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Efficacy of different chemical and biological products in the control of *Pseudomonas syringae* pv. *actinidiae* on kiwifruit

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Abstract The recent outbreak of bacterial canker on kiwifruit, caused by *Pseudomonas syringae* pv. *actinidiae,* has caused considerable damage to the international kiwifruit industry. Commercial products, and products under development, were evaluated over 2 years to assess their ability to control bacterial canker on kiwifruit under controlled conditions. The results were compared with two trials carried out in a kiwifruit orchard located in northern Italy during 2011 and 2012, to test the preventative efficacy of different copper formulations against *P. syringae* pv. *actinidiae*. In the greenhouse and orchard trials, copper hydroxide and the mixtures of copper hydroxide and copper oxychloride, significantly reduced the foliar symptoms by 70–80 % compared with the control, and showed low phytotoxicity. Similar efficacy was provided by acibenzolar-S-methyl, whose use has been temporarily extended to kiwifruit in Italy, with a maximum of four treatments per year. However, the product showed phytotoxicity on one-year old plants. The efficacy of fosetyl-Al was lower, particularly in the first orchard trials of 2012 and 2013. The efficacy of the other products tested never exceeded 30–40 %, and some products were not significantly different from the control. Kiwifruit plants grown in a steamed peat substrate mixed with compost obtained from digested organic matrices of municipal solid waste showed significantly fewer leaf spots compared with untreated controls. Copper compounds alternated with resistance inducers could be used in combination with compost, in order to develop new integrated control strategies to reduce the disease development and spread.

Keywords *Actinidia deliciosa* · Bacterial canker · Chemical control · Control strategies · Italy

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Introduction

Kiwifruit originated in China, but it was New Zealand that introduced its cultivation, which has been gradually adopted by other countries, becoming of primary importance in global fruit production. Kiwifruit is characterized by a high adaptability of the two species *Actinidia deliciosa* and *A. chinensis* (Ferguson and Huang 2007). The global production was over 1.4 million tons in 2012, and Italy produced around 384,000 t (FAOSTAT 2014). Italy produces more kiwifruit than any other country apart, possibly, from China (Testolin and Ferguson 2009). Piedmont (northwest Italy), where 6,050 ha of kiwifruit are cultivated (Piedmont Region - AGRISTAT 2011), is the largest Italian region responsible for kiwifruit storage and export, and the second largest producer of the fruit (ISTAT 2011).

Pseudomonas syringae pv. *actinidiae* (Psa) was first isolated and described in Japan in 1984 (Takikawa et al. 1989), then in Italy and South Korea (Koh et al. 1994; Scortichini 1994) on cv. Hayward (*A. deliciosa*). For more than 15 years this bacterial disease was considered to be of low importance on kiwifruit produced in Italy. However, since 2008, the first outbreaks of bacterial canker on kiwifruit cultivated in Central Italy, led to the removal of most orchards of *A. chinensis* in the region (Balestra et al. 2009; Ferrante and Scortichini 2009, 2010). During 2009–2011, *P. syringae* pv. *actinidiae* started to severely infect *A. deliciosa* cv. Hayward in the main fruit producing regions of Italy. During spring 2010, the first outbreaks were identified in Piedmont (northern Italy), where the

pathogen was most probably introduced by infected propagation material (Armentano 2010; Spadaro et al. 2010). Despite the strict application of preventative measures to avoid the spread of the disease, in 2011 the pathogen was present throughout the kiwifruit production area in northern Italy, with considerable yield losses and the removal of hundreds of hectares (Spadaro et al. 2011).

In 2010, the pathogen was also officially reported in New Zealand, where severe damage was caused to *A. chinensis* and *A. deliciosa* crops (Everett et al. 2011). The disease has reached pandemic proportions by spreading to France, Spain, Portugal, Switzerland, Chile, Turkey, South Korea and Japan (Vanneste et al. 2011a, b; Renzi et al. 2012; EPPO 2012).

The sudden disease outbreaks were caused by strains of *P. syringae* pv. *actinidiae* not derived from the pre-existing strains, but evolved separately from Chinese strains (Mazzaglia et al. 2012; Butler et al. 2013; McCann et al. 2013; Vanneste 2013). The more recent strains rapidly adapted to new hosts and environments through the gain or loss of mobile genetic elements and virulence factors (Scortichini et al. 2012).

