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Research Article

Kiwifruit under plastic covering: impact on fruit quality and on orchard microclimate

V. Chiabrando*, G. Giacalone

DISAFA – Department of Agriculture and Food Science, Largo Braccini 2 – 10095 Grugliasco (TO), Italy

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*Corresponding author. E-mail: valentina.chiabrando@unito.it

Abstract

Italy has a preeminent rank in kiwifruit industry, being the first exporter and the second largest producer after China. However, in the last few years kiwifruit yields and the total cultivated area considerably decreased, due to the spread of the bacterial canker caused by *Pseudomonas syringae* pv. *actinidiae* (Psa). Bacterial canker affected significantly the orchards productivity and consequently has caused severe economic losses in all major areas of kiwifruit cultivation, including Italy and considerable damage to the international kiwifruit industry.Several climatic conditions and cultural practices affect the development of the bacterial canker. Orchard hygiene, restrictions on the movement of nursery plants, orchard management and cultural practices, chemical and biological controls and breeding programs are being employed to limit the diffusion of the disease and help kiwifruit orchards to survive and produce. A possible way seems to be the modification of environmental conditions and it is being explored through the building of plastic covers over the orchards. This research work was focused on the impact of plastic cover on fruit quality, microclimate conditions and incidence of Psa. To evaluate the impact of plastic cover on microclimate, quality of fruits and disease incidence, the following parameters were evaluated: climatic parameters (temperature, PAR), quality parameters (color, fruit firmness, titratable acidity, °Brix, vitamin C) and disease incidence (%). The use of a permanent tunnel modifies light intensity and microclimate without any negative consequences on kiwifruits quality. Covering a kiwifruit orchard with a protective canopy reduces the spread of Psa throughout the orchard when covers are constructed over vines with low levels of infection. The use of plastic covers ensure the production of the current and future kiwifruit without compromising the quality of the fruit, but future trials will provide further information clarifying the effectiveness of the actual crop covers.

Keywords: *Actinidia chinensis; Actinidia deliciosa; Pseudomonas syringae* pv. *Actinidiae;* quality

Introduction

Kiwifruit, native to China, is a very economically important fruit crop in Italy. The first case of bacterial canker of kiwifruit (*Pseudomonas syringae* pv. *actinidiae*; Psa) in Italy was recorded in 1992 in Lazio (Scortichini, 1994) and Psa is now present throughout all the kiwifruit cultivation area with significant yield losses and the removal of hundreds of hectares (Balestra *et al.*, 2009). In the spring of 2008, the yield losses caused by Psa infection was estimated to a cost of 2 million Euros (Balestra *et al.*, 2009). Psa started to infects severely *Actinidia chinensis* on orchards cultivated in Central Italy and since 2009 new outbreaks have affected orchards of both A. *deliciosa* (cv Hayward) and A. *chinensis* in the main kiwifruit producing areas of Italy (Armentano 2010). To date, the bacterial disease is limiting the possibility of cultivating kiwifruit, in particular in the areas of northern Italy, where the climate pattern, creates favourable conditions for the pathogen spread (Monchiero *et al.*, 2015).

Psa is, at the moment the major source of losses in kiwifruit production because it is responsible for both economic losses in fruit production and plant mortality in the orchards (Monchiero *et al.*, 2015). For these reasons, researchers and kiwifruit industry have done considerable works and progresses in understanding Psa. Extensive research energies have focused on cultivar selection, agrichemical treatments and changes in management practices Balestra *et al.*, 2009; Armentano, 2010; Vanneste *et al.*, 2010; Vanneste *et al.*, 2011; Scortichini *et al.*, 2012; Monchiero *et al.*, 2015).

The eradication of the infection seems utopian so it is necessary to study how to live with the pathogen, to preventing or minimizing its spread and helping kiwifruit orchards to survive and produce. Primarily, more rigorous orchard hygiene and restrictions on the movement of nursery plants may help to limit the circulation of Psa. Additionally, orchard management has been different and breeding programs must consider selection for disease resistance as a priority. Consequently, it is important to develop innovative policies against Psa.

