmers and pyrimidine (6-4) pyrimidinone products in the genome. These could adversely affect the growth, development and morphology of plants, especially the productivity of sensitive crop species [21, 22]. Therefore, NIR spectroscopy may have collected the spectral signature of overly complex mechanisms.

The research on foliar pH is extremely limited. The "Foliar pH, and emerging plant functional trait: Biogeography and variability across northern China" [23] research involved carrying out a biogeographic study of characteristic and ubiquitous functional types in 22 districts throughout China. The extensive study resulted in a significant altitude gradient per km, that is, of -0.525 in woody plants and -0.731 in herbaceous plants, equal to -10% and -12%, respectively, with respect to the foliar pH averages. Since an increase in altitude entails an average increase in UVB during the year, which varies from 18 to 23% per 1000 m [23], our result is in agreement with the aforementioned ones, and the sense of the variation is confirmed, as the pH of the cress leaves dropped from 5.58 to 5.22 under a direct UV effect while applying 600 µW cm^{-2} for 8 hours. The pH also dropped in the outdoor group, to 5.33, when the solar UVs in those autumn days increased the amplitude from 300 µW cm^{-2} (h 9:00) to 1000 µW cm^{-2}.

From the agrarian point of view, this work confirms a well-known concern. The phytopathological framework of future crops will be very critical, because of the ongoing climate changes. The Agroinnova studies conducted in phytotrons [24-32] have in fact shown that, out of 12 combinations of crops x parasites, an increase in temperature was beneficial in 3 cases and negative in 8 other cases (75%), while an increase in CO_2, projected to 800-850 ppm, that is, double the current value, appeared negative in 10 out of 12 cases (83%) and strongly exacerbated the toxicity of the pathogens. In a previous work on foliar pH [5], it was specified that the pH is lowered by 0.07 units for an increase of 1 ° C in vines. In the aforementioned works conducted by Agroinnova, a temperature increase of 4° C was assigned to the phytotrons, which would correspond to a decrease of 0.28 pH units. In this work, the total UV effect was equal to -0.36 pH units, which, if transformed, would correspond to + 5 ° C. We have no direct or experimental elements to link the foliar pH to variations in pathologies. However, an examination of the historical pandemic events revealed that sunspots were numerically reduced and - consequently - UVs were abundant in the years mostly critical for crops .

Further studies are needed to clarify this matter. A candidate area for research is that of protected crops, in which the exclusive use of visible artificial light does not envisage administering UV to plants. Moreover, the sun, even though filtered by the transparent roofs of greenhouses, can have an effect on the leaves. By hypothesis, UV rays could be studied as modulators of resistance against parasitic fungi, bacteria and viruses fulfilling all the cautions required by the EN 62471: 2008 provisions, In this way, the foliar pH could become an easy and immediate indicator of the mechanisms that are triggered and developed as a result of the exposure of plants to ultraviolet radiation of various types. Recently Escobar Bravo et al[33] have shown that different supplemental UVAB is an important modulator of tomato defenses against Western flower thrips (Frankliniella occidentalis), but unfortunately did not check foliar pH reactions.

These results of this work corroborate the hypothesis of the influence of the solar cycle on the variation of the leaf pH of plants, which we believe may have been relevant in past agricultural pandemics.

From the environmental point of view, the foliar pH could be considered as an *in vivo* long-time indicator of the response of plants to climate changes.

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References

1. Masoero G, Cugnetto A, Sarasso G, Giovannetti G, Nuti M. (2019) Sunspots are correlated with foliar pH in grapevine. *Journal of Agronomy Research* 2 (3) 31-41.
2. Selås V, Hogstad O, Kobro S, Rafoss T. (2004) Can sunspot activity and ultraviolet–B radiation explain cyclic outbreaks of forest moth pest species? Proceedings of the Royal Society of London. Series B: *Biological Sciences* 271, 1897-901.
3. Eck, T. F., Bhartia, P. K., Kerr, J. B., Satellite estimation of spectral UVB irradiance using TOMS derived total ozone and UV reflectivity, *Geophysical Research Letters* 1995, 22, 611-614
4. Masoero G., Giovannetti G., 2015. In vivo Stem pH can testify the acidification of the maize treated by mycorrhizal and microbial consortium. *Journal of Environmental & Agricultural Sciences* 3, 23-30.
5. Masoero G, Cugnetto A. 2018 The raw pH in plants: a multifaceted parameter. *Journal of Agronomy Research* 1 (2), 18-34.
6. Masoero G., Peiretti P.G., Cugnetto A., Giovannetti G. 2018. Raw pH fall-out as a sign of a mycorrhizal modifier of Sorghum sudanensis. *Journal of Agronomy Research* 1 (2), 1-11.
7. Giovannetti G, Polo F, Nutricato S, Masoero G, Nuti M 2019. Efficacy of commercial symbiotic bio-fertilizer consortium for mitigating the Olive Quick Decline Syndrome (OQDS). *Journal of Agricultural Research*. 2 (1), 1-21.
8. Goldring, D., Sharon, D., 2016. Low-cost spectrometry system for end-user food analysis. *United States Patent* US009377396 B2.
9. Masoero G, Delmastro M. Cugnetto A. Giovannetti G, Nuti M. 2018 NIRS footprint of bio-fertilizers from hay litter-bags. *Journal of Agronomy Research* 1, 22 -33.
10. Peiretti PG, Masoero G, Tassone S. (2020) Near infrared reflectance spectroscopy (NIRS) evaluation of the nutritive value of leaf and green pruning residues of grapevine (*Vitis vinifera* L.). In: *Grapevines at a Glance*. Nova Science Publishers, Inc., NY, Chapter 3, 67-89.
11. Battaglini LM, Renna M, Lussiana C, Lombardi G, Probo M, Masoero G. 2017. Smart near infrared spectroscopy on frozen milk samples can discriminate grass-fed from conventional milk. *Italian Journal of Animal Science* 16, suppl. 1: 209-210.
12. Meineri G, Candellone A, Masoero G, Peiretti PG. (2020) Smart NIR tomoscopy to predict oxidative stress in rabbits. *Progress in Nutrition* 22(4).
13. Baldi E, Toselli M, Masoero G, Nuti M. (2020) Organic and symbiotic fertilization of tomato plants monitored by Litterbag-NIRS and Foliar-NIRS rapid spectroscopic methods. *Journal of Agronomy Research* 3(1), 9-26.
14. Volpato S, Masoero G, Mazzinelli G, Balconi C, Locatelli C, Lanzanova S, A. Ardigò A, Giovannetti G, Nuti M. (2019) Spectroscopic and foliar pH Model for Yield prediction in a Symbiotic Corn Production. *Journal of Agricultural Research* 2 (2), 1-18.

