Effects of foliage spray of phosphites on ripening of kiwifruit 'Hayward'

A.K. Yommi[1,a], C. Baeza[1], V. Quillehauquy[1], G. Fasciglione[1,4], M.E. Casanovas[1], M.P. Borrajo[1,4], P.A. Gomez Di Marco[2] and A. Andreu[3]

[1]UIB INTA-FCA, UNMdP, Balcarce, Argentina; [2]Institute of Plant Biotechnology, Universidad Politécnica de Cartagena, Murcia, Spain; [3]IIB-CONICET-FCEyN, UNMdP, Mar del Plata, Argentine; [4]CONICET, Argentina.

Abstract

Phosphites (Phi) are inducers of plant defense responses, although also other effects were reported. In kiwifruit (*Actinidia deliciosa* 'Hayward'), we demonstrated that Phi-treated fruit were more tolerant to gray mold development caused by *Botrytis cinerea*. The mechanisms are not yet well elucidated. The objective of this work was to evaluate the effect of Phi (30% P_2O_5, 20% K_2O), 0.3% (v/v) on 'Hayward' fruit maturity and quality indices, by application at different physiological stages of the fruit development: T1) one application at bloom; T2) six weekly Phi applications at the exponential phase of fruit growth; T3) combination of treatments 1 and 2; T4) control (without Phi). Kiwifruit of all treatments were harvested after physiological maturity (at least 6.2% total soluble solids content, SSC) and were analyzed at harvest in terms of firmness (N), SSC (%), titratable acidity (%) and color (CIELab). Also fruits were evaluated after 4 months of cold storage and shelf-life (7 days at 20°C). Ethylene production and respiration rate were determined at 20°C in fruit of all treatments. The experiment was conducted during two consecutive years. The results show that the Phi had an effect on firmness, variation between years, but no effects on other maturity indices. Firmness of Phi-treated fruits was higher than control ones at shelf-life, essentially when Phi was applied at the exponential phase of fruit growth. This change in the softening rate was associated with the ethylene production that was also affected by some Phi treatments. Phi treatments (T2 and T3) significantly affected the ethylene and respiration rates, reducing the values at the climacteric peak. This result of Phi on the softening of 'Hayward' kiwifruit in this study may have been mediated through salicylic acid that affects perception or biosynthesis of ethylene.

Keywords: *Actinidia deliciosa*, ethylene, firmness, elicitor, maturation

INTRODUCTION

Kiwifruit is a climacteric fruit where softening, increase in total soluble solids, starch degradation and flavor development are the major changes during ripening process. Firmness is the most important quality index for commercialization. Fruit is very firm at harvest (i.e., 60-70 N for 'Hayward') but it softens rapidly with losses of up to 80% after two months of cold storage (Arpaia et al., 1987). Recent studies reported that softening is mainly due to the pectin solubilization, soluble pectin depolymerization and loss of galactose (Atkinson et al., 2011). Autocatalytic production of ethylene triggers a chain of signals that regulates the expression of many genes involved in softening and other attributes such as taste, aroma and color (Solano et al., 1998).

Phosphite (Phi; $H_2PO_3^-$), also called phosphonate, is the anionic form of the phosphonic ($HPO(OH)_2$) or orthophosphorous (H_3PO_3) acid, usually found as salts. Although Phi is recognized as a fertilizer, also acts as a natural fungicide and elicitor. Phosphites have been shown to be effective for the control of diseases caused by oomycetes (Andreu et al., 2006; Miller et al., 2006; Machinandiarena et al., 2012; Lobato et al., 2011) and *Botrytis cinerea* in kiwifruit (Yommi et al., 2012, 2014). It might also affect ripening, since phosphite produces an increase in SA (Massoud et al., 2012) which activates the phenylalanine ammonia lyase

[a]E-mail: yommi.alejandra@inta.gob.ar

Acta Hortic. 1194. ISHS 2018. DOI 10.17660/ActaHortic.2018.1194.23
Proc. VIII International Postharvest Symposium:
Enhancing Supply Chain and Consumer Benefits – Ethical and Technological Issues
Eds.: F. Artés-Hernández et al.

(PAL) expression (Baenas et al., 2014) and reduces the enzymes involved in the ethylene synthesis (Babalar et al., 2007).

