Effect of bacterial canker caused by Pseudomonas syringae pv. actinidiae on postharvest quality and rots of kiwifruit 'Hayward'

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1   **Effect of bacterial canker caused by *Pseudomonas syringae* pv. *actinidiae* on postharvest quality**

2   **and rots of kiwifruit 'Hayward'**

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4   Simona Prencipe[1,2], Luca Nari[3], Graziano Vittone[3], Maria Lodovica Gullino[1,2], Davide Spadaro[1,2*]

5

6   [1] DISAFA – Dipartimento di Scienze Agrarie, Forestali ed Alimentari, Università degli Studi di

7   Torino, Largo P. Braccini 2, 10095 Grugliasco (TO), Italy.

8   [2] Centro di competenza per l'Innovazione in campo agro-ambientale (AGROINNOVA), Università

9   degli Studi di Torino, Largo P. Braccini 2, 10095 Grugliasco (TO), Italy.

10   [3] Agrion - Fondazione per la ricerca l'innovazione e lo sviluppo tecnologico dell'agricoltura

11   piemontese, Via Falicetto 24, 12030 Manta (Cn), Italy.

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19   *** Corresponding author**

20   Davide Spadaro

21   E-mail: davide.spadaro@unito.it

22   Telephone: +39-011-6708942

23   Fax: +39-011-6709307

Abstract

Kiwifruits produced from plants infected by *Pseudomonas syringae* pv *actinidiae* (*Psa*), the agent of bacterial canker, do not show visible disease symptoms and can be commercialized. Firmness, total soluble solids (TSS), and titratable acidity (TA) of *Actinidia deliciosa* 'Hayward' fruit, infected or not by *Psa*, were quantified, for two years, at harvest, at 90 days storage, and during shelf life. Analysis of dry matter (DM) and calcium at harvest, and postharvest rots, caused by *Botrytis cinerea*, during shelf life were also assessed. DM was higher and calcium content was lower in the kiwifruit from orchards infected by bacterial canker. During storage, firmness decreased and TSS increased rapidly in fruit from infected orchards. These parameters, together with the lower TA, favoured the higher susceptibility to grey mould rots. Besides the incidence of bacterial canker, climate conditions throughout the cropping seasons greatly affected the postharvest quality of fruit. Also the storage conditions modified the fruit quality: controlled atmosphere (CA) and 1-MCP treatment delayed fruit softening compared to normal atmosphere. TSS on fruit from healthy orchards was not influenced by the storage conditions. Fruit from *Psa* diseased orchards showed significantly lower TSS in CA storage and after 1-MCP treatment, compared with normal atmosphere storage. In this work, we showed that the presence of bacterial canker affects postharvest quality, shelf life, and susceptibility to postharvest rots of fruit.

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1. Introduction

The global production of kiwifruit was over 1.4 million tons in 2012, and Italy, with 384,000 tons and 24,800 hectares (FAOSTAT, 2014), produced more kiwifruit than any other country, apart from China. 'Hayward' is the most important kiwifruit cultivar, because of size, taste, longer storage, and high antioxidant capacity and vitamin C (Testolin and Ferguson, 2009).

Pseudomonas syringae pv. *actinidiae* (*Psa*), the agent of bacterial canker on kiwifruit, was first isolated and described in Japan in 1984 (Takikawa et al., 1989). In Italy, Scortichini (1994) first reported the presence of *Psa* on 'Hayward' kiwifruit in 1994, but the first epidemic outbreak of bacterial canker, caused by the highly virulent biovar 3 of *Psa,* occurred in 2008 (Balestra et al., 2009; Vanneste et al., 2013). During 2010-2014, around 1,922 hectares of kiwifruit were uprooted in the different regions of Italy due to bacterial canker (personal communication). The disease quickly reached pandemic proportions by spreading to France, Spain, Portugal, Switzerland, New Zealand, Chile, Turkey, South Korea, and Japan (EPPO, 2012). Kiwifruits from diseased plants do not show disease symptoms and can be commercialized. The presence of *Psa* could affect postharvest quality, storage shelf life and susceptibility to postharvest rots of fruit, which depend on the physiological state of the fruits, similarly as described for *Xylella fastidiosa* on grapevine (Choi et al., 2013) and citrus (Ribeiro et al., 2003).

In *Actinidia deliciosa* 'Hayward', total soluble sugar (TSS; percentage) is a physiological marker from fruit development to ripening, and a value of 6.2% has been established as optimal for harvest (Burdon et al., 2013). Also dry matter (DM) is an important parameter used as maturity marker predictive of fruit flavour (Patterson and Currie, 2011). DM and TSS are closely related parameters on ripe kiwifruit. Other chemical parameters, commonly used as markers of fruit quality, are firmness and sugar:acid ratio. The physiological parameters of kiwifruits are influenced by complex interactions between pedoclimatic conditions, agricultural practices, harvesting maturity, ethylene production, and accumulation of calcium (Montanaro et al., 2012; Burdon et al. 2014).