Another possible way seems to be the modification of environmental conditions and it is being explored through the construction of plastic covers over orchards. Moisture is essential for Psa multiplication and infection because is a bacteria and all bacteria need moisture to be able to travel on the plant surface and into the leaves. The plastic cover reduces leaf moisture and obstruct rainfall from spreading bacteria within the canopy. Further benefit is the protection from wind and frost. The concept of kiwifruit under coverage is to create a barrier between the plants and the adverse weather conditions in order to create an environment hostile to the growth of the bacteria. In Italy, this may seem like a radical change to the normal growing practices, despite it is already being used in Korea, Japan and New Zealand where much of the recent research in growing kiwifruit under cover is taking place (Welham 2013; Hampshire 2016).

This is a new technology, so researches will be required to help growers to understand how to grow kiwifruit in this new environment. The objectives of this work are to evaluate how the plastic roof system changes the microclimate of the orchard and consequently the quality of kiwifruit productions and how the plastic covering affects the Psa proliferation.

Material and methods

Orchards

The study was conducted for two consecutive years in two commercial orchards of *Actinidia chinensis* (Jingold™) located in north western Italy (Piedmont region).Kiwifruit vines were trained on T-bars and spaced 4 m between vines and 4.5 m between rows. In both the orchards, a drip line irrigation system was installed to 1 m height. All orchards were irrigated once in a day (63 L/day/vine) during spring and summer months. From April to July, soluble fertilizers were included in the irrigation water. All field practices were conducted as normal for commercial kiwifruit orchards in the area. Reference trees without cover were used as control. The areas with uncovered and covered trees were adjacent to each other.

Plastic Cover

The cover is a permanent type of structure with metal frames which support an overhead plastic film cover constructed over existing kiwifruit orchards structures. It is placed about 4meters above the growing canopy, provides a very good growing environment and can be managed to provide suitable ventilation and protection from rain. The plastic utilized was a high-transmission Ethylene-Vinil-Acetate (EVA) plastic (Gealite, G.E.A. Plast s.r.l., Massafra (TA), Italy) with a light (photosynthetically active radiation, PAR) transmission>75% and a IR thermal retention > 80 %.The initial cost of the protective canopy is around € 40.000 per hectare which includes the materials and construction.

Fruit samples

Kiwifruit samples were harvested at commercial maturity, following the guidelines provided by the Italian Kiwigold® consortium, on the same day from uncovered and covered trees. Fruits were selected for uniformity of size, maturity and without physical injuries or apparent infection.

Quality evaluations

Flesh color measurements were determined on each sample fruit (15) using a Minolta Chroma Meter CR-400 (Konica Minolta, Japan). The instrument was with the illumining D65 and an observation angle of 2° and calibrated with a standard white plate. The results were expressed in CIELAB system (L*,a*,b*) color space, proposed by the Commission Internationale de L'Eclaraige. The L* values describes the lightness, a* and b* values express respectively the red-greenness and blue-yellowness. In this system, a color representation is given by four parameters: luminosity, hue angle, chromaticity and color index. The hue or color angle (h°) shows the position of the color in a diagram where an angle of 0° corresponds to pure red, 90° to pure yellow, 180° to pure green, and 270° to pure blue. The h° is calculated with the equation $h° = \text{arctangent}\ [(b^*)(a^*)^{-1}]$, where a and b were the values acquired by the colorimeter reading. The chroma (C) indicates the color intensity and it is defined by the distance at the center of the three-dimensional diagram. It is calculated with the equation $C = [(a^*)^2 + (b^*)^2]^{1/2}$. The color index (C.I.) permits a direct correlation with the evaluation of visual appearance of the fruits color, which is calculated as C. I.= a/b (Francis 1980).

Flesh firmness was measured on two opposite sides of the fruit at 90° to each other at the equator using a hand-held Effegi penetrometer (model FT 327, Effegi, Alfonsine, Italy) (8 mm head) after peeling (1 cm^2). Firmness values were averaged for individual fruit and expressed in N.