It is hypothesized that phosphite causes a decrease in ethylene and respiration rates in kiwifruit, delaying ripening. The aim of the study was to determine the effects of phosphite treatments at different physiological stages on fruit ripening.

MATERIALS AND METHODS

Plant material and field treatments

A mature orchard of kiwifruit (*Actinidia deliciosa* 'Hayward') was used for the experiments (Sierra de los Padres, Argentina, 37°54'45"S; 57°48'09"W). Twelve plots of 3 single rows of vines (100 m) spaced 4 m were considered for both seasons (2014-2015). Each plot was randomly assigned to a total of 4 treatments. Only fruits of plants of the middle row of each plot were harvested. Treatments consisted of foliar applications (600 L ha^{-1}) of 0.3% (v/v) Afital™ (potassium phosphite containing 30% P_2O_5 and 20% K_2O, Phi; treated) as follows: one application at bloom (T1); six weekly applications during fruit growth (T2), from 100 to 150 days after bloom (dab); a combination of treatments T1 and T2 (T3). Non-treated plants were considered as control (T4).

Fruits for all treatments were harvested on the same day, when at least 6.2% of soluble solids content (SSC) was reached. Fruits were placed in plastic trays and stored at 0/1°C, 90% RH for 4 months. Half of them was analyzed at the end of cold storage and the other half was kept at 20°C for 7 days (shelf-life). Maturity indices (flesh color, firmness, SSC, titratable acidity) were determined at harvest, at the end of cold storage and shelf-life. Ethylene production and respiration rate (CO_2 emission) were measured in fruits at harvest.

Fruit quality and ripening measurements

1. Ethylene and respiration.

Ethylene and carbon dioxide production was determined at harvest on 24 individual fruits per treatment at 20°C, every 2 or 3 days until day 35. Rotten and disordered fruits (excessively soften compared with others from the same treatment) were discarded and not included for the analysis. Ethylene was measured by gas chromatography (Shimadzu GC-17A) fitted with a flame ionization detector (FID). Respiration (CO_2 production) was measured in the same GC, using a thermal conductivity detector (TCD).

2. Harvest and maturity indices.

Flesh color and firmness were determined at equatorial zone in 30 fruits per replicate. Color was measured using a chromameter (Minolta, model CR-300, Japan). CIELab* values were used to calculate color index (CI) (Vignoni et al., 2006). Fruit firmness was measured with a penetrometer (Effegi, Italy) with a 7.9-mm diameter tip. The fruit juice was extracted and SSC was determined with digital refractometer (Atago, Japan). Titratable acidity (TA) was analyzed by titratation of 10 mL of juice with 0.1 N NaOH until pH 8.1.

Data analysis

A completely randomized design with 3 replicates for 2 seasons (2014 and 2015) was performed. The significance of treatments was determined using ANOVA. Mean values were compared with the Tukey-Kramer test (α=5%). All statistical analyses were performed in R version 3.2.3. (R Development Core Team, 2008).

RESULTS AND DISCUSSION

Maturity indices at harvest

Firmness and flesh color (CI) were not affected by Phi treatments (data not shown). Instead, they significantly depended on the season. In the first season, treated and untreated fruits had lower CI (a darker green color) and lower firmness when compared to the second

season (CI: 2014=-8.94; 2015=-8.43 and firmness: 2014=71.5 N; 2015=84.3 N).

SSC and TA were affected by Phi treatments depending on the season (significant interaction; $p<0.05$). For the first season, Phi applications at bloom (T1) and during fruit growth (T2) delayed the SSC accumulation compared to control fruits. Fruits obtained from Phi-treated plants at bloom also had lower TA compared to control. However, no differences in SSC or TA between Phi treatments and control were detected in the second season (Figure 1). Considering a same level of Phi treatment, higher SSC and TA were found in 2015 compared to 2014.

