

The Raw pH in Plants: A Multifaceted Parameter

Giorgio Masoero^{1,*}, Alberto Cugnetto¹

¹Accademia di Agricoltura di Torino, Torino, Italy

Abstract

The measurement of the *in vivo* raw pH of vegetative organs is a unusual way obtaining plant knowledge. The authenticity of the pH parameter of the leaf and its independence from soil pH has already been highlighted. In the present work we observe how and to what extent water-temperature mechanisms as well as bio-fertilizers inocula can affect the raw pH and how great the biodiversity is in plants. A trial with *Arabidopsis thaliana* in a phytotrone has shown that, in the dark, the raw pH did not change from +18 to +35 °C ($b = -0.0027$ N.S.), while in the light, the regression coefficients were significant and negative, and the acidification in the leaves progressed from high (-0.0097) to normal (-0.0127) and then to low (-0.0370) water level. We have confirmed that warming induces a decrease of raw petiole pH of $-0.070 \text{ pH } ^\circ\text{C}^{-1}$ in grapevine leaves. In accordance with water-temperature mechanisms, the raw pH in grapevines has been found to be significantly higher in well-watered plants (pH = 4.29) than in stressed ones (4.12), with a pH decay of -3.9%. On the other hand, an average reduction of 0.10 units of raw pH would signal an increase in water stress of about -0.59 Mpa. Among the phenomena that can influence the raw pH, we have outlined three biotic factors: i) acidification as a result of a symbiotic farming fertilization i.e through the use of mycorrhizal and microbial fertilizers, with an average decay of around -3%, as a probable signature of symbiosis; ii) an "*acida plantarum natura*" scenario over 49 species, ranging from pH 3.06 to 6.38 ; iii) a strong ($R^2 = 0.9$) inverse polynomial pseudo-relationship of the number of fungicide sprays on the raw pH in a set of 15 species. It is suggested that this simple new multifaceted parameter can deserve interest.

Corresponding author: Giorgio Masoero, Accademia di Agricoltura di Torino; Torino, Italy,
Email: Giorgioxmasoero@gmail.com

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Introduction

The *in vivo* raw pH of vegetative organs is a unusual measurement in plant knowledge, that almost sounds like a paradox when compared to the resonant importance of soil pH¹ or pH and Eh² and moreover of pH and Eh in hydroponics systems^{3,4}. In fact the raw mass pH was an historic pivotal keystone, since AI Virtanen, NP in 1945, and still it is worldwide, everywhere silage fodder is adopted: in truth the present study is intended to deep inside the *in vivo* raw pH in crops and in orchards, that is *pre-* and not *post-mortem* phase. It must be pointed out that in the most studied vegetal species, *Arabidopsis thaliana*, the pH has been deeply discerned among intracellular components⁵ but the raw foliar pH, intended as the raw matrix of leaf tissues, remains neglected. In the framework of a study on mycorrhizal and microbial effects on maize⁶ a systematic significant response emerged in the *in vivo* raw pH of maize stems, showing an acidification de-gradient from roots (pH -7% in mycorrhized corn) to stem pH at ears height (-4%). After this first results, preliminary surveys in grapevine ascertained that the *Flavescence dorée*, a phytoplasma diffused in Latin EU-area⁷ attack determined an elevation of the pH in unhealthy sub-branch and petioles⁸. Raw pH fall-out in *Sorghum sudanensis* leaves has been recently recognised as a sign of mycorrhizal inoculation¹¹. So far, a relevant result was the appearance of acidic nature in the raw tissues of corn and grapevine that were lower than the previous leaf pH values determined in 92 species from the Cornelissen team^{9,10}.

Experimental Procedure

The aims of the present study were to pursuit the investigation of this paradox multifaceted parameter with address for either: i) a multi-species variation, and ii) a dependency on the aerial temperature and on the plant water status that, iii) a different susceptibility to fungal and or bacterial diseases. Four bodies of data were considered for the setup of the present work. Further comparative elaborations and suggested hypothesis are reported in the discussion.

Materials and methods

Measuring Raw pH

The *in-vivo* raw pH measurements were conducted using a BORMAC "XS pH 70" pH meter (www.giorgiobormac.com), range pH 0÷14, two decimals, provided with a combined plastic-glass electrode Hamilton Peek Double-PoreF, / Knick, dimensions (LxØ) mm 35×6, terminating with a very small and sensible tip sensor; other types were unstable and unreliable. The insertion of the tip in the petiole was facilitated by a small drill fitted with a 2 mm bit. In total 4181 leaves were examined for raw pH in the petiole axis, basal side. When the petiole was too thin for a tip insertion (olive, grape) then an axial cut by a lancet was executed, and the sensitive tip was wrapped by the two wings of petiole fork. For the *Arabidopsis thaliana* examination, several small packages of leaf blades were pierced with the tip. The maize pH was measured in the stem at the middle of 2nd internode⁶ and not in a part of the leaf.

Multispecies Variability

As a total forty-nine species (Table 1, Table 2) were sampled from twenty-nine botanic families, relevant to several orchards, trees, vegetables and ornamental species. The grapevine was the most represented species (No. 2190) suited by the maize (792). Geographic origins were Piedmont (vine, vorn and several other), Emilia (hazelnut, blueberry, cherry and kiwi) and Puglia (olive). The pH values were analysed according the species, by a one-way ANOVA, using PROC GLM, Tukey's HSD, by SAS V. 9 software (SAS Institute, Cary, NC, USA).