Damages caused by *P. syringae* pv. *actinidiae* are worsening the possibility of cultivating kiwifruit, particularly in areas such as northern Italy, which are closer to the thermal limits for *Actinidia* spp. cultivation (EPPO 2012). The climate pattern of northern Italy, characterized by cold winters with minimum temperatures lower than −15 °C, and fresh and humid spring and autumn seasons, creates favourable conditions for the pathogen spread.

Due to the virulence of the bacterial pathogen, preventative crop protection strategies play a major role and are mainly based on symptomatic plant removal and destruction, due to the high survival capacity of the pathogen in fallen leaves and pruning debris (Tyson et al. 2012), and on asymptomatic leaves as an epiphyte (Vanneste et al. 2011b). The pathogen can be found on the external surfaces of footwear, vehicles, and tools, which could be efficient vectors of the disease (Everett et al. 2012).

When purchasing plants for new orchards, it is important to check the production area of the scions and of the mother plants, to ensure plants are sourced from healthy nurseries. Among the agronomic practices, excessive nitrogen fertilization stimulates the pathogen development, while extensive pruning favours bacterial spread (Spadaro et al. 2011).

Preventative practices are fundamental, due to the prohibition of using antibiotic products and the reduced availability of active ingredients registered for use on kiwifruit. Currently, chemical control of *P. syringae* pv. *actinidiae* is mainly based on copper products, most of them authorized for winter treatment and effective against other bacterial pathogens on kiwifruit, such as *P. syringae* pv. *syringae* and *P. viridiflava* (Fratarcangeli et al. 2010). The first opportunity to use such products are as preventative applications during plant dormancy, such as after harvest and at leaf fall in autumn, and after pruning and at budding in winter, and also after every frost period. Moreover, during the growing season, other copper treatments could be used every time wounds are caused by hail or green pruning, by choosing formulates with a low copper content. However, the above mentioned strategies seem to be insufficient in controlling bacterial canker on kiwifruit, so new products have been evaluated and introduced into the market, such as formulations of copper with humic acids, amino acids or nitrates (Quattrucci et al. 2010), where the metal ions can be partially absorbed by the plant, reducing the runoff of the active ingredient.

Moreover, other interesting products are resistance inducers, such as fosetyl-Al, potassium phosphites, and acibenzolar-S-methyl, registered and used on other crops, and antagonistic microorganisms, such as *Bacillus subtilis* and *B. amyloliquefaciens*. Natural products, such as essential oils, could be used to control different diseases on fruit crops in the orchard (Lopez-Reyes et al. 2010; 2013). Together with commercial products, many other formulations can be found on the market, generally described as leaf fertilizers or plant strengtheners, to avoid the strict European regulations for registering and commercialising new agrochemicals. Another preventative strategy, which has previously been shown to have disease suppression characteristics, is to use soil amended with compost obtained from digested organic matrices of municipal solid waste (Pugliese et al. 2008; 2011).

The aim of the present work was to evaluate the efficacy of commercial products, and products under development, in controlling bacterial canker on kiwifruit under controlled conditions. The results were also compared with two trials carried out at a kiwifruit orchard located in Piedmont (northern Italy) during 2011 and 2012 to test the preventative efficacy of different copper formulations, used alone or in mixture with resistance inducers or mancozeb, against *P. syringae* pv. *actinidiae*.

Materials and methods

Plants and microorganisms

For the controlled conditions experiments, female plants of *Actinidia deliciosa* cv. Hayward were used. The plants were grown in black plastic pots containing 5 l of a sterilized peat mixture substrate (Irish peat : Swedish peat, 1:1 vol/vol) and were determined to be free of Psa symptoms following visual inspection. One leaf was sampled from every plant, DNA extracted and polymerase chain reaction (PCR) tests conducted according to the method described by Rees-George et al. (2010). All samples were negative for Psa.

Plants were spray-inoculated with strains of *Pseudomonas syringae* pv. *actinidiae* by directly spraying cell suspensions (10^8 cells ml^{-1}) on to the leaves of the kiwifruit plant. The strains used were isolated from kiwifruit leaves infected with *P. syringae* pv. *actinidiae,* harvested from orchards located in Piedmont. Inoculations were performed just after sunset, to exploit the overnight temperature reduction. After inoculation, plants were covered for 72 h with a transparent plastic film to maintain a humidity saturated environment.

Greenhouse trials in 2012

To test the efficacy of different products, trials were performed in two subsequent years, 2012 and 2013. In each trial, 25 treatments were compared, with each treatment being tested on 10 plants (Tables 1 and 2).

A set of 500 potted plants of *Actinidia deliciosa* cv. Hayward 30–40 cm high were divided into two 250 plant groups. In January 2012, the first group of plants was moved to the greenhouse where the temperature was kept at 20 °C.