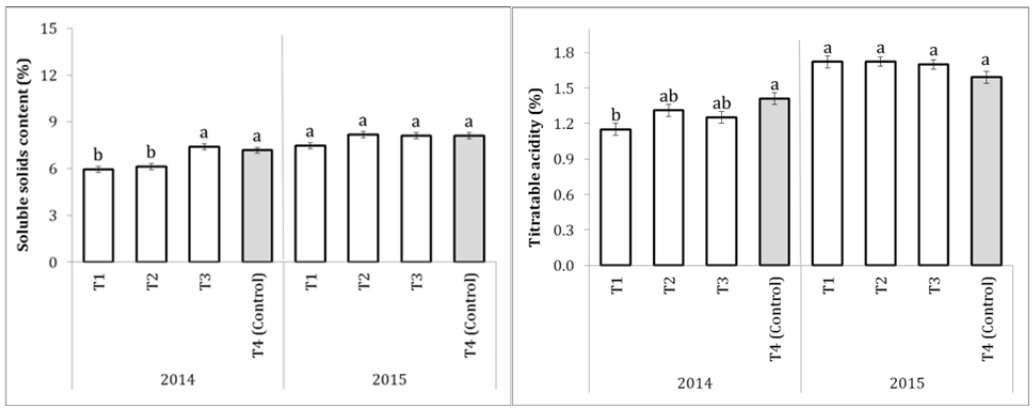

Figure 1. Soluble solids content (%) and titratable acidity (%) at harvest of Phi-treated kiwifruits: T1: one application at bloom; T2: six applications during fruit growth; T3: T1+T2, and control (T4), during two consecutive seasons (2014 and 2015). Lowercase letters indicate significant differences between treatments within season according to Tukey-Kramer test ($p=0.05$). Values represent means and errors bars indicate S.E. of three 30-fruit replicates.

As in others tree fruits, it is possible to find fruits in a wide range of maturity stages on the same plant. SSC values in the control fruits were similar in 2014 and 2015 seasons, showing that they were both harvested at a similar maturity stage. Some climatic conditions in 2014 affected SSC and TA in Phi-treated fruits, regardless of the physiological stage of the plants when Phi was applied.

Ethylene production and respiration rate

A great variability on ethylene and CO_2 production rates between individual fruits was found within each treatment, also fluctuating the day in which the peak of both of them was reached (Figure 2). Hyodo and Fukasawa (1985) found that ethylene emission ('Hayward') exponentially increased after reaching 0.1 $\mu L\ kg^{-1}\ h^{-1}$, corresponding to an important flesh softening. Feng et al. (2003) and Woodward (2007) reported that there is a considerable variability in the production of ethylene even in kiwifruit of similar firmness, and this is possibly related to the differences in the physiological stage of the fruit at harvest. Because the external color is not a useful harvest index in kiwifruit, all fruits are harvested together when the minimum SSC is achieved, resulting in a wide range of maturity as observed here.

When the values of maximum ethylene production for each fruit were analyzed, regardless the day it occurred, results showed that some Phi treatments affected the ethylene production but in different magnitude according to the season (significant interaction effect; $p=0.0036$). The combined treatment (T3) significantly affected the maximum ethylene production in both seasons (Table 1). Ethylene depletion was detected in treated-fruits when Phi was applied during fruit growth (T2). Ethylene production of Phi-treated fruits at bloom stage was similar to control (Table 1). The differences between seasons probably were related to climatic conditions, especially temperature, affecting fruit growth and maturation (Snelgar et al., 2005).