Postharvest quality and rots of kiwifruit could also be related to concentration of minerals, such as calcium, magnesium, and nitrogen (Spadaro et al., 2010). Calcium is an important macronutrient in plants, involved in development and softening of fruit and it influences their quality. It is an essential nutrient due to its function in stabilization of membrane systems, increase of cell turgor, resistance to cell wall degradation by enzymes, and reduction of fruit transpiration (Ghani et al., 2011). The calcium content in fruits is closely related to the microclimatic conditions around the fruits, which influence their transpiration (Xiloyannis et al., 2010).

Kiwifruit can be stored over 6 months at $0\pm1°C$ (Feng et al., 2006). During storage, fruit rots can cause serious economic losses (Spadaro et al., 2011), and *Botrytis cinerea*, causal agent of grey mould, is the most important postharvest pathogen (Pyke et al., 1994).

Fruit quality losses are mainly due to the fruit metabolic activity during postharvest, which is influenced by the storage conditions. Cold storage, controlled atmosphere (CA), and use of postharvest treatments based on ethylene inhibitors, such as 1-methylcyclopropene (1-MCP), could be used to increase the fruit storage life and to extend the ability to supply the market all year round, while maintaining the main fruit characteristics required by consumers (Watkins, 2006).

In order to evaluate the effects of *Psa* on postharvest quality and rots of kiwifruit, the quality parameters and the incidence of grey mould were assessed on kiwifruits harvested from healthy and *Psa* infected orchards, and stored under normal or controlled atmosphere. Orchards were considered healthy throughout the manuscript when bacterial canker symptoms were not visible or negligible. The physiochemical parameters considered were firmness, TSS, titratable acidity, DM, and calcium content. In addition, we verified the effects of 1-MCP treatment on postharvest quality and storage rots of kiwifruit from healthy and *Psa* infected orchards.

2. Materials and methods

2.1. Fruit and bacterial canker incidence

4

97 Twelve orchards of *A. deliciosa* 'Hayward', six infected by *Psa* and six healthy, were selected in

98 north western Italy (Piedmont region) for harvesting kiwifruit for two years (2013 and 2014).

99 The number of vines, the age, and the agronomic practices carried out are reported for each orchard

100 of *Actinidia deliciosa* "Hayward" (supplementary Table 1). Three repetitions of 100 plants were

101 observed in each orchard to calculate the incidence (%) of *Psa*-infected plants. Plants were considered

102 symptomatic when spots with a chlorotic halo were observed on at least two leaves (Table 1). To

103 verify the presence of *Psa*, from symptomatic leaves collected from all orchards, isolation was carried

104 out by using Pseudomonas Selective Agar with CFC supplement (Merck, Germany). Single colonies

105 were grown in Luria Bertani broth (Merck) on a rotary shaker (150 rpm) at 27°C for two days. DNA

106 was extracted by using Instagene™ Matrix (Biorad, United States) according to the manufacturer's

107 protocol. Primers PsaF1/PsaR2 and PsaF3/PsaR4 were used for PCR amplification, following Rees-

108 George et al. (2010). The obtained amplicons were run on a gel and compared with positive controls.

109 The genomic DNA was also used to amplify the 16S ribosomal gene with the universal primers 27f

110 and 1492r (Weisburg *et al.*; 1991) and the PCR products were sequenced and compared with those

111 deposited in GenBank using the BLAST programme (Zhang et al., 2000). We also determined the

112 virulence and the haplotype through *cts* sequencing, according to Vanneste et al. (2010).

113 Five fruit were harvested from 100 vines per orchard. Fruits were stored in controlled conditions or

114 in commercial packinghouses.

115

116 *2.3. Trials in controlled conditions*

117 Kiwifruits from every orchard (500 fruit, around 45 kg) were divided in three boxes (15 kg each).

118 Fruits were stored – within 60 h from harvest – in experimental cold chambers for 90 days at 1.0°C

119 ±1 and RH 98%. After 90 days, fruits were transferred at 10°C for 20 days shelf life, to favour the

120 pathogen development. Firmness, total soluble sugar (TSS), and titratable acidity (TA) were

121 measured at harvest, end of storage, and after shelf life. Grey mould incidence was assessed after

shelf life. The trial was carried out for two years (2013 and 2014). Statistical analysis was performed using Student's t-test at 95%, 99%, and 99.9% confidence level.