Temperature Timeline Effects

Four *Barbera* grapevines were grown in-pot outdoor at the DISAFA experimental implant (45°03'58.6"N 7°35'23.8"E). The pH was measured on fifty-six leaves, without automatic correction for temperature, and sampled at three different timepoints (8-9; 12-13 and 19-20) during October 2016. In this part of the study were comprised other raw pH measurements recorded on *Barbera* grapevines from the same implant and sampled in July on the late morning. The external temperature was considered as the independent variable in a regression study of pH. The hour of the day was also plotted to consider the daily

timeline effect.

Water Stress Effects

The pH was measured on 176 leaves from 15 *Grenache* variety grapevines grown in pot at 25 ± 2 °C in a greenhouse at the same implant as above. The leaves belonged from a control well watered and from a water-stresses thesis, monitored by Ψ_{stem} . The pH and the hydronium $[H^+]$ were related with the stress condition by a mixed model considering the plant random within stress group, and fixed the watering factor. Further regression analyses considered the pH and $[H^+]$ individual values on the Ψ_{stem} of the plant, established on some protected leaves at the moment when the plant was cut for the sap extraction.

Water-Thermo-Light Combined Effects

Sixteen pots of *Arabidopsis thaliana* forty-days old were conditioned for thermo-light in a small phytotrone set at $+18^\circ\text{C}$ and $+30^\circ\text{C}$ with 12 h day-night photoperiod. A first run was conducted at high level of watering where half of the pots were kept in dark for 24 h before the raw pH measurements, at the end of the daylight period. A following run was developed at normal watering, without dark period and a third run interested the residual plants, that were inducted in water stress at the two temperature levels in a normal daylight period. An IR pyrometer established the leaf temperature, while the pH-meter was disconnected for the thermal compensation. A total of 104 measurements were developed in the three runs.

Symbiotic (Mycorrhizal and Microbial) Effects

In a framework of symbiotic biofertilization on fields experiments, a complex commercial consortium mycorrhizal-based (Micosat F ®, by CCS Aosta s.r.l.)¹¹ (S) was used and compared to internal control not inoculated (C). Different systems were adopted for root inoculation. In adult vineyards and other orchards was practiced a cut depth 30 cm at a distance of 50 cm from the row, distributing 20 kg ha⁻¹ of bio-fertilizer. In a very minor cases, with young orchards, the plants have been pudding-treated. As a total seven species, were collected from four farms in 2014 (corn) and from nineteen orchards in 2015 (vine, hazelnut, blueberry, cherry, kiwi and olive); geographic origins were Piemonte (vine and corn), Emilia (hazelnut, blueberry, cherry and kiwi) and Puglia (olive). A two way ANOVA with interaction was

used for statistical analysis. The model included the species and symbiotic factors

Pseudo-Relationship with the Fungicide Sprays.

In the report published from the EFSA EU-ERA network¹², in the original Table 17¹² were exposed the frequencies of the fungicide and insecticide sprays for 13 orchards and crops. Because no data were available for pear, a key species for pH involvement, and coffee, some specialist colleagues were asked about. These mean values were colligated with the mean pH of the species by using polynomial models. A special study concerned the differential outbreaks of the fire blight (*Erwinia amylovora*) in apple and pear, as meticulously monitored in the Bolzano Province¹³ from 1999 to 2016 in some 8.185 farms; the relative incidence and odds ratio referred to farms and hectares.

Results

Multispecies Variability

The raw pH appeared as a very wide-distributed parameter across the species (Figure 1)

An apparent scenario of acidity, (Table 1, Table 2) spaced from *Vitis riparia* (pH = 3.06) to pumpkin (*Sechium edule*, 6.38) with a wide range of 974 to 1 in term not in potenz but in hydronium $[H^+]$ concentration, respectively. The grapevine, *Vitis vinifera*, appeared as the most acidic species (3.68), followed by the maize (4.84), oak (4.87), apple (4.99), apricot (5.03), peach (5.09), plum (5.17), cherry (5.24), olive (5.26), pepper (5.32), coffee (5.31), tomato (5.46), aubergine (5.48), poplar (5.50), pear (5.52), potato (5.77), lettuce (5.97), basil (6.08), cauliflower (6.10), and pumpkin (6.38).

Temperature and Water-Stress Effects

In the experiment with grapevine aimed to an assessment of the pH to the aerial temperature, it was observed a saddle trend from morning to evening (Figure 2). The value was stable overnight and it depended on the combination between sunlight and aerial temperature. Otherwise during the dark period the pH remained stable. Such rise of acidity in the middle day can be ascribed both to a thermal factor or to a sun irradiance available abundance.

Where regressing the pH and the aerial temperature (Figure 3) the two variables appeared as

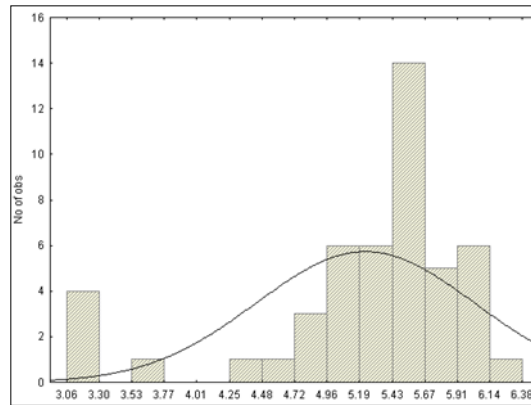


Figure 1. Histogram of the raw pH from the 49 species

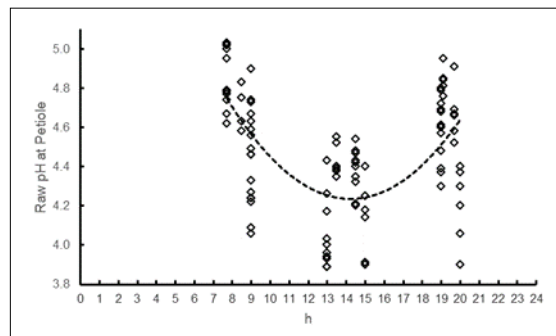


Figure 2. Plot of the raw pH at Petiole in different hours of the day.