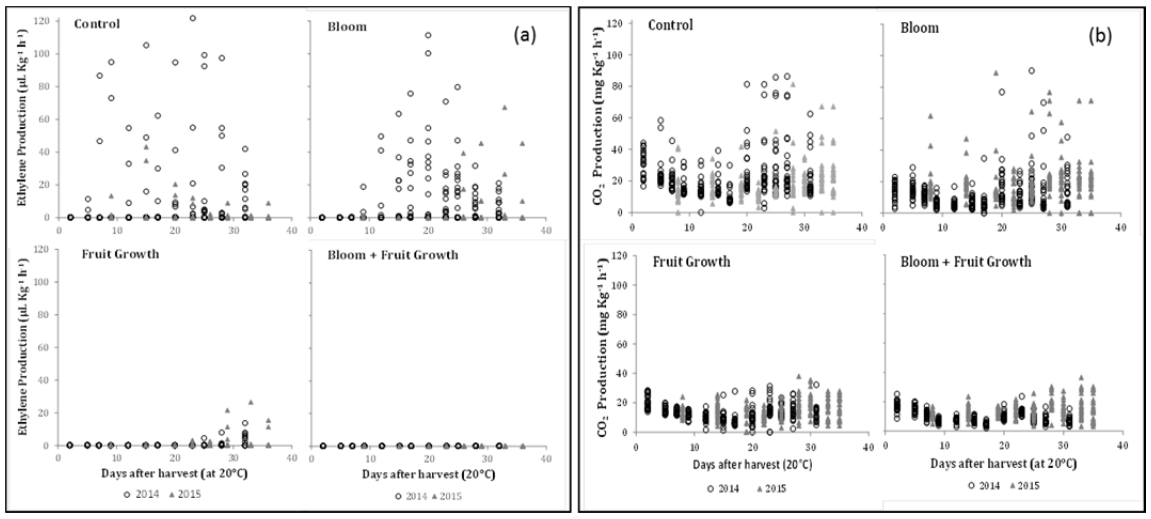

Figure 2. (a) Ethylene and (b) carbon dioxide production (20°C) for Phi-treated and untreated plants at different physiological stages: bloom (T1), fruit growth (T2), bloom + fruit growth (T3) and control (T4) during two consecutive seasons (2014 and 2015) (n=1895 for ethylene; n=1996 for CO_2).

Table 1. Maximum ethylene and carbon dioxide emission rates (20°C) of kiwifruits from Phi treated (T1, T2 and T3) and untreated plants (T4), during two consecutive seasons (2014 and 2015).

Maximum production	Seasons	Treatments			
		T1	T2	T3	T4 (control)
Ethylene (µL kg^{-1} h^{-1})	2014	27.75±7.4 a[1]	2.60±0.7 b	0.17±0.1 c	26.10±8.0 a
	2015	5.87±3.0 z	1.64±0.9 y	0.17±0.1 x	5.28±3.1 yz
CO_2 (mg kg^{-1} h^{-1})	2014	25.78±4.0	22.45±1.2	17.89±0.9	38.37±3.4
	2015	32.31±4.0	21.01±1.2	22.65±0.9	29.28±3.4
Mean CO_2 (2014-2015)		29.04±2.8 ab	22.58±0.9 b	19.45±0.6 c	33.83±2.5 a

[1]Values with different letters within the same line indicate significant differences between treatments according to Tukey-Kramer test (p=0.05).

Phi was applied at the following physiological stages: T1: bloom, T2: fruit growth, T3: bloom and fruit growth, T4: non-treated (control). Values represent means and S.E. (n=171 for ethylene; n=191 for CO_2).

Non-significant (p=0.0531) interaction was detected for the maximum respiration rate (measured by CO_2 production). In fact, there was no significant main effect due to year (p=0.0748). Maximum CO_2 production rate was significantly (p<0.0001) affected by Phi treatments. As shown in Table 1, Phi-treated fruits at bloom and during fruit growth (T3) or during fruit growth (T2) presented a lower maximum respiration rate than compared to control.

Fruit quality at the end of cold storage and shelf-life

Non-significant differences in CI, SSC and TA among Phi treatments were observed at the end of cold storage and shelf-life (data not shown). There was a significant (0.0001<p<0.0260) season effect on these variables. The results are presented as main effects for each season (Table 2).

'Hayward' is a worldwide grown cultivar with excellent characteristics. It is considered that the physiological maturity is reached when SSC values are around 6.2%. That stage represents a minimum maturity, but not the optimal, since sensory parameters are better in later stages (Burdon et al., 2004). High dry matter at harvest is associated with greater flavor and consumer acceptance at ripe stage, as dry matter at harvest is correlated with SSC (Burdon et al., 2004). A higher dry matter detected at the vineyard in 2015 (18.5%) respect

to 2014 (16.4%) would explain the differences in SSC and TA between seasons. Although, Phi-treated fruits (T1 and T2) for the first season had lower SSC at harvest than the minimum associated with physiological maturity, they ripened during cold storage reaching similar SSC values than the control.

Table 2. Seasonal effect, after 4 months of cold storage (0/1°C) plus shelf-life (7 days at 20°C), on maturity indices of kiwifruits. Each value is the mean of all Phi treated and untreated-fruits.

	Seasons	Maturity indices		
		CI	SSC (%)	TA (%)
End of the cold storage	2014	-9.90±0.14 b[1]	13.72±0.12 b	1.08±0.01 b
	2015	-9.18±0.14 a	16.50±0.12 a	1.22±0.01 a
Shelf-life	2014	-9.23±0.11 b	13.81±0.13 b	1.06±0.02 b
	2015	-8.09±0.11 a	16.93±0.13 a	1.21±0.02 a

[1]Values within the same column followed by the same letter are not significantly different between seasons at the end of cold storage or shelf-life according to Tukey-Kramer test (p=0.05).