2.4. Trials in packinghouses

During the first year, the fruit from one healthy orchard (Healthy 6 in Table 1) and one *Psa* diseased orchard (Diseased 6 in Table 1) were divided in three replications of 15 kg boxes. The fruit boxes were stored – one day after harvest – in two commercial packinghouses located in Piedmont, northern Italy (Sanifrutta, Castigliole Saluzzo (CN) and Rosatello, Lagnasco (CN)), under three conditions: normal atmosphere (1.0±1 °C; RH 98%), controlled atmosphere alone (-0.8 ±0.8 °C; RH 98%; O_2 2.5±0.5kPa, CO_2 3.5±0.5kPa), and controlled atmosphere after treatment with SmartFresh™ Powder (a.i.: 3.3%, 1-MCP; Rohm *&* Hass). Kiwifruit were treated in 37 L plastic barrels with 0.5 µL L^{-1} SmartFresh™ according to manufacturer's instruction at room temperature for 24 hours before closing the storage room. Fruits were stored in cold chambers for 90 days, and later they were kept in simulated shelf life conditions at 10°C for 20 days. Firmness and total soluble sugar (TSS) were measured at harvest, end of storage, and shelf life. Statistical analysis was carried out using Student's t-test at 95%, 99% and 99.9% confidence level, depending on the experiment.

2.5. Quality parameters and chemical analysis

Firmness, expressed in N, was measured using penetrometer FRUIT TEST™ FT 327 (EFFEGI, Alfonsine, Italy) with a 8 mm diameter plunger tip on two sides of fruits. TSS (%) was measured using the digital refractometer NR-151 (DBR95, Singapore), by squeezing one drop of juice from each end of kiwifruit. TA was obtained by titration with NaOH 0.1 M (pH 8.0) using 6 mL clear juice diluted with distilled water up to 30 mL final volume. Acidity was calculated as percent of citric acid. Each parameter was measured in four fruit per box (n = 12) per orchard. The analyses were performed twice. At harvest, percentage of calcium and dry matter (Table 1) were determined by Laboratorio Agrochimico Regionale of Piedmont Region (Ceva, Italy).

2.6. Phytopathological analysis

The incidence of *Botrytis cinerea* was assessed on the fruits of the trial in controlled conditions. Fruit showing stem-end rots were used to isolate *B. cinerea* on Potato dextrose agar (PDA, Merck, Germany) with 0.025 g L^{-1} streptomycin (Applichem, Germany). Isolations were made from 10 fruits per repetition and 3 repetitions per orchard. Morphological identification of the pathogen species, from monoconidial cultures, was confirmed with PCR amplification of ITS region using primer ITS1/ITS4 (White et al., 1990) following standard protocols. The sequences obtained were compared with those already deposited in GenBank.

3. Results

3.1. Incidence of Psa *and chemical analyses at harvest*

The average incidence (%) of bacterial canker on kiwifruits plants, in 2013, was 1.3% for healthy orchards and 86.5% for diseased ones, while in 2014 it was 0.5% and 58.0% for healthy and diseased orchards, respectively (Table 1). No differences between healthy and diseased orchards were found with respect to agronomic practices, including vine spacing, irrigation, training system, pesticide treatments, shelter, rootstock, number of pollinators, and girdling (Supplementary Table 1). Also the average age and height were not significantly different between healthy and diseased orchards. The fertilizer regime on healthy orchards was 80 to 90 nitrogen unit per hectare, while it was reduced in *Psa*-diseased orchards to 50-60 nitrogen unit per hectare. The lower nitrogen input did not affect the final nitrogen content of fruit, which was 0.78% and 0.71% in healthy and diseased fruit in 2013, and 0.83% and 0.79%, respectively, in 2014 (data not shown). Plants showing bacterial canker symptoms were confirmed for the presence of *Psa*. Bacterial colonies isolated from symptomatic tissues were used for amplification and sequencing of the 16S ribosomal gene. All the sequences showed 100%

174   identity with other sequences of *Psa* and two of them were deposited in GenBank (accession numbers

175   KP794939 and KP794940). The isolates confirmed their virulence on plantlets of kiwifruit 'Hayward'

176   and they showed to belong to the haplotype I of gene *cts* (data not shown).

177   At harvest, fruit from diseased orchards showed on average higher DM and lower calcium content,

178   in both years, compared with those from healthy orchards (Table 1). In 2013, DM was 13.8% for

179   healthy fruits and 16.3% for diseased ones ($P \leq 0.001$), while in 2014 it was 14.7% and 15.6%

180   ($P \leq 0.05$), respectively. Calcium content (%) in 2013 was 0.32% for healthy fruits and 0.24% for

181   diseased ones ($P \leq 0.05$), while in 2014 it was 0.39% and 0.34%, respectively.

182   Differences in climate between the two years of study were relevant (Supplementary Figures 1 and

183   2). The cropping season 2013 was characterized by lower minimum temperatures in winter, ranging

184   from -3°C to 1°C, and higher relative humidity, with precipitation concentrated in spring, with a total

185   of 343 mm. In autumn and summer, precipitation and temperatures were similar to the average of the

186   last years.In contrast, the cropping season 2014 showed higher minimum temperatures in winter,

187   ranging from -1°C to 3°C, without frosts, and warmer minimum and maximum pre-harvest

188   temperatures in autumn. The harvesting period was characterized by higher precipitation, with 404

189   mm, and a maximum of 290 mm in November 2014.