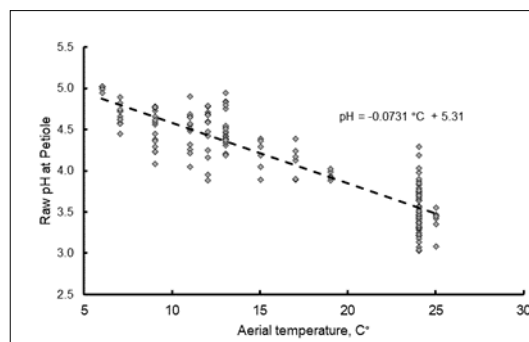


Figure 3. Regression of the raw pH in petiole of grapevines on the aerial temperature.

Table 1. Raw pH values in the 1-30th (49) species, ordered by values

Species	Botanic Family	No.	Mean	Rank	SD
01 <i>Vitis riparia</i>	Vitaceae	23	3.06	a	0.22
02 <i>Vitis rupestris</i>	Vitaceae	18	3.1	a	0.22
03 <i>Vitis berlandieri</i>	Vitaceae	16	3.21	a	0.28
04 <i>Vitis riparia</i> 183G× <i>Vitis cinerea</i>	Vitaceae	12	3.27	a	0.18
05 grape- <i>Vitis vinifera</i>	Vitaceae	2190	3.69	b	0.37
06 <i>Ginkgo biloba</i>	Ginkgoaceae	10	4.41	c	0.13
07 <i>Platanus acerifolia</i>	Platanaceae	10	4.66	d	0.14
08 maize- <i>Zea mais</i>	Graminaceae	792	4.84	d	0.39
09 oak- <i>Quercus Robur</i>	Fagaceae	10	4.87	d	0.10
10 maple- <i>Acer Platanoides</i>	Sapindaceae	13	4.89	de	0.13
11 apple- <i>Malus Domestica</i>	Rosaceae	76	5.04	e	0.27
12 orange- <i>Citrus Sinensis</i>	Rutaceae	12	4.99	ef	0.17
13 apricot- <i>Prunus Armeniaca.</i>	Rosaceae	17	5.03	ef	0.27
14 raspberry- <i>Rubus Spp.</i>	Rosaceae	11	5.25	ef	0.34
15 peach- <i>Prunus Persica</i>	Rosaceae	19	5.09	fg	0.2
16 plum- <i>Prunus Insititia</i>	Rosaceae	19	5.17	efgh	0.26
17 cherry- <i>Prunus Avium</i>	Rosaceae	31	5.24	fghi	0.24
18 olive- <i>Olea Europaea</i>	Oleaceae	121	5.26	gi	0.26
19 pepper- <i>Capsicum Annuum</i>	Solanaceae	14	5.32	gi	0.16
20 coffee- <i>Coffea Arabica</i>	Rubiaceae	121	5.31	gij	0.12
21 <i>Ailanthus altissima</i>	Simaroubaceae	30	5.35	gijk	0.19
22 <i>Hoya carnosa.</i>	Apocinaceae	10	5.41	gijkl	0.10
23 <i>Magnolia grandiflora</i>	Magnoliaceae	13	5.44	gjkl	0.05
24 tomato- <i>Lycopersicon esculentum</i>	Solanaceae	16	5.46	gjkl	0.19
25 <i>Tilia platyphyllos</i>	Malvaceae	11	5.46	gjkl	0.11
26 aubergine- <i>Solanum melongena</i>	Solanaceae	10	5.48	gjklm	0.07
27 kiwi- <i>Actinidia chinensis</i>	Actinidiaceae	30	5.49	jklm	0.12
28 lemon- <i>Citrus limon</i>	Rutaceae	14	5.49	jklmn	0.09
29 poplar- <i>Populus nigra</i>	Salicaceae	21	5.5	jlmn	0.24
30 Sage- <i>Salvia pratensis</i>	Lamiaceae	10	5.51	jlmn	0.15

a..n Means followed by the same letter are not significantly different, after Tukey's HSD test at P < 0.05