Total soluble solid content was measured in the undiluted filtered juice extracted from each fruit using a handheld refractometer (ATAGO-1; Atago Co. Ltd., Tokyo, Japan), calibrated using distilled water. The results were averaged for individual fruit and expressed as °Brix. Titratable acidity and pH were determined by adding 50 mL of distilled water into 10 ml of filtered juice and titrated with 0.1N NaOH to pH 8.2 with an automatic titrator (Compact 44–00, Crison Instruments SA, Barcelona, Spain). Titration data were expressed as meq/l. To evaluate the dry matter content, 2 mm thick fruit slice with the skin were cut and dried in an oven at 105°C to constant weight (approximately 72 h). The determination of dry matter content was calculated as percentage weight difference (AOAC 1996).

Extraction and evaluation of Vitamin C

Ten g of fresh kiwifruit was homogenized with an Ultra-Turrax T25 (IKA-Werke, Germany) for 2 min with 10 ml of MeOH/H_2O (5:95 v/v), citric acid (0.1M), acidethylenediaminetetraacetic acid (0.5 g/l) and sodium fluoride (4 mM NaF). The homogenate was filtered and the pH was adjusted to 2.2–2.4 by adding HCl (4 N). Acidified extract was centrifuged for 5 min at 4 °C and the supernatant was filtered through a C18 Sep-Pak cartridge (Waters, Milford, Mass., USA) and a 0.45 µM polytetrafluoroethylene filter (Titan filter 17 mm membrane, SUN-SR). Then, 250 µL of freshly prepared o-phenylenediamine dehydrochloride solution (OPDA, 18.8 mM/L) was add to 750 µL of extract. After 37 min in the dark, the sample was analyzed with HPLC. Ascorbic acid (AA) and dehydro ascorbic acid (DHAA) content was investigated (Gonzalez-Molina et al., 2008). HPLC analysis of vitamin C (AA+DHAA) was carried out using an Agilent HPLC 1200 Series (Agilent, Waldbronn, Germany) system consisting of a manual injection valve, a G1311A quaternary pump, a 20-µL sample loop, a diode array detector GI315D UV-vis and controlled by Agilent ChemStation software B.03.02. Separations of DHAA and AA were realized with a column Eclipse XDB-C18 (150 x 4,6 mm; 5 µm particle size; Sigma Italiana SRL, Ozzano Emilia, Italy). The mobile phase was MeOH/H_2O (5:95, v/v), 5 mM cetrimide, 50 mM potassium dihydrogen phosphate (pH 4.5). The total run time was 10 min with a flow-rate of 0.9 ml/min and the detector wavelengths were 348 nm for DFQ detection and 261 nm for AA detection.The vitamin C content considered AA and DHAA contents and was expressed as mg/100 g of fresh kiwifruit weight. Reported results values are the mean ± SE of three replicates at day 0 and at the end of storage period (8 days) for each treatment. All standards and reagents were of analytical purity "pro-analysis" and were purchased from SIGMA (Sigma Italiana SRL, Ozzano Emilia, Italy).

Di-seaseincidence

To determine the effect of covers on the progression of the disease, symptoms were observed throughout the growing season in four areas of the orchard with six sample plants in each orchard to calculate the incidence (%) of Psa-infected plants following visual inspection. Plants were considered symptomatic when spots with a chlorotic halo were observed on at least two leaves. After each monitoring round, canes with dieback were marked and removed. Therefore, the count of canes reflected the amount of material that was removed as a result of Psa infection.

Leaf Relative Chlorophyll Content (SPAD evaluation)

The relative levels of total chlorophyll were estimated with a portable chlorophyll meter (SPAD-502 Minolta, Osaka, Japan) on30 sample leaves per treatment. Three SPAD measurements(37.8 mm^2 total measurement area) were averaged per leaf to represent one observation. The Minolta SPAD meter (Minolta Camera Co., Ltd., Japan) is a hand-held spectrophoto meter used to measure the relative greenness of plants in a rapid and nondestructive manner. SPAD readings have been found to correlate positively with leaf N and leaf chlorophyll concentration (Schepers *et al.*, 1992).