190

191   *3.2. Fruit quality in controlled conditions*

192   The quality parameters measured for the trials performed in 2013 and 2014 in controlled conditions

193   are reported in Table 2 and Table 3, respectively. Firmness and TA values decreased from harvest to

194   shelf life, while TSS increased following fruit ripening. Fruits from diseased orchards showed lower

195   firmness and TA, and higher TSS, compared with healthy fruits. Significant differences in TSS

196   ($P \leq 0.001$) were found at the end of storage in 2013 (Table 2) between the fruits from healthy (11.9%)

197   and *Psa* infected orchards (13.8%). Also TA was significantly different ($P \leq 0.001$) between fruit from

198   healthy (0.84%) and diseased (0.48%) orchards. During simulated shelf life, TSS on healthy and

199   diseased kiwifruit increased, respectively, to 11.6% and 14.2% ($P \leq 0.001$).

200 During the second year of experimentation (Table 3), significant differences ($P \leq 0.001$) were found

201 at the end of storage with TSS values of 12.2% for healthy and 13.5% for diseased orchards, and in

202 shelf life ($P \leq 0.05$) with, respectively, 13.4% and 14.2%. For TA, significant differences occurred

203 only at harvest ($P \leq 0.001$) with values of 2.68% and 1.46% respectively, on fruits from healthy and

204 diseased orchards. Firmness was significantly different at the end of storage with values of 6.94 N

205 and 5.36 N for fruits from healthy and diseased orchards ($P \leq 0.01$), and in shelf life with values,

206 respectively, of 2.87 N and 1.76 N ($P \leq 0.05$).

207

208 *3.3. Fruit quality in semi-commercial conditions*

209 The results of the trials performed in commercial packinghouses are shown in Table 4. In normal

210 atmosphere storage (NA), no differences were found for firmness at the end of storage and in shelf

211 life, with values of 22.5 N and 12.5 N for healthy fruits and 18.9 N and 12.1 N for fruits from diseased

212 orchards.In contrast, significant differences ($P \leq 0.001$) were observed for TSS at the end of storage

213 and in shelf life, with values of 11.5% and 12.0% for healthy fruits and 13.5% and 14.4% for diseased

214 fruits, respectively.

215 The results under controlled atmosphere (CA) showed, the same trend, with a decrease of firmness

216 and increase of TSS. Firmness values were significantly different after simulated shelf life ($P \leq 0.05$),

217 with 5.49 N for healthy fruits and 4.61 N for diseased ones. Significant TSS differences were observed

218 both at the end of storage and after shelf life, with values of 11.6% and 12.8% for healthy fruits, and

219 of 12.4% and 13.7% for fruits from diseased orchards, respectively ($P \leq 0.001$).

220 The fruits stored under controlled atmospheres after treatment with 1-MCP showed significant

221 differences both for firmness ($P \leq 0.05$) and TSS ($P \leq 0.001$) at the end of storage and after simulated

222 shelf life. Firmness of healthy fruits, with values of 69.1 N at the end of storage and 18.9 N after shelf

223 life, were significantly higher compared with fruits from diseased orchards, with values of 65.2 N and

224 16.7 N, respectively. TSS was 11.7% for healthy fruits and 12.4% for diseased fruits at the end of

225 storage, while, after shelf life it was 12.6% and 13.8%, respectively.

226

3.4. Postharvest rots

Phytopathological survey showed higher incidence of postharvest rots in fruit from diseased orchards
with 23% rotten fruit in the first year and 45% in the second one, compared with 2% and 16% in fruit
from healthy orchards ($P \leq 0.001$). Some sequences obtained by PCR amplification of the internal
transcribed spacer (ITS1, 5.8S gene and ITS2) of the fungal pathogen isolated from diseased fruit
(Accession numbers KP794937 and KP794938) showed 100% identity to sequences deposited in
GenBank as *Botryotinia fuckeliana*, the teleomorph of *B.cinerea*, which is the commonly accepted
name of the pathogen.

4. Discussion

Kiwifruit quality and diseases could be influenced by climatic conditions during the cropping season.
As a consequence of a colder winter and rainy spring, 2013 was characterized by a higher incidence
of *Psa*, compared to 2014, with milder winter and drier spring. According to Serizawa and Ichikawa
(1993a, b), favourable climatic conditions for *Psa* are cool temperatures, persistent rain, and high
humidity, which characterized the 2013 season.

Changes in physiochemical parameters were observed during kiwifruit storage. Kiwifruit TA
decreased gradually during storage (Park et al., 2006), because of organic acid consumption for
respiration (Moing et al., 2001), while TSS increased over storage, due to starch conversion into sugar
(Wills and Rigney, 2007).

