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This is the author's manuscript

Original Citation:

Availability:

This version is available http://hdl.handle.net/2318/1581072 since 2017-05-12T13:42:47Z

Published version:

DOI:10.1016/j.postharvbio.2015.11.010

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13 May 2024

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

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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.

Keywords:

Actinidia deliciosa, bacterial canker, *Botrytis cinerea*, controlled atmosphere, 1-methylcyclopropene, *Pseudomonas syringae*.

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±1°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

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).

148

149 *2.6. Phytopathological analysis*

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

157

158 **3. Results**

159

160 *3.1. Incidence of* Psa *and chemical analyses at harvest*

161

162 The average incidence (%) of bacterial canker on kiwifruits plants, in 2013, was 1.3% for healthy
163 orchards and 86.5% for diseased ones, while in 2014 it was 0.5% and 58.0% for healthy and diseased
164 orchards, respectively (Table 1). No differences between healthy and diseased orchards were found
165 with respect to agronomic practices, including vine spacing, irrigation, training system, pesticide
166 treatments, shelter, rootstock, number of pollinators, and girdling (Supplementary Table 1). Also the
167 average age and height were not significantly different between healthy and diseased orchards. The
168 fertilizer regime on healthy orchards was 80 to 90 nitrogen unit per hectare, while it was reduced in
169 *Psa*-diseased orchards to 50-60 nitrogen unit per hectare. The lower nitrogen input did not affect the
170 final nitrogen content of fruit, which was 0.78% and 0.71% in healthy and diseased fruit in 2013, and
171 0.83% and 0.79%, respectively, in 2014 (data not shown). Plants showing bacterial canker symptoms
172 were confirmed for the presence of *Psa*. Bacterial colonies isolated from symptomatic tissues were
173 used for amplification and sequencing of the 16S ribosomal gene. All the sequences showed 100%

identity with other sequences of *Psa* and two of them were deposited in GenBank (accession numbers KP794939 and KP794940). The isolates confirmed their virulence on plantlets of kiwifruit 'Hayward' and they showed to belong to the haplotype I of gene *cts* (data not shown).

At harvest, fruit from diseased orchards showed on average higher DM and lower calcium content, in both years, compared with those from healthy orchards (Table 1). In 2013, DM was 13.8% for healthy fruits and 16.3% for diseased ones ($P \leq 0.001$), while in 2014 it was 14.7% and 15.6% ($P \leq 0.05$), respectively. Calcium content (%) in 2013 was 0.32% for healthy fruits and 0.24% for diseased ones ($P \leq 0.05$), while in 2014 it was 0.39% and 0.34%, respectively.

Differences in climate between the two years of study were relevant (Supplementary Figures 1 and 2). The cropping season 2013 was characterized by lower minimum temperatures in winter, ranging from -3°C to 1°C, and higher relative humidity, with precipitation concentrated in spring, with a total of 343 mm. In autumn and summer, precipitation and temperatures were similar to the average of the last years.In contrast, the cropping season 2014 showed higher minimum temperatures in winter, ranging from -1°C to 3°C, without frosts, and warmer minimum and maximum pre-harvest temperatures in autumn. The harvesting period was characterized by higher precipitation, with 404 mm, and a maximum of 290 mm in November 2014.

3.2. Fruit quality in controlled conditions

The quality parameters measured for the trials performed in 2013 and 2014 in controlled conditions are reported in Table 2 and Table 3, respectively. Firmness and TA values decreased from harvest to shelf life, while TSS increased following fruit ripening. Fruits from diseased orchards showed lower firmness and TA, and higher TSS, compared with healthy fruits. Significant differences in TSS ($P \leq 0.001$) were found at the end of storage in 2013 (Table 2) between the fruits from healthy (11.9%) and *Psa* infected orchards (13.8%). Also TA was significantly different ($P \leq 0.001$) between fruit from healthy (0.84%) and diseased (0.48%) orchards. During simulated shelf life, TSS on healthy and diseased kiwifruit increased, respectively, to 11.6% and 14.2% ($P \leq 0.001$).

During the second year of experimentation (Table 3), significant differences ($P \leq 0.001$) were found at the end of storage with TSS values of 12.2% for healthy and 13.5% for diseased orchards, and in shelf life ($P \leq 0.05$) with, respectively, 13.4% and 14.2%. For TA, significant differences occurred only at harvest ($P \leq 0.001$) with values of 2.68% and 1.46% respectively, on fruits from healthy and diseased orchards. Firmness was significantly different at the end of storage with values of 6.94 N and 5.36 N for fruits from healthy and diseased orchards ($P \leq 0.01$), and in shelf life with values, respectively, of 2.87 N and 1.76 N ($P \leq 0.05$).

3.3. Fruit quality in semi-commercial conditions

The results of the trials performed in commercial packinghouses are shown in Table 4. In normal atmosphere storage (NA), no differences were found for firmness at the end of storage and in shelf 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 orchards.In contrast, significant differences ($P \leq 0.001$) were observed for TSS at the end of storage and in shelf life, with values of 11.5% and 12.0% for healthy fruits and 13.5% and 14.4% for diseased fruits, respectively.