Flesh softening at values around 5-8 N is necessary to reach an adequate eating stage (Beever and Hopkirk, 1990). Usually, kiwifruits are harvested starting from physiological maturity and they complete the ripening process during cold storage. This fact allows exporters to sell the fruit to distant markets or defer the sales in the domestics. Cold storage reduces the rate of flesh softening and is recommended to extend shelf life. Previous research with 'Hayward' indicated that it can be stored 4 to 5 months at 0°C and high relative humidity for (Rushing, 2004).

As described above, Phi treatments did not significantly affect firmness at harvest, however, a significant ($p<0.0001$) interaction was found at the end of cold storage. Results showed that Phi treatments kept firmness only in the second season. While all the fruit showed a similar firmness in 2014 (around 9.1 N), Phi-treated fruits during fruit growth (T2) were firmer than the untreated control (T4) ones in 2015 (Figure 3).

All or some Phi treatments delayed softening during shelf-life, depending on the season (significant interaction; $p=0.0041$). For the first season, all Phi treatments (T1, T2 and T3) significantly delayed flesh softening during the shelf-life (Figure 3). In the second, T2 and T3 were the most effective treatments to maintain fruit firmness.

Phosphite enters the cell via Pi transporters and show acropetal and basipetal movement when is applied over the foliage (Danova-Alt et al., 2008). Plant response varies with the physiological stage at the phase of application, increasing during the exponential growth period, when the metabolism is more active (Baenas et al., 2014). Therefore, Phi movement within the plant and towards the fruit would increase during fruit growth, expecting a better response to the treatment at that physiological stage. In our study, the Phi applications in the period of 100 to 150 dab (T2), a phase related to an active fruit growth and starch accumulation (Richardson et al., 2004), was more effective for delaying ripening. Probably, a higher number of applications (six instead of one) involved in T2 respect to bloom treatment (T1) is another reason to explain the better results found for T2 and T3.

As others elicitors, Phi would induce physiological changes in the plant. Plants respond to Phi treatment increasing total SA content (Massoud et al., 2012). Also, it was reported that SA inhibits the ethylene biosynthesis (Leslie and Romani, 1988; Zhang et al., 2003). Moreover, a high positive correlation was found in kiwifruit between free SA content and firmness (Zhang et al., 2003). In this work we demonstrate that Phi reduces ethylene emission and respiration rate in 'Hayward' kiwifruit, delaying fruit softening. The increase in SA content in Phi-treated fruits might be the biochemical mechanism operating on the depletion in the maximum ethylene rate and therefore, the cause of observed delayed fruit softening.

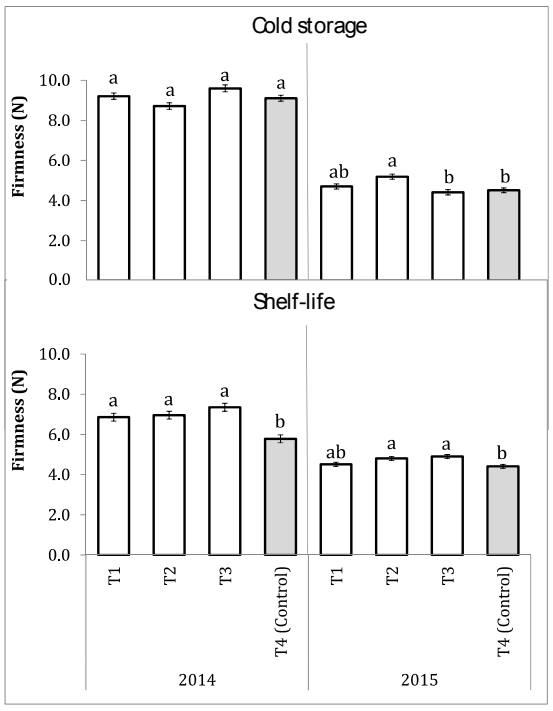

Figure 3. Firmness (N) of Phi-treated kiwifruits: T1: one application at bloom; T2: six applications during fruit growth; T3: T1+T2, and control (T4), during two consecutive seasons (2014 and 2015), after cold storage (4 months at 0°C) and shelf-life (7 days at 20°C). Lowercase letters indicate significant differences between treatments within season according to Tukey-Kramer test (p=0.05). Values represent means and errors bars indicate S.E. (*n*=30).