Plants respond to biotic stresses, i.e a plant pathogen, with modifications in the physio-chemical
parameters of sink organs, such as fruit or tuber, as reported for potato infected by *Erwinia carotovora*
(Nourian et al., 2002). Also kiwifruit showed an influence in the chemical composition and the quality
of fruits due to the presence of *Psa*. The chemical analyses performed revealed substantial differences
between kiwifruit from orchards infected or not by bacterial canker. Higher DM and lower calcium

concentration were observed in fruit from diseased plants. DM modification could be related to lower water uptake from roots linked to the presence of *Psa* in the vascular system of kiwifruit (Spinelli et al., 2011; Donati et al., 2014). The correlation between the presence of a plant pathogen in the vascular system and its influence in plant transpiration is reported for several pathosystems (Ribeiro et al., 2003; Choi et al., 2013). DM is also related to climatic conditions, orchard location, fertilization, and harvest time (Spadaro et al., 2010).

Lower calcium content contributes to favourable conditions to infections and physiochemical disorder and, consequently, higher incidence of postharvest diseases (Biggs, 1999). Gerasopoulos et al. (1996) showed positive correlation between calcium and firmness in many fruits. Lower calcium content in kiwifruit from diseased plants could indirectly compromise the cell wall integrity of fruits, increasing the susceptibility to postharvest pathogens (Quiles et al., 2004). Calcium could also contribute to lower susceptibility to pathogens, by reducing the germination of fungal species, as reported by Droby et al. (1997) for *Penicillium digitatum*. For these reasons, probably, a higher presence of *B. cinerea* rots was observed in fruit from diseased orchards, both in 2013 and 2014, similarly to Barboni et al. (2010).

In the fruit from *Psa* diseased orchards, TA was lower and TSS was higher compared to the fruit from healthy orchards. The bacterial pathogen presence could affect the fruit, which has higher respiration rate, higher sugar solubilisation, and, consequently, higher TSS (Shahkoomahally and Ramezanian, 2015).

During the second year, TA in fruits, particularly in the healthy ones, was significantly higher compared to 2013, due to higher temperatures. Organic acid metabolism occurring from harvest to storage is influenced by temperatures. Higher temperatures induce higher fruit respiration rate and enzymatic activity (Walton and De Jong, 1990), that reduce TA (Marsh et al., 2004).

Moreover, higher grey mould incidence and lower firmness in the second year is related to the abundant precipitation of November 2014. As reported by Lysiak (2013), high precipitation at harvest

favoured postharvest diseases on fruit, because the rain contributed to reduce the firmness and the wet environment could favour the pathogen spore penetration.

Significant differences in fruit quality were also related to storage conditions. CA delays fruit softening (Manolopoulou and Papadopoulou, 1998), which is related to the combined effect of lower respiration and transpiration (Hertog et al., 2004). Kiwifruit treated with 1-MCP had, as expected, even higher firmness compared with CA-stored fruit, as reported by Watkins (2006). TSS was not significantly different on healthy fruit stored under NA, CA or after 1-MCP treatment. Fruit from *Psa* diseased orchards showed significantly lower TSS in CA storage and after 1-MCP treatment, compared with normal atmosphere storage. Throughout the experiment in different storage conditions, firmness was lower and TSS was higher in fruit from *Psa* diseased orchards compared with fruit from healthy orchards.

In conclusion, kiwifruit produced from plants infected by bacterial canker did not show visible disease symptoms. For the first time, we showed that the presence of *Psa* greatly affects the postharvest quality, the shelf life, and the susceptibility to postharvest rots of fruit. Dry matter was higher and calcium content was lower in the kiwifruit from diseased orchards. During storage faster firmness decrease and TSS increase were registered. These parameters and the lower TA favoured the susceptibility to postharvest rots, and particularly to *B. cinerea*. Besides the incidence of bacterial canker, also climate conditions throughout the cropping season greatly affected the postharvest quality of fruit. The choice of the storage conditions, including controlled atmosphere and 1-MCP treatment, could help to maintain the shelf life of kiwifruit, with higher firmness and lower TSS. Future work will focus on the effect of bacterial canker on some physiological parameters of kiwifruit, such as respiration and transpiration rates.

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Tables

Table 1 – Geographical location and incidence of plants infected by bacterial canker (%) of twelve *Actinidia deliciosa* "Hayward" orchards considered in this study (2013 and 2014). Average dry matter (DM; %) and calcium content (Ca^{2+}; %) are reported for the kiwifruits harvested from these orchards.