The principle of measurement of the SPAD is based on the difference in light attenuation at 650 and 940 nm. In this work, the instrument was used to estimate chlorophyll content in kiwifruit leaves under plastic cover and control leaves. Results were calculated as index in SPAD units ranging from 0 to 80(means of 30 leaves ±SE) (Azia and Stewart 2001; Richardson et al., 2002).

Climatic Parameters

Temperature

Portable data-logger (Hobo® system, Onset Computer Corporation, USA) was used in each orchad to collect air temperature data during the study period. The data logger was located in the kiwifruit canopy 2 m above the ground both under the cover and on control (without cover). The data loggers were programmed to collect data at 6 hours intervals and the average, the maximum and the minimum temperature were recorded.

Photosynthetic active radiation

The photosynthetically active radiation (PAR) measurements for the orchards were monitored monthly during the vegetative stage under the plastic cover and in control orchard without the cover. Measurements of PAR were collected through a portable light-bar LI-COR Model LI-1800 (Ha-Li, EIC, Madrid, Spain) with ten PAR sensors approximately 1.5 m above ground level. The radiation was determined between 12 am and 1:00 pm (local time) on sunny days when clouds were not there. Photosynthetic active radiation (PAR, µmol $m^{-2}s^{-1}$) data presented are the average of the 10 sensors.

Statistical analysis

Where possible, results were expressed as mean ± SE. Data were subjected to analysis of variance (ANOVA) and the means were compared by Tukey's HSP test (honest significant differences). Differences between mean values were considered significant when $p \leq 0.05$. STATISTICA software was used for all data analyses (version 6.0, StatSoft Inc., Tulsa, USA).

Results

Temperature

Daily temperatures showed that the use of covers exerted a limited influence (Fig. 1). In particular, mean temperatures tended to be higher under cover compared with the uncovered control throughout the evaluation period (April 2015-April 2016) with variations of about + 1.38°C between the cover and the control condition in the Orchard 1 (Fig. 1) and of about + 0.58°C between the cover and the control condition in the Orchard 2 (Fig. 2).

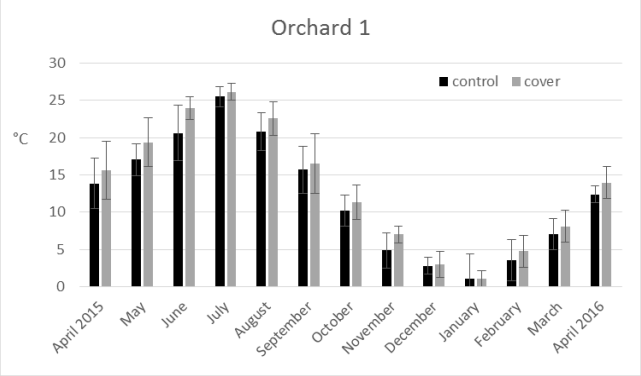

Fig. 1. Mean temperature (°C) under plastic cover and control recorded from April 2015 to April 2016 in Orchard 1. Data are reported as the mean of 30 days of daily cumulates (mean ± SE).

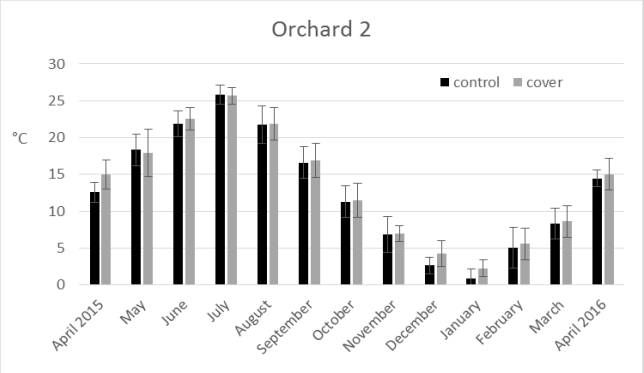

Fig. 2. Medium temperature (°C) under plastic cover and control recorded from April 2015 to April 2016 in Orchard 2. Data are reported as the mean of 10 days of daily cumulates (mean ± SE).