Table 2. Raw pH values in the 31-49th (49) species, ordered by values

Species	Botanic Family	No.	Mean	Rank	SD
_31_thistle- <i>Cynara cardunculus L. altili</i>	Asteraceae	14	5.51	jlmn	0.22
_32_pear- <i>Pyrus communis L.</i>	Rosaceae	23	5.52	jlmn	0.11
_33_artichoke- <i>Cynara cardunculus L. scolymus</i>	Asteraceae	12	5.69	mn	0.49
_34_onion- <i>Allium cepa.</i>	Amaryllidaceae	46	5.58	mno	0.13
_35_beet- <i>Beta vulgaris L. var. cruenta</i>	Amaranthaceae	10	5.73	mnop	0.16
_36_curcuma- <i>Curcuma longa</i>	Zingiberaceae	10	5.66	mnopq	0.18
_37_celery- <i>Apium graveolens</i>	Apiaceae	16	5.71	mopq	0.10
_38_leek- <i>Allium porrum</i>	Amaryllidaceae	25	5.77	oq	0.23
_39_potato- <i>Solanum tuberosum.</i>	Solanaceae	58	5.77	oq	0.23
_40_chicory- <i>Cichorium intybus</i>	Asteraceae	33	5.81	oq	0.11
_41_fig- <i>Ficus carica</i>	Moraceae	14	5.87	oq	0.28
_42_Arabidopsis- <i>thaliana</i>	Crucifere	24	5.96	r	0.09
_43_lettuce- <i>Lactuca sativa</i>	Asteraceae	43	5.97	r	0.14
_44_carrot- <i>Daucus carota</i>	Apiaceae	31	5.99	rs	0.21
_45_Zamioculcas- <i>zamiifolia</i>	Araceae	11	6.05	rs	0.22
_46_basil- <i>Ocimum basilicum</i>	Labiatae	21	6.08	rs	0.28
_47_fennel- <i>Foeniculum vulgare</i>	Apiaceae	26	6.08	rs	0.15
_48_cauliflower- <i>Brassica oleracea.</i>	Crucifere	33	6.1	s	0.21
_49_pumpkin- <i>Sechium edule</i>	Cucurbitaceae	31	6.38	t	0.34
R ² = 0.86		4181	4.39		0.34

inversely and closely related with a linear regression coefficient of $-0.073 \pm 0.003 \text{ pH } ^\circ\text{C}^{-1}$ (Eq. A, $P < 0.0001$ in Table 3).

The raw petiole pH was also sensible to divergent conditions in the soil moisture. In the pot experiment, with grapevines from *Grenache* vineyard (Table 4 and Figure 4) the pH was significantly different in the well watered ($\text{pH} = 4.29$) and in the stressed conditions (4.12). The pH decay was 3.9%, but in terms of $[\text{H}^+]$ the increase was 47%.

When looking at the individual values (Table 5) the relationships direct and inverse appeared significant but weak ($P = 0.04$): in the direct sense the pH regressed on the Ψ_{stem} as $+0.05 \text{ Mpa}^{-1}$, but on averages the increase would be $+0.17 \text{ Mpa}^{-1}$. In the reverse sense, an average reduction of 0.10 unit of pH would signal an increase in water stress about -0.59 Mpa .

The experiment with *Arabidopsis thaliana* in a phytotrone raised highly significant level (Table 6) showing that in the dark the raw pH did not change in the interval from $+18$ to $+35$ $^\circ\text{C}$ ($b = -0.0027 \text{ N.S.}$; Figure 5; Table 7) while under the light, the regression coefficients were significant and negative, progressing in acidification from a water levels high (-0.0097), to a normal (-0.0127), and to a low (-0.0370).

Symbiotic (Mycorrhizal and Microbial) Effects

The main factors were significant, with the two factor interaction near significant (Table 8). With reference to Table 9 the symbiotic factor was highly significant in all species except kiwi, with maximum effect in cherry (-7%), high effect in olive and hazelnut (-4%), medium effect in corn and blueberry (-3%), minor effect in vine (-2%).

Pseudo-Relationship with the Fungicide Sprays

According to the EFSA Report on Collection of Pesticide Application Data in EU12 in 2013 the average number of active ingredients, formulations, products and sprays (spray rounds/spray passes) applied to each of the environmental field crops was very variable among crops. As far as apple, grapes and carrots crops are concerned in Italy, Poland, UK, and Spain the number of fungicides sprays applied were on average 16.67, 12.03 and 2.25 respectively (Table 10).

When looking at the fungal pest occurrences a strong inverse polynomial relationship ($R^2 = 0.9$) was interpolated among the mean pH of 15 species and the number of fungicide sprays (Table 11 and Figure 6).

The apple ($\text{pH} = 4.99$, Table 10) appeared as highly inclined to fungal attacks ($\text{NFS} = 13.8$) while the pear (5.52) was much less damaged by fungal diseases ($\text{NFS} = 4$). As opposite, for a bacterial disease (Table 12) the propensity of the farms to fire blight was 11.3% in pear vs. 0.89% in apple ($P < 0.0001$), but when calculated per hectare the incidence were 22.48% vs. 0.35%, respectively. If one want to express the degree of relative risk, in the two species, we refer to the Odds Ratio (OR) method, then we see that a farm of pear will have an OR of 13 to be concerned with fire blight than a farm of apples. But according to the small size of the two kind of farms (0.45 vs. 2.55 ha for pear and apple, respectively) the OR will grow to 65 when considering a single plant of fruit, apple or pear.

Discussion

"*De acidula plantarum natura*" subtitle could have shaped this paper, when looking at the low pH in leading crops and orchards. The model species *Arabidopsis thaliana* appeared as a low acidic one: the average raw pH value of the leaves at 25°C was 5.96. This value is near the vacuole pH (5.2) and the Golgi apparatus (6.3-6.8) but quite below the nucleus, plastid stroma and cytoplasm (7.2-7.3) and far below the mitochondria matrix (8.1)⁵. The first measurement results of pH on finely cut leaves of avocado were published long ago: Haas¹⁴ stated that no significant change in the pH of finely cut leaves or juice was noted over a wide pH range in the soil of avocado cultures; however, he did not consider the water status of the avocado trees. The mean observed value in avocado was 5.53 ± 0.13 , thus it could have been placed at the 33th 50-1 position, just after pear in the ranking shown in Table 1. When regarding the literature, the raw pH has been ignored, except in the work of the proponents of the "Acid Growth Theory"¹⁵. A rare observation¹⁶ of leaves along a highway regarded the surface pH, which was found to be lower than that of the leaves at a distance from the road, but the pH level was found to increase when dust was deposited on the leaves. In fact, the acidic status of the plant depends on