The trial included two treatments with resistance inducers and one treatment of all other products, such as protectants, disinfectants or bactericides prior to spray-inoculation with Psa. Plants were treated with resistance inducers on March 13, 2012, and then with all the products 7 days later. The products were applied by spraying a 25 ml suspension per plant. On the following day, all plants were spray-inoculated with a suspension of bacterial cells (10^8 cells ml^{-1}); each plant was sprayed with a 30 ml suspension. Seven days after inoculation, when the first symptoms occurred, plants were treated again. After the second application of all treatments, pots were moved outside under a shadow net and treatments were performed by following the calendar indicated in Table 1. In the case of compost treatments, kiwifruit plants were planted in a mixture of steamed peat mixture substrate (Irish peat:Swedish peat, 1:1 vol/vol) (80 %) and compost (20 %), but no other treatments

Table 1 Treatments and dates of glasshouse trials (2012) to evaluate the efficacy of strategies to control *P. syringae* pv. *actinidiae* on kiwifruit

Active ingredients	Dosage a.i. (ml/g/hl)	Dosage c.p. (ml/g/hl)	Dates of application Trial 1*	Dates of application Trial 2**
Untreated	–	–	–	–
Copper oxychloride	60	300	3, 4, 5, 6, 7, 8, 9, 10	2, 3, 4, 5, 6, 7, 8, 9
Copper hydroxide	30	200	3, 4, 5, 6, 7, 8, 9, 10	2, 3, 4, 5, 6, 7, 8, 9
Copper oxychloride + Copper hydroxide	30.8	110	3, 4, 5, 6, 7, 8, 9, 10	2, 3, 4, 5, 6, 7, 8, 9
Copper oxychloride + C. hydroxide (IRF 120)	30	100	3, 4, 5, 6, 7, 8, 9, 10	2, 3, 4, 5, 6, 7, 8, 9
Copper oxychloride + C. hydroxide (IRF 155)	30	300	3, 4, 5, 6, 7, 8, 9, 10	2, 3, 4, 5, 6, 7, 8, 9
Acibenzolar-S-methyl	10	20	2, 3, 4, 5, 6, 7, 8, 9, 10	1, 2, 3, 4, 5, 6, 7, 8, 9
Acibenzolar-S-methyl	20	40	2, 3, 4, 5, 6, 7, 8, 9, 10	1, 2, 3, 4, 5, 6, 7, 8, 9
Copper oxychloride +MnO_2 + ZnO (Kendal TE)	103.5+2.25+2.25	300	2, 3, 4, 5, 6, 7, 8, 9, 10	1, 2, 3, 4, 5, 6, 7, 8, 9
Fosetyl-Al	16	200	2, 3, 4, 5, 6, 7, 8, 9, 10	1, 2, 3, 4, 5, 6, 7, 8, 9
Oil of *Satureja*	100	100	2, 3, 4, 5, 6, 7, 8, 9, 10	1, 2, 3, 4, 5, 6, 7, 8, 9
Oil of thyme	100	100	2, 3, 4, 5, 6, 7, 8, 9, 10	1, 2, 3, 4, 5, 6, 7, 8, 9
Propolis	200	200	2, 3, 4, 5, 6, 7, 8, 9, 10	1, 2, 3, 4, 5, 6, 7, 8, 9
Sodium silicate	10	10	2, 3, 4, 5, 6, 7, 8, 9, 10	1, 2, 3, 4, 5, 6, 7, 8, 9
ZnSO4 + CH3CO3H + H2O2 (BIOBACTER PLUS)	7.5+42.5+90	250	3, 4, 5, 6, 7, 8, 9, 10	2, 3, 4, 5, 6, 7, 8, 9
Bacillus subtilis	62.68	400	2, 3, 4, 5, 6, 7, 8, 9, 10	1, 2, 3, 4, 5, 6, 7, 8, 9
Bacillus amyloliquefaciens	37.5	150	2, 3, 4, 5, 6, 7, 8, 9, 10	1, 2, 3, 4, 5, 6, 7, 8, 9
Gluco humates - NP fertilizer (leaf treatment)	24+108	600	2, 3, 4, 5, 6, 7, 8, 9, 10	1, 2, 3, 4, 5, 6, 7, 8, 9
Gluco humates - NP fertilizer (root treatment)	24+108	600	2, 5, 7, 8, 10	1, 4, 7, 9
Gluco humates - NP fertilizer (leaf treatment)	20+90	500+	2, 3, 4, 5, 6, 7, 8, 9, 10	1, 2, 3, 4, 5, 6, 7, 8, 9
Gluco humates - NP fertilizer (root treatment)	20+90	500	2, 5, 7, 8, 10	1, 4, 7, 9
Potassium silicate	10	10	2, 3, 4, 5, 6, 7, 8, 9, 10	1, 2, 3, 4, 5, 6, 7, 8, 9
Copper chelate	12	40	3, 4, 5, 6, 7, 8, 9, 10	2, 3, 4, 5, 6, 7, 8, 9
Synthetic zeolites	950	1,000	3, 4, 5, 6, 7, 8, 9, 10	2, 3, 4, 5, 6, 7, 8, 9
Compost 1 (Maturation 6 months)	20 %		1	1
Compost 2 (Maturation 4 months)	20 %		1	1