Considering the temperatures detected at different times of the day, results showed different trends: at 8 a.m. in the months from May to July of evaluated period (2015-2016) the temperature under plastic covers was lower (average of 3.8 ° C), while, in the same months, at 8 p.m. became more than 2°C higher.These results indicated that the plastic covers determined, in the first warm months, a delay in the daily heat. However, the daily heating did not produce a warming during the hottest hours of the day. In the winter months, the covers determined a little increase in temperature all the day, in particular when the incidence of sunlight is most vertical. Higher temperatures under the plastic cover caused a slight advanced ripening, in accordance with the findings analyzed in the qualitative parameters.

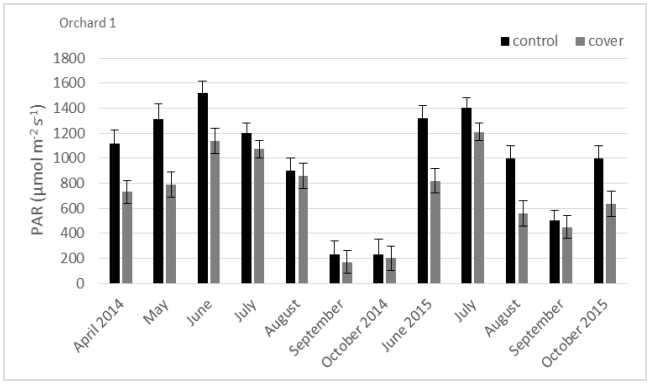

Fig. 3. Photosynthetic active radiation (µmol m^{-2} s^{-1}) under plastic cover and open air from April 2014 to October 2015 in Orchard 1. Data are reported as the mean of 3 measures (mean ± SE).

Photosynthetic active radiation

Results indicated a 25 % reduction in PAR under the plastic covers compared to PAR of control orchard (Fig. 3). On a typical sunny day in June in Orchard 1, the photo-synthetically active radiation interception values under the plastic cover were, an average of 25 % for 2014 and 37 % for 2015, compared with light conditions in the adjacent field (control).On the same days, in Orchard 2, the PAR at the plant level was intercepted as 57% in 2014 and an average of 44 %in June 2015 by the plastic cover (data not shown). Lower PAR reductions were found in the days at the beginning of autumn in both considered orchards, according with the results of Iglesias and Alegre (Iglesias and Alegre, 2006) in an orchard covered with anti-hail nets. The reduction in PAR and light quantity under plastic covers observed in the current study could, therefore, be attributed to the light blocking properties of the materials used. Although the use of covers lowered PAR reaching the crop, the quantities received by the crop still remained within acceptable range and did not have a major impact on the plants.

Leaf Relative Chlorophyll Content (SPAD evaluation)

Leaves under plastic covers showed higher chlorophyll content than control (Fig. 4) and consequently a better efficiency for photosynthesis (Taiz and Zeiger, 2006).

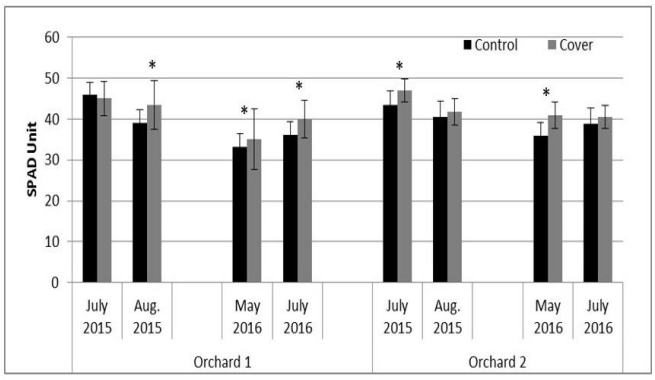

Fig. 4. Leaf Relative Chlorophyll Content (SPAD units) on leaves under plastic cover and control leaves Orchard 1 and 2. Data are reported as the mean of 30 measures (mean ± SE).* Statistically significant differences at the 5% level between treatments