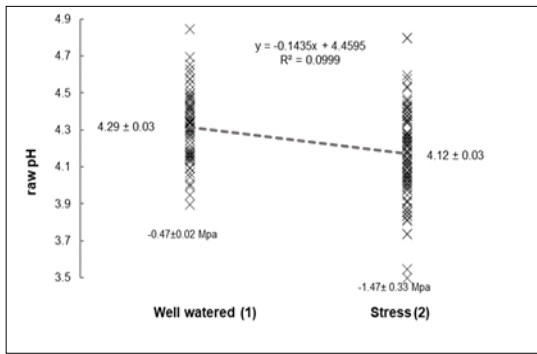


Figure 4. Petiole raw pH regressed on the Well watered (1) or Stressed (2) conditions.

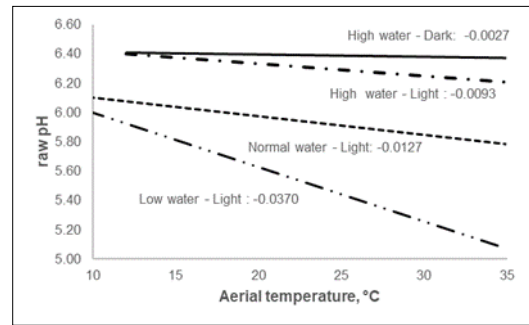


Figure 5. Regression of raw pH of leaves in *Arabidopsis thaliana* (Y Axis) on the aerial temperature (Y Axis) for three water availability levels (High, Normal, Low) and in the Dark (in Water High).

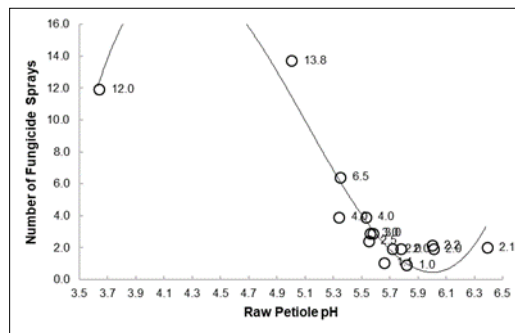


Figure 6. Relationships between the Number of Fungicide Sprays and the mean Raw pH of the 15 species considered in Tab. 10. Equations parameters reported in Tab. 11

Table 3. Regression of the pH on the aerial temperature (No. 158).

Equations	R ²	Intercept	b(°C)	SE	Prob	b(°C ²)	SE	Prob
A	0.797	5.31	-0.073	0.003	<.0001	.	.	.
B	0.800	4.80	.	.	.	-0.00219	0.00009	<.0001
C	0.800	4.99	-0.027	0.023	0.24	-0.00139	0.00069	0.045

Table 5. Regression of the raw petiole pH on the ψ stem and the inverse relationship among the Δw and pH in control and water stressed vines (No. 175)

Variable	R ²	b(ΔW)	SE	Prob(b)
pH	0.024	0.05	0.024	0.0416

Table 4. LSmeans of the raw pH in control and water stressed vines (No. 175)

Variable	Control well watered		Stressed		Prob
	LSMean	SE	LSMean	SE	
Raw pH	4.29	0.03	4.12	0.03	0.0002
Hydronium [H ⁺]	58.05	5.61	84.81	5.37	0.0007

Table 6. Effects of the water level, and light regimen, and the aerial temperature on the raw pH in *Arabidopsis thaliana* leaves (No. 104)

Factor	R ²	CV%	RMSE	Mean	Prob.
Model	0.84	2.70	0.16	6.07	<.0001
Water level [High \ Normal \ Low]					0.0563
Light regimen [Light \ Dark]					0.4894
Temperature (Water * Light)					<.0001

Table 7. Regression Coefficients of the Raw pH in *Arabidopsis* leaves on the aerial temperature, according the water level, and light regimen (No. 104)

Water	Light	b	sb	Prob.
High	Dark	-0.0027	0.0037	0.4698
High	Light	-0.0097	0.0035	0.0069
Normal	Light	-0.0127	0.0039	0.0015
Low	Light	-0.0370	0.0035	<.0001

Table 8. Two way ANOVA with species factor, combined with symbiotic factor and their interaction for *in-vivo* raw pH (No. 2133)

RMSE	P Species	P Symbiotic	P Interaction
0.35	<.0001	0.0004	0.0523

Table 9. *In vivo* raw pH of Conventional (C) and Symbiotic (S) species and the relative decrease.

Species	Raw pH ..						[H ⁺]
	No.	rank	C	S	Prob	S/C	Mean
Vine	1083	a	3.64	3.57	0.008	-2%	229
Corn	807	b	4.9	4.75	<.0001	-3%	13
Hazelnut	56	c	5.16	4.94	<.0001	-4%	7
Blueberry	46	cd	5.17	5	0.004	-3%	7
Cherry	40	de	5.43	5.05	0.014	-7%	4
Kiwi	29	e	5.44	5.54	0.474	2%	4
Olive	72	f	5.78	5.57	<.0001	-4%	2
Total/Means	2133		5.07	4.92		-3%	38

Table 10. Tabulated values from EFSA ¹² of the Number of Fungicide Sprays (NFS) and raw pH for selected fifteen species.