ACKNOWLEDGEMENTS

This work has been financially supported by INTA, projects PNFRU1105083 and BASUR1272103. We wish to thank to Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). We thank to Agro-EMCODI S.A., Argentina, for supplying the Afital™. The present paper constitutes part of the Doctoral thesis of Alejandra Yommi at the FCA-UNMdP.

Literature cited

Andreu, A.B., Guevara, M.G., Wolski, E.A., Daleo, G.R., and Caldiz, D.O. (2006). Enhancement of natural disease resistance in potatoes by chemicals. Pest Manag. Sci. *62* (*2*), 162–170 https://doi.org/10.1002/ps.1142. PubMed

Arpaia, M.L., Labavitch, J.M., Greve, C., and Kader, A.A. (1987). Changes in the cell wall components of kiwifruit during storage in air or controlled atmosphere. J. Am. Soc. Hortic. Sci. *112*, 474–481.

Atkinson, R.G., Gunaseelan, K., Wang, M.Y., Luo, L., Wang, T., Norling, C.L., Johnston, S.L., Maddumage, R., Schröder, R., and Schaffer, R.J. (2011). Dissecting the role of climacteric ethylene in kiwifruit (*Actinidia chinensis*) ripening using a 1-aminocyclopropane-1-carboxylic acid oxidase knockdown line. J. Exp. Bot. *62* (*11*), 3821–3835 https://doi.org/10.1093/jxb/err063. PubMed

Babalar, M., Asghari, M., Talaei, A., and Khosroshahi, A. (2007). Effect of pre-and postharvest salicylic acid treatment on ethylene production, fungal decay and overall quality of Selva strawberry fruit. Food Chem. *105* (*2*), 449–453 https://doi.org/10.1016/j.foodchem.2007.03.021.

Baenas, N., García-Viguera, C., and Moreno, D.A. (2014). Elicitation: a tool for enriching the bioactive composition of foods. Molecules *19* (*9*), 13541–13563 https://doi.org/10.3390/molecules190913541. PubMed

Beever, D.J., and Hopkirk, G. (1990). Fruit development and fruit physiology. In Kiwifruit: Science and Management, I.J. Warrington, and G.C. Weston, eds. (Auckland, NZ: Ray Richards Publisher and NZ Society for Horticultural Science), p.97–126.

Burdon, J., McLeod, D., Lallu, N., Gamble, J., Petley, M., and Gunson, A. (2004). Consumer evaluation of 'Hayward'

kiwifruit of different at-harvest dry matter contents. Postharvest Biol. Technol. *34* (*3*), 245–255 https://doi.org/10.1016/j.postharvbio.2004.04.009.

Danova-Alt, R., Dijkema, C., DE Waard, P., and Köck, M. (2008). Transport and compartmentation of phosphite in higher plant cells–kinetic and P nuclear magnetic resonance studies. Plant Cell Environ. *31* (*10*), 1510–1521 https://doi.org/10.1111/j.1365-3040.2008.01861.x. PubMed

Feng, J., Maguire, K.M., and MacKay, B.R. (2003). Factors affecting ethylene production of Hayward kiwifruit. Acta Hortic. *610*, 203–209 https://doi.org/10.17660/ActaHortic.2003.610.27.

Hyodo, H., and Fukasawa, R. (1985). Ethylene production in kiwi fruit. J. Japanese Society Horticultural Science *54*, 209–215 https://doi.org/10.2503/jjshs.54.209.

Leslie, C.A., and Romani, R.J. (1988). Inhibition of ethylene biosynthesis by salicylic Acid. Plant Physiol. *88* (*3*), 833–837 https://doi.org/10.1104/pp.88.3.833. PubMed

Lobato, M.C., Machinandiarena, M.F., Tambascio, C., Dosio, G.A.A., Caldiz, D.O., Daleo, G.R., Andreu, A.B., and Olivieri, F.P. (2011). Effect of foliar applications of phosphite on post-harvest potato tubers. Eur. J. Plant Pathol. *130* (*2*), 155–163 https://doi.org/10.1007/s10658-011-9741-2.

