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Post-harvest quality and health of kiwifruit 'Hayward' affected by *Pseudomonas syringae* pv. *actinidiae*

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Abstract

**   *Pseudomonas syringae* pv. *actinidiae* (*Psa*) is the causal agent of bacterial canker of kiwifruit. The presence of *Psa* could affect the postharvest quality and health of fruit, which depends on the physiological state of the fruits and the techniques used for storage. In order to evaluate the effects of the presence of the pathogen on post-harvest quality, the fruits from twelve orchards of *Actinidia deliciosa* 'Hayward', affected or not by *Psa*, were examined. Firmness, total soluble sugar (TSS), titratable acidity (TA) and postharvest rots were measured in different storage conditions, such as normal and controlled atmosphere, at three time points: at harvest, after 90 days storage, and in shelf life (after 120 days storage). The experiments were performed for two years. A further pre-storage treatment with 1-MCP was applied to evaluate the effects on fruit quality and storage. Significant differences between healthy and diseased samples were found for all the parameters analyzed. Fruits coming from diseased orchards showed lower firmness and TA values and higher TSS compared to healthy fruits. The fruits from diseased plants were much more susceptible to *Botrytis* rot. The results confirmed that the presence of *Psa* in orchards is associated to lower shelf life of kiwifruits and higher incidence of postharvest rots.**

INTRODUCTION

Italy with 384,000 tons/years (FAOSTAT, 2014), is one of the most important countries involved in kiwifruit production, apart from China (Testolin and Ferguson, 2009). For the high antioxidant capacity, presence of vitamin C, size, taste, and longer storage 'Hayward' represents the most important kiwifruit cultivar (Franco et al., 2006; Tavarini et al., 2008). Kiwifruit can be stored over 6 months at 0±1°C (Antunes and Sfakiotakis, 2002) and the major economic losses in postharvest are mainly due to grey mould caused in postharvest by *Botrytis cinerea* (Pike et al., 1994). Concentration of minerals, as nitrogen and calcium, is an important parameter involved in postharvest quality and health of kiwifruit (Spadaro et al., 2010). Calcium plays a crucial role in quality because of its function in reduction of fruit transpiration, stabilization of resistance to cell wall degradation, and increase in cell turgor (Ghani et al., 2011). As reported by Watkins (2006), in order to increase storage life and supply the market all year is important the use of cold storage, controlled atmosphere (CA), and postharvest treatment like 1-methylcycloprophene (1-MCP).

The agent of bacterial canker, *Pseudomonas syringae* pv. *actinidiae* (*Psa*), was first isolated in Japan in 1984. In Italy the first outbreaks of bacterial canker, causing serious economic losses, were reported in 2008 and quickly it reached pandemic proportions (EPPO, 2012). The presence of *Psa* could affect the postharvest quality though kiwifruit from diseased plants do not show symptoms and can be commercialized.

In order to evaluate the effects of *Psa* on postharvest quality and health of kiwifruit, the firmness, the total soluble sugar, the titratable acidity, and the incidence of Botrytis rot on kiwifruits harvested from *Psa* infected and healthy orchards, stored under normal or controlled atmosphere, were measured. Further analyses of dry matter (DM) and calcium content were performed at harvest. 1-MCP treatment was also applied in order to evaluated postharvest quality on kiwifruit.

MATERIALS AND METHODS

Kiwifruit were harvested from twelve orchards, six infected by *Psa* and six healthy, of *Actinidia deliciosa* 'Hayward' in north western Italy (Piedmont region) during 2013 and 2014. Fruits were stored in different conditions: in experimental cold chambers in normal atmosphere (NA) (1.0°C ±1 and RH 98%) and in semi-commercial packinghouse in normal atmosphere (1.0±1 °C; RH 98%), controlled atmosphere (CA) 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 1-MCP (SmartFresh™ Powder, a.i.: 3.3%, 1-MCP; Rohm & Hass) for 90 days. For trial in experimental cold chamber fruit were transferred in shelf life at 10°C for 20 days. Fruits from one healthy orchard and one from *Psa* diseased orchard were selected for a semi-commercial packinghouse trial.

Firmness and total soluble sugar (TSS) were measured at three time points: at harvest, at the end of storage, and in shelf life. A further measure of titratable acidity (TA) was analysed for trial in experimental cold chamber.