Species	No.	pH	pH ²	pH ³
	Fungicide			
	Sprays			
Grape	12.0	3.63	13.18	47.83
Apple	13.8	4.99	24.9	124.25
Coffee	4.0	5.33	28.41	151.42
Tomato	6.5	5.34	28.52	152.27
Pear	4.0	5.52	30.47	168.2
Artichoke	2.5	5.54	30.69	170.03
Leek	3.0	5.55	30.8	170.95
Onion	3.0	5.57	31.02	172.81
Beet	1.1	5.65	31.92	180.36
Celery	2.0	5.71	32.6	186.17
Potato	2.0	5.77	33.29	192.1
Chicory roots	1.0	5.81	33.76	196.12
Carrot	2.2	5.99	35.88	214.92
Cavolfiore	2.0	6.00	36.00	216.00
Pumpkins	2.1	6.38	40.72	259.81

Table 11. Polynomial regressions of the Number of Fungicide Sprays (NFS) on the raw pH in 15 species, or 14 species, grape excluded.

Equation	Terms	No.	R ²	RMSE	Mean	Coeff.	SE	Prob
D	Intercept	15	0.636	2.41	4.08	31.53	5.79	0.0001
	pH					-4.97	1.0432	0.0004
E	Intercept	15	0.877	1.35	4.08	-847.83	159.48	0.0002
	pH					539.37	98.77	0.0002
	pH ²					-109.29	19.86	0.0002
	pH ³					7.16	1.3	0.0002
F	Intercept	14	0.52	2.36	3.51	42.92	10.94	0.002
	pH					-6.97	1.93	0.0036
G	Intercept	14	0.9	1.04	3.51	437.31	55.27	<.0001
	pH					-146	19.43	<.0001
	pH ²					12.21	1.7	<.0001

Table 12. Outbreaks of the Fire Blight (*Erwinia amylovora*) in apple and pear, in the Bolzano Province (1999 to 2016), and relative incidence referred to farms and hectares

Species	Mean y ⁻¹	SD	No. Farms	Incidence	Ha	Ha Incidence
Pear	19	53	171	11.31%	86	22.48%
Apple	72	158	8014	0.89%	20598	0.35%
Odds Ratio (Pear/Apple)				13		65
z test				9.34		30.4
Prob.				<0.0001		<0.0001

the sugars circulating in the phloem. An increasing level of sugars in sap extracted from non-functional poplars vessels was correlated to a decrease in the pH to around 5.5, while values of 6÷7 were registered in the functional vessels, which only contained traces of sugar¹⁷.

In an acidic environment, around 4.9 pH values, young barley leaves equipped by microprobes for [H⁺] and infected by powdery mildew fungus, apoplastic pH showed a rapid increase towards 5.4, then a more stable decrease around 4.8 at 2 h after infection, but at longer-term pH increased at 5.2 values (+6%)¹⁸. According the Authors, alkalization process seems to have the general goal to minimize and obstruct fungal growth biochemically.

In a broad scenario of plants the pH of phloem exudate is characteristically alkaline (pH 8.0 to 8.5) and belong from non-reducing sugars (sucrose), amides (glutamine and asparagine), amino acids (glutamate and aspartate) and organic acids¹⁹. Acidity of the whole green tissues has longely been utilized in silage operations; methods adopted to improve silage feeding value include rapid wilting and acidification, either by acids products or use of anaerobic inoculants²⁰; however the pH of the raw material is not considered, at most the buffering power is taken into account.

The team of Cornelissen^{9,10} elicited the leaf pH as a new plant trait and explored it as featuring in the carbon or nutrient cycling context in the framework of 92 species (Table 11) which quintile distribution appears quite similar to that of the present 49 species but discarded towards less acidic values (Figure 7).

The authenticity of the pH parameter has been highlighted¹⁰, upsetting the myth of the soil pH: as shown in Figure 8 in fresh leaves the leaf pH will rise only of 0.036 pH_{soil}⁻¹ (about 0.7%) but in pre-dried herbage the inverse occurred (-0.4%). Important to note the differences in pHs: after pre-drying at 60°C for 48 h and rehydrating, the increase of pH was about 4.3%. This confirm the sense of the relationships pH-temperature-humidity (Table 13).

As to the positive relationships between the pH and the water availability a primary apparent sign of stress is the rise in leaf temperature, an infrared sensed measurement basic for plant-water relations, and

specifically for stomatal conductance monitoring²¹. In the present work we have commensurate the drop in petiole raw pH to a water stress condition, but chiefly the linear drop was accounted for by the increase of aerial temperature (-0.07 pH °C⁻¹); in fact in the equations C (Table 3) when the quadratic term was considered, the fit did not improve. It is well known that when the temperature rises over 25°C, the pH declines in force of the normal autoprotolysis - autoionization of the water. But it should be pointed out that the acidic solutions are affected less by this phenomenon than the alkaline ones, and especially when natural buffers are present. In eighteen selected deep eueptic green solvents (DES, i.e. glycerol, ethylen glycol, lactic acid, malic acid .. etc.) the regression of the pH on the temperature over a range +25 ÷ +60°C was -0.018 ± 0.006²². These values are near our data from *Arabidopsis thaliana* in normal watering, but in high watering the value was reduced to -0.093 and, especially, in the dark the value was grown to zero, a null autoprotolysis – autoionization mechanism. Our findings highlight that the pH adjustment of the plant to a water-thermal-light environment is an active dynamic and not a passive process, which demand the presence of the light and may be different according the species. Lindenthal et al.²³ showed in cucumber that downy mildew initial development shifted down -0.8°C the leaf temperature and this cooling was due to a correlated increase of transpiration rate (r = - 0.762).