Orchard	Geographical location	Geographical coordinates	2013			2014		
			DM (%)	Ca^{2+}(%)	Infected plants(%)	DM (%)	Ca^{2+}(%)	Infected plants(%)
Healthy 1	Campiglione Fenile (TO)	44° 48' 10" N, 7° 19' 29" E	13.53	0.35	0	13.98	0.31	0
Healthy 2	Campiglione Fenile (TO)	44° 48' 10" N, 7° 19' 29" E	13.23	0.28	4	14.09	0.35	2
Healthy 3	Campiglione Fenile (TO)	44° 48' 10" N, 7° 19' 29" E	13.87	0.29	1	15.36	0.37	0
Healthy 4	Campiglione Fenile (TO)	44° 48' 10" N, 7° 19' 29" E	13.58	0.32	2	14.5	0.47	1
Healthy 5	Campiglione Fenile (TO)	44° 48' 10" N, 7° 19' 29" E	14.13	0.34	0	15.01	0.5	0
Healthy 6	Envie (CN)	44° 40' 59" N 7° 22' 19" E	14.21	0.32	1	15.45	0.32	0
		Mean	13.76	0.32	1.3	14.73	0.39	0.5
Diseased 1	Bagnolo Piemonte (CN)	44° 45' 42" N 7° 18' 54" E	15.17	0.32	66	16.62	0.39	26
Diseased 2	Envie (CN)	44° 40' 59" N 7° 22' 19" E	17.12	0.22	86	15.72	0.35	49
Diseased 3	Envie (CN)	44° 40' 59" N 7° 22' 19" E	17.23	0.26	90	15.08	0.3	58
Diseased 4	Revello (CN)	44° 39' 20" N 7° 23' 34" E	16.09	0.22	94	16.18	0.32	63
Diseased 5	Revello (CN)	44° 39' 20" N 7° 23' 34" E	16.48	0.26	95	15.64	0.37	65
Diseased 6	Envie (CN)	44° 40' 59" N 7° 22' 19" E	15.3	0.16	88	14.58	0.34	55
		Mean	16.23	0.24	86	15.64	0.34	58
		P value	***	*	***	*	ns	***

*, **, *** Differences between given mean values with Student's t-test are significant for *P* value ≤ 0.05, ≤ 0.01, ≤ 0.001. Not significant (ns, P$\geq$0.05).**Table 2** – Firmness, total soluble sugar, and titratable acidity of kiwifruit 'Hayward' harvested from six healthy and six *Psa* infected orchards

at harvest, end of storage, and shelf life. Fruit were harvested on 23 October 2013. The trial was performed under experimental conditions during

2013. Each value of firmness, total soluble sugar and titratable acidity is the mean of n=12 replicates.

	Firmness [N]			Total soluble sugar [%]			Titratable Acidity [%]			Grey mould
Orchard	Harvest	End of storage	Shelf life	Harvest	End of storage	Shelf life	Harvest	End of storage	Shelf life	(%)
Healthy 1	95.7 ±6.37	8.04 ±2.45	5.29 ±0.39	5.80 ±0.25	10.2 ±0.91	11.4 ±0.73	0.94 ±0.03	0.95 ±0.21	0.96 ±0.13	0
Healthy 2	71.2 ±6.96	6.86 ±2.25	5.29 ±0.69	5.70 ±0.32	12.0 ±0.41	10.9 ±0.55	0.95 ±0.14	1.05 ±0.37	0.79 ±0.19	3
Healthy 3	80.0 ±8.23	8.53 ±3.14	5.29 ±0.69	4.80 ±0.45	12.3 ±0.73	12.0 ±0.57	0.81 ±0.02	0.98 ±0.24	0.75 ±0.09	3
Healthy 4	73.3 ±7.64	9.41 ±5.00	5.19 ±0.39	7.30 ±0.33	12.2 ±0.51	11.4 ±0.58	0.81 ±0.04	0.93 ±0.24	0.81 ±0.06	1
Healthy 5	70.9 ±7.06	11.0 ±4.41	5.29 ±0.39	6.80 ±0.28	12.4 ±0.41	11.8 ±0.56	0.82 ±0.08	0.78 ±0.14	0.79 ±0.09	4
Healthy 6	81.8 ±7.84	10.3 ±2.55	7.64 ±0.98	6.06 ±0.36	12.1 ±0.85	12.0 ±0.79	0.83 ±0.07	0.74 ±0.15	0.93 ±0.08	2
Healthy Average	78.8 ±7.35	9.02 ±3.30	5.67 ±0.59	6.08 ±0.88	**11.9** ±0.84	**11.6** ±0.45	0.86 ±0.07	0.90 0.12	**0.84** ±0.09	**2**
Diseased 1	80.7 ±6.37	14.3 ±2.16	5.29 ±0.69	6.60 ±0.41	13.0 ±0.58	12.9 ±0.76	1.17 ±0.06	0.87 ±0.16	0.53 ±0.01	29
Diseased 2	86.3 ±6.86	6.66 ±2.55	5.59 ±0.59	6.40 ±0.35	14.4 ±0.43	15.3 ±0.58	0.88 ±0.12	1.01 ±0.22	0.48 ±0.14	26
Diseased 3	75.7 ±6.17	8.13 ±1.67	5.19 ±0.49	6.20 ±0.44	14.9 ±0.67	15.9 ±0.82	0.86 ±0.03	0.76 ±0.14	0.48 ±0.04	15
Diseased 4	73.2 ±7.45	9.21 ±1.57	6.08 ±0.59	6.20 ±0.4	13.5 ±0.81	14.3 ±0.61	1.01 ±0.04	0.75 ±0.19	0.51 ±0.03	15
Diseased 5	72.4 ±7.94	11.4 ±5.19	5.78 ±0.69	7.60 ±0.32	13.7 ±0.64	14.1 ±1.12	0.82 ±0.06	0.92 ±0.21	0.50 ±0.07	28
Diseased 6	77.9 ±7.06	10.4 ±3.82	7.55 ±1.37	6.60 ±0.26	13.3 ±0.83	12.7 ±0.76	1.07 ±0.17	0.84 ±0.13	0.35 ±0.06	22
Diseased Average	77.7 ±6.97	10.0 ±2.83	5.91 ±0.74	6.60 ±0.52	**13.8** ±0.70	**14.2** ±1.28	0.97 ±0.14	0.86 ±0.10	**0.48** ±0.06	**23**
P value	ns	ns	ns	ns	***	***	ns	Ns	***	***