The results under controlled atmosphere (CA) showed, the same trend, with a decrease of firmness and increase of TSS. Firmness values were significantly different after simulated shelf life ($P \leq 0.05$), with 5.49 N for healthy fruits and 4.61 N for diseased ones. Significant TSS differences were observed both at the end of storage and after shelf life, with values of 11.6% and 12.8% for healthy fruits, and of 12.4% and 13.7% for fruits from diseased orchards, respectively ($P \leq 0.001$).

The fruits stored under controlled atmospheres after treatment with 1-MCP showed significant differences both for firmness ($P \leq 0.05$) and TSS ($P \leq 0.001$) at the end of storage and after simulated shelf life. Firmness of healthy fruits, with values of 69.1 N at the end of storage and 18.9 N after shelf life, were significantly higher compared with fruits from diseased orchards, with values of 65.2 N and 16.7 N, respectively. TSS was 11.7% for healthy fruits and 12.4% for diseased fruits at the end of storage, while, after shelf life it was 12.6% and 13.8%, respectively.

226

227 *3.4. Postharvest rots*

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

235

236 **4. Discussion**

237

238 Kiwifruit quality and diseases could be influenced by climatic conditions during the cropping season.
239 As a consequence of a colder winter and rainy spring, 2013 was characterized by a higher incidence
240 of *Psa*, compared to 2014, with milder winter and drier spring. According to Serizawa and Ichikawa
241 (1993a, b), favourable climatic conditions for *Psa* are cool temperatures, persistent rain, and high
242 humidity, which characterized the 2013 season.
243 Changes in physiochemical parameters were observed during kiwifruit storage. Kiwifruit TA
244 decreased gradually during storage (Park et al., 2006), because of organic acid consumption for
245 respiration (Moing et al., 2001), while TSS increased over storage, due to starch conversion into sugar
246 (Wills and Rigney, 2007).
247 Plants respond to biotic stresses, i.e a plant pathogen, with modifications in the physio-chemical
248 parameters of sink organs, such as fruit or tuber, as reported for potato infected by *Erwinia carotovora*
249 (Nourian et al., 2002). Also kiwifruit showed an influence in the chemical composition and the quality
250 of fruits due to the presence of *Psa*. The chemical analyses performed revealed substantial differences
251 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

277 favoured postharvest diseases on fruit, because the rain contributed to reduce the firmness and the
278 wet environment could favour the pathogen spore penetration.
279 Significant differences in fruit quality were also related to storage conditions. CA delays fruit
280 softening (Manolopoulou and Papadopoulou, 1998), which is related to the combined effect of lower
281 respiration and transpiration (Hertog et al., 2004). Kiwifruit treated with 1-MCP had, as expected,
282 even higher firmness compared with CA-stored fruit, as reported by Watkins (2006). TSS was not
283 significantly different on healthy fruit stored under NA, CA or after 1-MCP treatment. Fruit from *Psa*
284 diseased orchards showed significantly lower TSS in CA storage and after 1-MCP treatment,
285 compared with normal atmosphere storage. Throughout the experiment in different storage
286 conditions, firmness was lower and TSS was higher in fruit from *Psa* diseased orchards compared
287 with fruit from healthy orchards.
288 In conclusion, kiwifruit produced from plants infected by bacterial canker did not show visible disease
289 symptoms. For the first time, we showed that the presence of *Psa* greatly affects the postharvest
290 quality, the shelf life, and the susceptibility to postharvest rots of fruit. Dry matter was higher and
291 calcium content was lower in the kiwifruit from diseased orchards. During storage faster firmness
292 decrease and TSS increase were registered. These parameters and the lower TA favoured the
293 susceptibility to postharvest rots, and particularly to *B. cinerea*. Besides the incidence of bacterial
294 canker, also climate conditions throughout the cropping season greatly affected the postharvest
295 quality of fruit. The choice of the storage conditions, including controlled atmosphere and 1-MCP
296 treatment, could help to maintain the shelf life of kiwifruit, with higher firmness and lower TSS.
297 Future work will focus on the effect of bacterial canker on some physiological parameters of kiwifruit,
298 such as respiration and transpiration rates.
299

300 **Acknowledgments**

301 The Authors wish to thank the financial support of Piedmont Region Union in the framework of the
302 project “PRO.ACT.IN.” (PSR FEASR 2007/2013, European Fund for Rural Development, Measure

124, Action 1). The Authors gratefully acknowledge the orchards and packinghouses owners, and Michele Giraudo who contributed to the experimental trials. The Authors acknowledge also Prof. Angelo Garibaldi for his accurate revision of the manuscript.

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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.

			2013			2014		
Orchard	**Geographical location**	**Geographical coordinates**	**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≥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.

	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	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	localized irrigation	Normal fertilizer regime: 80-90 N unit/ha	pergola (V-trellis)	Copper	no	1.6	no	1:4	no
Healthy 2	780	460	14				Copper	no	1.5	no	1:4	no
Healthy 3	460	480	17				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	localized irrigation	Reduced fertilizer regime: 50-60 N unit/ha	pergola (V-trellis)	Copper	no	1.6	no	1:4	no
Diseased 2	910	470	19				Copper	no	1.5	no	1:4	no
Diseased 3	560	510	17				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.