Firmness (N) was measured using penetrometer FRUIT TEST™ FT 327 (EFFEGI, Alfonsine, Italy) with a 8 mm diameter plunger tip. TSS (%) was obtained using the digital refractometer NR-151 (DBR95, Singapore) by squeezing one drop of juice. 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 expressed as percent citric acid. Each parameter was measured in three replicates. The analyses were performed twice. Percentage of calcium and dry matter were determined by Laboratorio Agrochimico Regionale of Piedmont Region (Ceva, Italy).

The incidence of *Psa* was assigned by observing the visual symptoms of bacterial canker infection. Botrytis rot incidence (%) was evaluated after shelf life.

The PCR amplification of 16S region, by using species-specific primers PsaF1/PsaR2 and PsaF3/PsaR4 (Rees-George et al., 2010), and the ITS region using primer ITS1/ITS4 (White et al., 1990) were used to detect the presence of *Psa* in field and of *Botrytis cinerea* on fruit, respectively.

The obtained sequences were compared with those already deposited in GenBank using BLAST program (Zhang et al., 2000).

Statistical analysis was carried out using T-student test at 95%, 99%, and 99.9% confidence.

RESULTS AND DISCUSSION

Incidence of *Psa* and post-harvest rot

Presence of *Psa* in orchard was higher in 2013 compared to 2014. Climate conditions affect the presence of *Psa* in field, as reported by Serizawa and Ichikawa (1993). In 2013 lower minimal temperature in winter, higher relative humidity, and higher level of precipitation in spring favored the spread of the bacteria with 86.5% infected plants. The cropping season of 2014 was characterized by warmer temperature, both in winter and pre-harvest period, and precipitation concentrated in the harvest period, which are unfavourable for the development of the pathogen, with only 58% of infected plants found (Table 1).

DM and calcium content were different between fruits from healthy and diseased orchards. Higher average of DM, 16.3% diseased orchards in 2013 (P≤0.001) and 15.6% in 2014 (P≤0.05), instead of 13.8% (2013) and 14.7% (2014) for healthy fruits, were found. Percentage of calcium content in 2013 and 2014 was 0.32% and 0.39% for healthy fruits, and 0.24% and 0.34%, for diseased ones. Phytopathological analyses revealed higher incidence of postharvest rots in fruit from diseased

orchards compared to fruit from healthy orchards ($P\leq0.001$) for both years (Table 1). Higher incidence was found in the second year with 45% of rotten fruit compared to 23% in the first year, probably, as a result of precipitation concentrated at harvesting (Lysiak, 2013) and lower calcium content, that compromises cell wall integrity and increases susceptibility to postharvest pathogens (Quiles et al., 2004).

Fruit quality in experimental condition

Differences in TSS and TA were found between fruit from diseased and healthy orchards. In 2013 (Fig.1b and Fig.1c) TSS values resulted significantly different ($P\leq0.001$) at the end of storage with value of 11.9% for healthy and 13.8% for *Psa* infected orchards, and in shelf-life with 11.6% and 14.2%, respectively. Values of TA ($P\leq0.001$) were higher after 120 days in healthy fruit (0.84%) compared to diseased ones (0.48%). No differences were found for firmness (Fig.1a). In 2014, fruit from healthy orchards resulted firmer than fruit from diseased ones at the end of storage ($P\leq0.01$), with value of 6.94 N and 5.36 N, and in shelf life ($P\leq0.05$), with values of 2.87 N and 1.76 N, respectively. TSS were higher for diseased orchards, in 2013, with values of 12.2% for healthy and 13.5% for diseased at the end of storage, and 13.4% and 14.2% in shelf life. The TA values were statistically different ($P\leq0.001$), during the harvesting period with values of 2.68% for healthy and 1.46% for diseased orchards. As reported by several authors the presence of a plant pathogen involves changes in plants and in chemical and quality parameters, linked to changes in respiration, transpiration, organic acid consumption, and rate of enzymatic activity (Choi et al., 2013; Shahkoomahally and Ramezanian, 2015).