15. Köhler H, Contreras RA, Pizarro M, Cortés-Antíquera R, Zúñiga GE. (2017). Antioxidant Responses Induced by UVB Radiation in *Deschampsia antarctica* Desv. *Frontiers in plant science* 8, 921.

16. Rao MV, Paliyath G, Ormrod DP. (1996) Ultraviolet-B- and ozone induced biochemical changes in antioxidant enzymes of *Arabidopsis thaliana*. *Plant Physiol*. 110, 125–136.

17. Strid A, Chow WS, Anderson JM. (1990). Effects of supplementary ultraviolet-B radiation on photosynthesis in *Pisum sativum*. *Biochem. Biophys. Acta* 1020, 260-268.

18. Rivas-San Vicente M, Plasencia J. (2011) Salicylic acid beyond defence: its role in plant growth and development. *Journal of experimental botany* 62 (10), 3321-38.

19. Day TA, Ruhland CT, Xiong FS. (2001) Influence of solar ultraviolet-B radiation on Antarctic terrestrial plants: results from a 4-year field study. *Journal of Photochemistry and Photobiology B: Biology*. 62 (1-2), 78-87.

20. Reddy KR, Prasad PV, Singh SK. (2010) Effects of ultraviolet-B radiation and its interactions with climate change factors on agricultural crop growth and yield. In *UV radiation in global climate change* (pp. 395-436). Springer, Berlin, Heidelberg.

21. Mpoloka SW. (2008) Effects of prolonged UV-B exposure in plants. *African Journal of Biotechnology* 7 (25), 4874-4883.

22. Fina J, Casadevall R, AbdElgawad H, Prinsen E, Markakis MN, Beemster GT, Casati P. (2017) UV-B inhibits leaf growth through changes in growth regulating factors and gibberellin levels. *Plant physiology* 174, 1110-26.

23. Liu S, Yan Z, Chen Y, Zhang M, Chen J, Han W. (2019) Foliar pH, an emerging plant functional trait: Biogeography and variability across northern China. *Global Ecology and Biogeography* Mar, 28 (3), 386-97.

24. Blumthaler M, Ambach W, Ellinger R. (1997) Increase in solar UV radiation with altitude. *Journal of photochemistry and Photobiology B*: *Biology* Jun 1, 39(2), 130-134.

25. Gullino ML, Gilardi G, Garibaldi A. (2017) Evaluating severity of leaf spot of lettuce, caused by *Allophoma tropica*, under a climate change scenario. *Phytopathologia Mediterranea* 56, 2, 235-241.

26. Gullino ML, Gilardi G, Garibaldi A. (2017) Effect of a climate change scenario on *Fusarium equiseti* leaf spot on wild rocket and radish under phytotron simulation. *Phytoparasitica* Jul 1, 45 (3), 293-298.

27. Gilardi G, Pugliese M., Chitarra W, Ramon I, Gullino ML, Garibaldi A. (2016) Effect of elevated atmospheric CO_2 and temperature increases on the severity of basil downy mildew caused by *Peronospora belbahrii* under phytotron conditions. *Journal of Phytopathology* 164 (2), 114–121.

28. Gilardi G, Pugliese M, Gullino ML, Garibaldi A. (2016) Simulated elevated atmospheric CO_2 and temperature affect the severity of bean and pelargonium rust. *Phytoparasitica* Jul 1, 44(3), 325-32.

29. Pugliese M, Gullino ML, Garibaldi A. (2010) Effects of elevated CO_2 and temperature on interactions of grapevine and powdery mildew: first results under phytotron conditions. *Journal of Plant Diseases and Protection* 117, 9–14.

30. Pugliese M, Cogliati E, Gullino ML, Garibaldi A. (2012a) Effect of climate change on *Alternaria* leaf spot of rocket salad and black spot of basil under controlled environment. *Communications in Agricultural and Applied Biological Sciences* 77, 241–4.

31. Pugliese M, Liu J, Titone P, Garibaldi A, Gullino ML. (2012b) Effect of elevated CO2 and temperature on interactions of zucchini and powdery mildew. *Phytopathologia Mediterranea* 51, 480–487.

32. Ferrocino I, Chitarra W, Pugliese M, Gilardi G, Gullino ML, Garibaldi A. (2013) Effect of elevated atmospheric CO_2 and temperature on disease severity of *Fusarium oxysporum f.*sp. lactucae on lettuce plants. *Applied Soil Ecology* 72, 1–6.

33. Escobar Bravo R, Chen G, Grosser K, Van Dam NM, Leiss KA, Klinkhamer PG. (2019) Ultraviolet radiation enhances salicylic acid-mediated defense signaling and resistance to *Pseudomonas syringae* DC3000 in

a jasmonic acid-deficient tomato mutant. *Plant signaling & behavior* 14, e1581560.