***Differences between given mean values with Student's t-test are significant for *P* value ≤ 0.001. Not significant (ns, P≥0.05).

Table 3 – Firmness, total soluble sugar, and titratable acidity of kiwifruits 'Hayward' harvested from six healthy and six *Psa* infected orchards at harvest, end of storage, and shelf life. Fruit were harvested on 16 October 2014. The trial was performed under experimental conditions during 2014. Each value of firmness, total soluble sugar and titratable acidity is the mean of n= 12 replicates.

| Orchard | Firmness [N] | | | | | | Total soluble sugar [%] | | | | | | Titratable Acidity [%] | | | | | | Grey mould (%) |
	Harvest		End of storage		Shelf life		Harvest		End of storage		Shelf life		Harvest		End of storage		Shelf life		
Healthy 1	54.1	±5.59	7.64	±2.74	3.53	±2.06	7.59	±0.40	12.2	±0.63	13.2	±0.50	2.92	±0.12	1.39	±0.02	1.66	±0.29	17
Healthy 2	59.8	±4.21	7.64	±2.94	3.72	±2.35	7.48	±0.53	11.6	±0.71	12.1	±0.60	2.94	±0.30	1.32	±0.02	1.30	±0.03	12
Healthy 3	59.6	±4.90	5.19	±0.98	2.94	±2.06	7.62	±0.44	12.2	±0.58	13.1	±0.52	2.91	±0.12	1.44	±0.03	1.34	±0.03	17
Healthy 4	54.4	±6.86	7.84	±2.35	2.55	±1.76	7.88	±0.65	12.0	±0.47	13.6	±0.53	2.65	±0.36	1.41	±0.03	1.48	±0.02	10
Healthy 5	54.8	±5.00	7.94	±3.23	2.35	±1.27	8.53	±0.37	12.8	±0.73	13.9	±0.49	2.52	±0.14	1.50	±0.01	1.43	±0.01	19
Healthy 6	59.7	±5.78	5.39	±2.16	2.16	±0.88	8.17	±0.43	12.1	±0.52	14.3	±0.47	2.14	±0.07	1.42	±0.08	1.39	±0.02	23
Healthy Average	57.1	±5.39	**6.94**	±2.40	**2.87**	±1.73	7.88	±0.40	**12.2**	±0.39	**13.4**	±0.77	**2.68**	±0.32	1.41	±0.06	1.43	±0.13	**16**
Diseased 1	45.8	±6.47	4.90	±2.25	1.27	±0.39	8.21	±0.57	13.7	±1.02	13.9	±0.55	1.72	±0.29	1.38	±0.04	1.39	±0.03	48
Diseased 2	59.7	±4.31	6.37	±2.35	1.96	±0.98	8.03	±0.31	13.8	±0.25	13.8	±0.46	1.52	±0.26	1.45	±0.04	1.48	±0.06	34
Diseased 3	54.0	±2.84	6.27	±2.45	1.57	±0.69	7.83	±0.20	13.3	±0.26	14.3	±0.70	1.53	±0.03	1.40	±0.01	1.44	±0.04	56
Diseased 4	52.2	±5.88	5.19	±2.35	2.55	±1.27	7.92	±0.53	13.4	±1.24	14.1	±0.47	1.39	±0.12	1.45	±0.03	1.44	±0.01	53
Diseased 5	54.8	±4.21	5.68	±2.55	-	-	8.01	±0.42	14.0	±1.38	15.3	±1.20	1.42	±0.16	1.55	±0.06	1.42	±0.05	38
Diseased 6	60.4	±6.86	3.72	±2.65	1.47	±0.49	7.67	±0.45	13.3	±1.17	14.0	±0.70	1.20	±0.12	1.34	±0.04	1.23	±0.04	38
Diseased Average	54.5	±5.10	**5.36**	±2.43	**1.76**	0.76	7.95	±0.19	**13.5**	±0.29	**14.2**	±0.54	**1.46**	±0.17	1.43	±0.07	1.40	±0.09	**45**
P value	ns		**		*		ns		***		*		***		ns		ns		***