An objective reason for the lack of studies is that measurement of the *in vivo* raw pH of green vegetable organs is not easy and, essentially, this measurement cannot be automated by chemical or (till now) spectroscopy. Instead, several attempts have been made in the liquid background to optimize the ingredients of the nutritive solution. Ferentinos and Albright³ in a hydroponic system, modelled the pH and the electrical conductivity in deeptrough devices by mean of a neural network. Domingues et al.⁴ developed an automated system to control the pH and concentration of nutrient solution in hydroponic crops. However, no monitoring or responsible feed-back from the plant was considered in terms of pH response. Yu et al.²⁴ made several attempts to predict the so-called "apoplastic pH", using agar or bead indicators, measurements of juices, weak acid influxes,

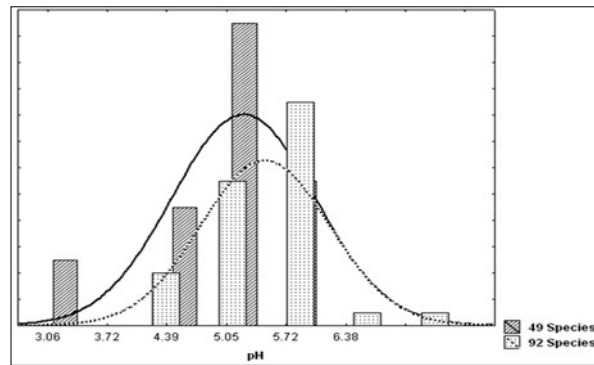


Figure 7. Distribution in quintiles of the pH in the 49 Species of the present work compared with the 92 Species described by the Cornelissen team^{9,10}.

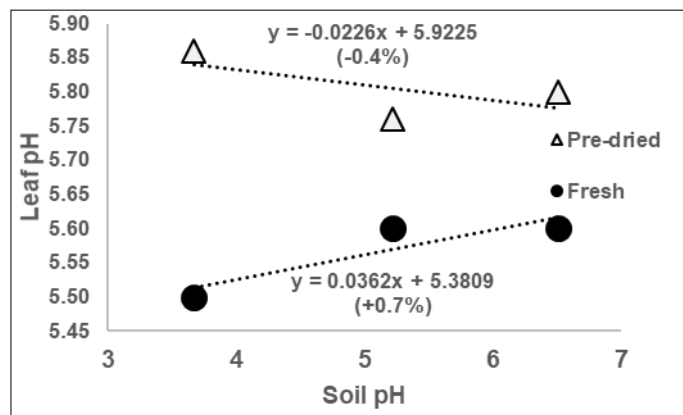


Figure 8. Regression of the average Leaf pH of fresh or pre-dried herbage on the soil pH (data elaborated from Cornelissen et al.¹⁰).

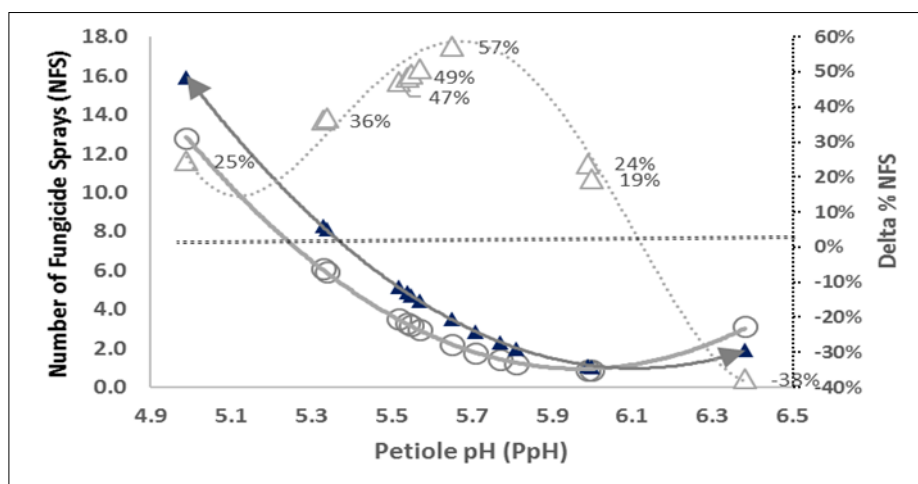


Figure 9. Hypothetical co-evolution of the raw pH (X axis) and the number of fungicide sprays after a very strong climatic change. A rebound of +1.8°C in aerial temperature decrease (-0.13) the pH and this shift of the Number of Fungicide Sprays (NFS) in absolute (▲, left Y axis) or relative % (Δ, right Y axis) from the base relationship NFS * pH (○).