The higher values of chlorophyll content seem to be a common feature of trees covered by netting or plastic covers because it is a clear indication of the response of leaves to the light environment. Similar acclimation responses to shading were reported by Mierowska *et al.* (2002) and Dai *et al.* (2009). Lobos *et al.* (2012) showing that leaf relative chlorophyll content increases linearly as the % of intercepted PAR decreases.

Fruit quality

In the first season of testing (2014), the analyses of fruits quality showed that the fruits under the plastic covers had a more advanced maturity stage compared to control, in term of a significantly higher values of dry matter (+2.33% in orchard 1 and +2.93% in orchard 2),sugar content (+1.63° Brix in orchard 1and +2.65°Brix in orchard 2)and hue angle (-3.41°h in orchard 1 and -3.6°h in orchard 2) (Table 1).The higher TSSC confirmed the advanced ripening in kiwi fruits under covers because it has been shown that kiwi fruits contains sucrose phosphate synthase (SPS), a keyenzyme in sucrose biosynthesis, whose activity increased during ripening (MacRae *et al.*, 1992).Moreover, TSSC of kiwifruit is considered as an index of fruit maturity because it corresponds to a conversion of starch to soluble sugars (Macrae *et al.*, 1989).

The firmness of fruits did not differ significantly between treatments and the trend is contradictory between the two considered orchards (Table 1). Titratable acidity values showed nearly the same values in both treatments and orchards. In the summer of 2014 vitamin C content was also evaluated in both treatments and orchards. Results showed significantly higher values in covered kiwi fruits (141.45 mg/100g and 106.33 mg/100g in orchard 1 and 2 respectively) compared to control (124.86 mg/100g and 79.17 mg/100g in orchard 1 and 2 respectively). The accumulation of AA during ripening depends on type of fruit; Lee and Kader (2000) reported that AA content increased with ripening in apricot, peach and papaya, but decreased in apple and mango. Higher level of vitamin C confirmed the advanced ripening in kiwifruits under the covers and it was probably also correlated with higher level of leaf relative chlorophyll content under the covers (Hayat *et al.*, 2010).

In the 2015 kiwifruit samples, significant differences between treatments ($P \leq 0.05$) were observed in dry matter content and titratable acidity (13 % higher in control fruits)in orchard 1 and in the flesh firmness of the fruits harvested in the second orchard (Table 1).In orchard 1, control kiwifruits showed also higher values of firmness and lower values of colorimetric parameters (h° and C) compared to the covered fruits. A different trend was observed in orchard 2, where the control fruits showed higher values of colorimetric parameters and lower values of dry matter, TSSC and titratable acidity. Considering the lower values of flesh firmness and the higher values of TSSC, also in the summer 2015, the samples fruit under plastic cover had a more advanced maturity stage compared to the control. Considering the results of Campbell and Marini [1992], the lower flesh firmness of sample fruits under covered environments might also be the result of a reduced cell wall development and the high water influx to the cortex cells of the fruit. This indicates that shading the kiwifruit plants with different covers conditions might reduce structural (cell wall

Table 1. Fruit quality under plastic cover and control in Orchard 1 and 2 in 2014 and 2015. Data are reported as the mean of 30 measures for dry matter, flesh firmness, hue angle (mean ± SE), as mean of 3 measures for TSSC, TA and pH (mean ± SE). * in rows denote statistically significant differences between cover and control, at the 5% level.