*, **, *** Differences between given mean values with Student's t-test are significant for *P* value ≤ 0.05, ≤ 0.01, ≤ 0.001. Not significant (ns, P≥0.05).

Table 4 - Firmness and total soluble sugar (TSS) at three time points (harvest, end of storage and shelf life) of kiwifruits 'Hayward' harvested from healthy and *Psa* infected orchards and stored under normal atmosphere (1.0°C ±1 and RH 98%), controlled atmosphere alone (-0.8±0.8°C; RH 98%; O_2 2.5±0.5%, CO_2 3.5±0.5%), and controlled atmosphere after treatment with SmartFresh™ Powder (a.i.: 3.3%, 1-MCP; Rohm *&* Hass). Each value of firmness, total soluble sugar and titratable acidity is the mean of n=12 replicates.

	Firmness [N]						
	Normal atmosphere			**Controlled atmosphere**		**Controlled atmosphere + 1-MCP**	
	Harvest	**End of storage**	**Shelf life**	**End of storage**	**Shelf life**	**End of storage**	**Shelf life**
Healthy	78.8 ±9.51	22.5 ±8.62	12.5 ±5.10	56.4 ±6.37	17.0 ±5.49	69.1 ±4.51	18.9 ±3.14
Diseased	77.7 ±5.19	18.9 ±6.27	12.1 ±2.35	57.1 ±6.17	14.7 ±4.61	65.2 ±6.76	16.7 ±2.06
P value*	ns	ns	ns	ns	*	*	*

	Total soluble sugar (TSS) [%]						
	Normal atmosphere			**Controlled atmosphere**		**Controlled atmosphere + 1-MCP**	
	Harvest	**End of storage**	**Shelf life**	**End of storage**	**Shelf life**	**End of storage**	**Shelf life**
Healthy	6.08 ±0.88	11.5 ±0.79	12.0 ±0.62	11.6 ±0.48	12.8 ±0.73	11.7 ±0.67	12.6 ±0.49
Diseased	6.60 ±0.52	13.5 ±0.78	14.4 ±0.65	12.4 ±0.87	13.7 ±0.68	12.4 ±0.79	13.8 ±0.86
P value*	ns	***	***	***	***	***	***

*, **, *** Differences between given mean values with Student's t-test are significant for *P* value ≤ 0.05, ≤ 0.01, ≤ 0.001. ns. Not significant (P≥0.05).

Supplementary material

Supplementary Table 1. Number of vines, age and agronomic practices of the twelve *Actinidia deliciosa* "Hayward" orchards considered in this
study.

Orchard	Vines (n°)	Vine spacing (vines/ha)	Age	Irrigation	Fertilisers	Training system	Pesticide treatments	Shelter	Average height (m)	Rootstock	Pollinators (n°)	Girdled trunk
Healthy 1	880	500	20				Copper	no	1.6	no	1:4	no
Healthy 2	780	460	14		Normal fertilizer regime: 80-90 N unit/ha	pergola (V-trellis)	Copper	no	1.5	no	1:4	no
Healthy 3	460	480	17	localized irrigation			Copper	no	1.6	no	1:3	no
Healthy 4	860	520	15				Copper	no	1.6	no	1:4	no
Healthy 5	430	500	17				Copper	no	1.6	no	1:3	no
Healthy 6	590	460	21				Copper	no	1.5	no	1:4	no
Diseased 1	740	480	15				Copper	no	1.6	no	1:4	no
Diseased 2	910	470	19		Reduced fertilizer regime: 50-60 N unit/ha	pergola (V-trellis)	Copper	no	1.5	no	1:4	no
Diseased 3	560	510	17	localized irrigation			Copper	no	1.6	no	1:4	no
Diseased 4	580	490	21				Copper	no	1.6	no	1:3	no
Diseased 5	630	500	20				Copper	no	1.5	no	1:4	no
Diseased 6	960	475	18				Copper	no	1.6	no	1:4	no

Supplementary Figure captions

Supplementary Fig. 1 – Minimum, mean and maximum temperatures [°C] and precipitation [mm]

of Piedmont region, northern Italy during 2013.

Supplementary Fig. 2 - Minimum, mean and maximum temperatures [°C] and precipitation [mm]

of Piedmont region, northern Italy during 2014.