Fruit quality in semi-commercial condition

For the trial in NA similar results, compared to those obtained in trial in 2013 in experimental conditions, were found. No differences in firmness, with values of 22.5 N for healthy and 18.9 N for diseased fruits at 90 days, and 12.5 N for healthy and 12.1 N for fruits from diseased orchards in shelf-life, were found. TSS was higher in diseased orchards ($P\leq 0.001$) both at the end of storage and in shelf life, with values of 11.5% and 13.5%, and 12.0% and 14.4% for healthy and diseased fruits, respectively.

For trial in CA, firmness resulted significantly different in shelf life ($P\leq0.05$), with 5.49 N for healthy fruits and 4.61 N for diseased ones. Significant TSS differences ($P\leq0.001$) were observed both at the end of storage with values of 11.6% and 12.4% for healthy and diseased fruits respectively, and after shelf life with values of 12.8% for healthy and 13.7% for diseased fruits.

For trial in CA with 1-MCP treatment, firmness values were higher compared to the other conditions, both for fruit from healthy and diseased orchards. Significant differences ($P\leq0.05$) were found at the end of storage with values of 69.1 N for healthy compared to 65.2 N for diseased fruits, and in shelf life with values of 18.9 N and 16.7 N for healthy and diseased fruits respectively. TSS ($P\leq0.001$) was 11.7% and 12.6% for healthy fruits and 12.4% and 13.8% for diseased ones, at the end of storage and in shelf life respectively.
As reported by Watkins (2006), CA and 1-MCP are helpful to preserve higher values of firmness.

CONCLUSIONS
Changes in quality parameters were reported during kiwifruit storage. Fruit from diseased orchards showed premature ripening, with lower firmness and TA, and higher percentage of TSS, even if they did not show symptoms of the disease. Furthermore fruits from diseased orchards showed higher susceptibility to postharvest rots, and higher dry matter and lower calcium content. Crucial factors for differences found are ascribed to the presence of *Psa* in orchards and to combined meteorological variables.
The choice of storage methods is important as well as the use of 1-MCP treatment in order to

maintain higher value of firmness and great value of TSS and TA. Moreover, an important role is given to climate conditions. In relationship with temperature, relative humidity and precipitation, quality and chemical parameters showed significant differences within fruits from healthy and diseased orchards. Weather conditions influence fruits from development to harvest. Future activities will evaluate the rate of transpiration and production in ethylene for fruits from affected orchards in cool and controlled atmosphere, exploring methods to reduce pathological breakdown, and maintain quality for longer storage time.

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Tables

Table 1 - Geographical location, incidence of plants infected by bacterial canker (%) and incidence of Botrytis rot after 120 days storage (%) of 12 *Actinidia deliciosa* "Hayward" orchards considered in this study (2013 and 2014).

Orchard	Geographical location	2013		2014	
		Infected plants (%)	Botrytis rot (%)	Infected plants (%)	Botrytis rot (%)
Healthy 1	Campiglione Fenile (TO)	0	0	0	17
Healthy 2	Campiglione Fenile (TO)	4	3	2	12
Healthy 3	Campiglione Fenile (TO)	1	3	0	17
Healthy 4	Campiglione Fenile (TO)	2	1	1	10
Healthy 5	Campiglione Fenile (TO)	0	4	0	19
Healthy 6	Envie (CN)	1	2	0	23
	Mean	**1.3**	**2**	**0.5**	**16**
Diseased 1	Bagnolo Piemonte (CN)	66	29	26	48
Diseased 2	Envie (CN)	86	26	49	34
Diseased 3	Envie (CN)	90	15	58	56
Diseased 4	Revello (CN)	94	15	63	53
Diseased 5	Revello (CN)	95	28	65	38
Diseased 6	Envie (CN)	88	22	55	38
	Mean	**86**	**23**	**58**	**45**
	P value	***	***	***	***

*** Differences between given mean values with T-student test are significant for *P* value ≤ 0.001.

Figures

Figure 1 - Firmness (1a), total soluble sugar (1b), titratable acidity (1c) of kiwifruit 'Hayward' harvested from 12 *Actinidia deliciosa* "Hayward" orchards considered in this study, at harvest, end of storage, and shelf life. The trial was performed under experimental conditions during 2013. Each value is the mean of n=12 replicates. * Differences between given mean values with T-student test are significant for *P* value ≤ 0.001.

1a

1b

1c