Table 13. Leaf pH Values in 92 species examined by Cornelissen team, ordered by values

Cornelissen et al.¹⁰ Species	pH	Cornelissen et al.⁹ Groups	pH
<i>Ra, Rumex acetosella</i>	3.85	<i>Gymnosperm</i>	4.10
<i>Am, Achillea millefolium</i>	5.20	<i>Ericoid Myc</i>	4.10
<i>Ga, Galium aparine</i>	5.20	<i>Moss</i>	4.50
<i>Ca, Chamerion angustifolium</i>	5.25	<i>Ecto Myc</i>	4.65
<i>To, Taraxacum officinale</i>	5.30	<i>Carnivorous</i>	4.90
<i>Tc, Tanacetum vulgare</i>	5.40	<i>Fern allies</i>	5.10
<i>Eh, Epilobium hirsutum</i>	5.50	<i>Sedges and rushes</i>	5.18
<i>Pr, Papaver rhoea</i>	5.50	<i>Graminoids</i>	5.25
<i>Cs, Centaurea scabiosa</i>	5.55	<i>Dicot NMyc</i>	5.30
<i>Pl, Plantago lanceolata</i>	5.55	<i>Hemiparasite</i>	5.35
<i>Km, Koeleria macrantha</i>	5.85	<i>Nitrogen fixers</i>	5.49
<i>Fo, Festuca ovina</i>	5.90		
<i>Lp, Lolium perenne</i>	5.93		
<i>Bp, Brachypodium pinnatum</i>	5.95		
<i>Ae, Arrhenatherum elatius</i>	6.00		
<i>Pa, Poa annua</i>	6.00		
<i>Dp, Digitalis purpurea</i>	6.01		
<i>Ev, Eriophorum vaginatum</i>	6.02		
<i>Ac, Agrostis capillaris</i>	6.05		
<i>Dg, Dactylis glomera</i>	6.05		
<i>Lc, Lotus corniculatus</i>	6.05		
<i>Lh, Leodonton hispidus</i>	6.27		
<i>Ao, Anthoxanthum odoratum</i>	7.60		

ion-selective electrodes and optical probes, but no reliable method was found. Ten years later, Mc Millan et al.²⁵, made new attempts to conduct inferential measurements, solution temperature correction, efficient calibration, noise minimization, location optimization, and predictive maintenance, by taking advantage of smart features and wireless communication. As published in *New Frontiers in Plant Science*²⁶ the development and properties of genetically encoded pH sensors is a progredient research theme.

Faced with a multifaceted nature of the raw pH acidic wide range, it is possible to ask which lowest common denominator (a large entity) or which greatest common divisor (a small entity) should be considered? In the authors' opinion, assuming the pH as a common divisor, in the pseudo-relationships NFS / pH (Figure 6) a common denominator is the propensity towards fungal attack when the pH is more acidic, with vinegrape and apple as driving species, but also the propensity towards bacterial attack when the pH is less acidic, where a clear demonstration outcomes from a balance among the apple vs. pear diseases (Table 12). Maize is a very acidic species; in Table 1 it is positioned as 8th /49, but it is excluded from the Table 10 because the number of fungicide treatments is very variable according the insecticide necessities; in fact, the resistance to fungal attacks is normally higher than what is actually recognized in the intensively cropped orchards and no one or few fungicide treatments are necessary; nevertheless, mycotoxin in cereals is a historical problem, but also an emergency in exceptionally warm and dry summers. Otherwise the occurrence of bacterial diseases in plants is much less frequent than in animals, and one reason could be exactly the acidic nature of the green matrices. Severe cases of bacterial *Pseudomonas* in Italy concerned the kiwifruit²⁷. Note that the kiwi has a 5.49 pH, ranked as 27th /49, a not highly acidic band. On the other hand the *Xylella fastidiosa* rounous attacks²⁸ on Salento olive trees thrive in a intermediate raw pH band (olive is 13 / 49) and moreover the Flavescence dorée of grapevine⁷ is an incursion of phytoplasmas in a very acid environment.

In perspective, the warming from climate change scenarios, could affect a rise in raw acidity of plants, fearing that fungicide sprays could be increased by one per C° degree rise. In the Figure 9 we have

hypothesized a very high increase of +1.8°C in the average temperature; the major incidences as percentage of NFS increase, reside in the median 5.5 ÷ 5.9 pH range.

The rise in aerial temperature will grew fungal as well as bacterial disease pressure. In a grapevine district Salinari et al.²⁹ have calculated that two more fungicide sprays were necessary under the most negative climate scenario, compared with present management regimes. The increase of presumed 1.8 C° will correspond in the present paper (Table 3, eq. A) at about -0.13 units of raw petiole pH and consequently the elevation in fungicide treatments could be estimated by Eq. D (Table 7) as 13.8 when the actual reference was 12.0. This means that in spite of the the external events regarding the life of the fungi and bacteria out of the crops, it could be introduced an internal category of biological mechanism, leading to increase the acidity circulating in the petiole and leaves, and - unfortunately - this event is favourable to a further increase of fungal diseases. Indeed already now vinegrape are being subjected to an ever increasing problem from fungi. A notable increasing trend of fungicide treatments in France³⁰ is apparent, as the national vineyard district showed 12.4 ± 3.3 NFS in 2013 vs 9.7 ± 2.7 in 2010, which means a rise of +10% per year.

Conclusion

At precision farming operational plan, the raw pH could be considered as metabolic signal of water stress; moreover, vibrational spectroscopy in NIR range could be correlated with it beyond the thermal infra-red (IR) signature.

Symbiotic farming is a sustainable and resilient ausilium to preserve and improve soil fertility, where the raw pH could be a response sign of efficiency for microbial biota evolution in the rhizosphere.

In perspective, the warming of plants from climate change scenarios, could push a rise in plant acidity, fearing that fungicide sprays could be increased in future, moreover when considering the consequences from a strong negative impact of the water stress on the raw pH.

A suggestive paradoxal challenge is given by the acidifying reaction of arbuscular mycorrhizae which instead of favouring the conditions for development of

fungus damages, apparently trigger defensive mechanisms against the mycotoxins³¹.

The raw pH is candidate to become a multifaceted parameter in front of a dilemma: if the atmosphere, the oceans and the soils are acidifying why not the plants?

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