Parameters	2014				2015			
	Orchard 1		Orchard 2		Orchard 1		Orchard 2	
	control	cover	control	cover	control	cover	control	cover
Dry matter (%)	14.1±1.2*	16.4±1.4	14.7±0.9*	17.6±1.1	20.7±1.9*	18.9±1.1	19.9±2.1	20.3±0.9
Flesh firmness (N)	44.7±2.4	48.9±2.3	52.4±3.1	50.6±2.5	50.1±2.9	48.1±3.1	53.8±1.9*	43.7±2.7
Total Soluble Solid Content (°Brix)	6.2±0.5*	7.8±0.4	7.4±0.8*	10.1±1.1	12.2±0.4	12.5±0.8	11.9±1.1	12.5±1.3
Titratable Acidity (meq/l)	229.8±8.4	220.2±6.6	186.5±7.4	191.4±6.1	239.7±5.6*	212.1±4.9	208.7±7.4	215.2±7.1
pH	3.5±0.02	3.1±0.1	3.2±0.08	3.5±0.07	3.2±0.09	3.4±0.08	3.4±0.08	3.3±0.08
Hue angle (h°)	102.1±2.1*	98.7±3.2	104.7±3.3*	101.1±4.1	101.1±2.5	102.5±2.8	103.8±2.3	101.2±3.1
Chroma	32.5±2.1	31.1±3.1	28.9±1.8	29.9±2.6	27.9±2.9	28.2±2.9	27.9±3.6	27.3±2.9
Color Index	0.21	0.15	0.26	0.19	0.19	0.17	0.21	0.2

and middle lamellae constituents) and storage carbohydrates, leading to a lower flesh firmness and higher TSSC kiwi fruits at commercial maturity stage. Moreover, in the two years of investigation, the fruits under covers were also more uniform in size and shape. This result suggested that under plastic covers, being the temperature more constant without extreme peaks of maximum and minimum temperatures, could affect the uniformity of fruit weight.

Pathological evaluation

During summer 2015, approximately 50% - 80 % of the plants were infected by Psa.In most cases and in both orchards, the disease expressed in vines derived from infections that have taken place before the covers were installed. Canes with dieback were marked and removed by the orchard manager; therefore the count of canes reflects the amount of material that has been removed as a result of Psa infection.

The next year of testing, a reduction of endophytic Psa population of about 25 % was observed in plants under tunnels from both orchards as a result of the removal of Psa-infected canes throughout the trial and because the Psa infection did not progress in healthy plant material placed under covers. Therefore, the permanent cover did not remove the bacteria but slowed down the progression of the disease in already infected vines whilst new vines are established. The preliminary results of this research suggested that when clean plants are placed under covers they remainrelatively disease free and have fewer visible symptoms of Psa infection. Moreover, the warmer temperatures under the cover also limited the bacteria's development and kept the leaves dry, limiting the pathogen as it needs water to multiply and move (Black *et al.*, 2015).

Conclusion

This research work focused on the impact of plastic covers on the kiwifruits quality, microclimate and the incidence of bacterial canker. Early indications are that the plastic covers influenced the physiochemical characteristics of fruits, in general the samples under covers showed lower firmness values and higher TSSC compared to control, with consequent small changes in the maturation period. Covering kiwifruit orchard with a protective canopy reduced the spread of Psa throughout the orchard when covers were constructed over vines, with low levels of infection. It may be possible to slow the progression of the disease in already infected vines whilst new vines are established. In general, the Psa infection did not grow in healthy plant material placed under covers. The canopy keeps the kiwifruit leaves dry from rain, which is thought to help preventing the disease from entering the vine. Other benefits include protection from wind, frost, hail, possible improvements in fruit size and fruit reject rates. In conclusion, the results suggest that the use of plastic covers ensure the production of the current and future kiwifruit without compromising the quality of the fruit, but future trials will provide further information clarifying the effectiveness of the actual crop covers. Pollination, chemical usage, irrigation and pest management will also need to be investigated to evaluate the sustainability of growing under protective plastic covers. Although the covers are a high initial capital cost the profits, particularly from high values kiwifruit production, do justify this new technology and looks like a possible opportunity to protect against the Kiwifruit industries greatest threat.

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