Machinandiarena, M.F., Lobato, M.C., Feldman, M.L., Daleo, G.R., and Andreu, A.B. (2012). Potassium phosphite primes defense responses in potato against *Phytophthora infestans.* J. Plant Physiol. *169* (*14*), 1417–1424 https://doi.org/10.1016/j.jplph.2012.05.005. PubMed

Massoud, K., Barchietto, T., Le Rudulier, T., Pallandre, L., Didierlaurent, L., Garmier, M., Ambard-Bretteville, F., Seng, J.M., and Saindrenan, P. (2012). Dissecting phosphite-induced priming in *Arabidopsis* infected with *Hyaloperonospora arabidopsidis.* Plant Physiol. *159* (*1*), 286–298 https://doi.org/10.1104/pp.112.194647. PubMed

Miller, J.S., Olsen, N., Woodell, L., Porter, L.D., and Clayson, S. (2006). Post-harvest applications of zoxamide and phosphite for control of potato tuber rots caused by oomycetes at harvest. Am. J. Potato Res. *83* (*3*), 269–278 https://doi.org/10.1007/BF02872163.

R Development Core Team. (2008) R: a Language and Environment for Statistical Computing (Vienna, Austria: R Foundation for Statistical Computing), http://www.R-project.org.

Richardson, A.C., Marsh, K.B., Boldingh, H.L., Pickering, A.H., Bulley, S.M., Frearson, N.J., Ferguson, A.R., Thornber, S.E., Bolitho, K.M., and Macrae, E.A. (2004). High growing temperatures reduce fruit carbohydrate and vitamin C in kiwifruit. Plant Cell Environ. *27* (*4*), 423–435 https://doi.org/10.1111/j.1365-3040.2003.01161.x.

Rushing, J.W. (2004). Kiwifruit. In Agriculture Handbook Number 66. The Commercial Storage of Fruits, Vegetables and Florist and Nursery Stocks, K.C. Gross, C.Y. Wang, and M. Saltveit, eds. http://www.ba.ars.usda.gov/hb66/kiwifruit.pdf.

Snelgar, W.P., Hall, A.J., Ferguson, A.R., and Blattmann, P. (2005). Temperature influences growth and maturation of fruit on 'Hayward' kiwifruit vines. Funct. Plant Biol. *32* (*7*), 631–642 https://doi.org/10.1071/FP05062.

Solano, R., Stepanova, A., Chao, Q., and Ecker, J.R. (1998). Nuclear events in ethylene signaling: a transcriptional cascade mediated by ETHYLENE-INSENSITIVE3 and ETHYLENE-RESPONSE-FACTOR1. Genes Dev. *12* (*23*), 3703–3714 https://doi.org/10.1101/gad.12.23.3703. PubMed

Vignoni, L.A., Césari, R.M., Forte, M., and Mirábile, M.L. (2006). Determinación de indice de color en ajo picado. Inf. Tecnol. *17* (*6*), 63–67 https://doi.org/10.4067/S0718-07642006000600011.

Woodward, T.J. (2007). Variation in 'Hayward' kiwifruit quality characteristics. Ph.D. thesis (The University of Waikato), http://hdl.handle.net/10289/2640.

Yommi, A., Quillehauquy, V., Fasciglione, G., Guzzo, C., Casanovas, M., and Andreu, A. (2012). La aplicación de fosfitos en kiwi retrasó el ablandamiento de la fruta y mejoró componentes del sabor y la tolerancia a la podredumbre gris. In Cap. V. Calidad Poscosecha de Frutas y Hortalizas. Avances en Poscosecha de Frutas y Hortalizas, I. Recasens, J. Graell, and G. Echeverria, eds. (Lleida, Spain: Ediciones de la Universidad de Lleida), p.477–483.

Yommi, A., Feldman, M., Olivieri, F., Guzzo, M.C., Suarez, P., Quillehauquy, V., and Andreu, A. (2014). Phosphites improve tolerance to gray mold and induce defense mechanisms in the 'Hayward' kiwifruit. Paper presented at: VIII International Symposium of Kiwifruit (Dujiangyan, Chengdu, China: ISHS).

Zhang, Y., Chen, K., Zhang, S., and Ferguson, I. (2003). The role of salicylic acid in postharvest ripening of kiwifruit. Postharvest Biol. Technol. *28* (*1*), 67–74 https://doi.org/10.1016/S0925-5214(02)00172-2.