*Trial 1: 1: 12/03; 2: 13/3; 3: 20/3; 4: 28/3; 5: 12/4; 6: 26/4; 7: 9/5; 8: 22/5; 9: 5/6; 10: 19/6

**Trial 2: 1: 04/04; 2: 11/04; 3: 18/04; 4: 23/04; 5: 30/04; 6: 08/05; 7: 22/05; 8: 05/06; 9: 19/06

Table 2 Treatments and dates of glasshouse trials (2013) to evaluate the efficacy of strategies to control *P. syringae* pv. *actinidiae* on kiwifruit

Active ingredients	Dosage a.i. (ml/g/hl)	Dosage c.p. (ml/g/hl)	Dates of application Trial 1*	Dates of application Trial 2**
Untreated	–	–	–	–
Copper oxychloride	60	300	3, 4, 5, 6, 7, 8, 9, 10	2, 3, 4, 5, 6, 7, 8, 9, 10
Copper hydroxide	30	200	3, 4, 5, 6, 7, 8, 9, 10	2, 3, 4, 5, 6, 7, 8, 9, 10
Copper oxychloride + Copper hydroxide	30.8	110	3, 4, 5, 6, 7, 8, 9, 10	2, 3, 4, 5, 6, 7, 8, 9, 10
C. oxychloride + C. hydroxide (IRF 155)	45	450	3, 4, 5, 6, 7, 8, 9, 10	2, 3, 4, 5, 6, 7, 8, 9, 10
C. oxychloride + C. hydroxide (IRF 155)	30	300	3, 4, 5, 6, 7, 8, 9, 10	2, 3, 4, 5, 6, 7, 8, 9, 10
Acibenzolar-S-methyl	10	20	2, 3, 4, 5, 6, 7, 8, 9, 10	1, 2, 3, 4, 5, 6, 7, 8, 9, 10
Acibenzolar-S-methyl	5	10	2, 3, 4, 5, 6, 7, 8, 9, 10	1, 2, 3, 4, 5, 6, 7, 8, 9, 10
(Organic + ureic N) + K oxide (KENDAL)	10.5+46.5	300	2, 3, 4, 5, 6, 7, 8, 9, 10	1, 2, 3, 4, 5, 6, 7, 8, 9, 10
Fosetyl-Al	16	200	2, 3, 4, 5, 6, 7, 8, 9, 10	1, 2, 3, 4, 5, 6, 7, 8, 9, 10
Oil of *Satureja*	100	100	2, 3, 4, 5, 6, 7, 8, 9, 10	1, 2, 3, 4, 5, 6, 7, 8, 9, 10
Oil of thyme	100	100	2, 3, 4, 5, 6, 7, 8, 9, 10	1, 2, 3, 4, 5, 6, 7, 8, 9, 10
Propolis	200	200	2, 3, 4, 5, 6, 7, 8, 9, 10	1, 2, 3, 4, 5, 6, 7, 8, 9, 10
Potassium silicate (leaf treatment)	10	10	2, 3, 4, 5, 6, 7, 8, 9, 10	1, 2, 3, 4, 5, 6, 7, 8, 9, 10
Ureic N + P_2O_5 (Bio PROTEK)	25+25	250	3, 4, 5, 6, 7, 8, 9, 10	2, 3, 4, 5, 6, 7, 8, 9, 10
Bacillus subtilis	62.68	400	2, 3, 4, 5, 6, 7, 8, 9, 10	1, 2, 3, 4, 5, 6, 7, 8, 9, 10
Bacillus amyloliquefaciens	37,5	150	2, 3, 4, 5, 6, 7, 8, 9, 10	1, 2, 3, 4, 5, 6, 7, 8, 9, 10
Gluco humates - NP fertilizer (leaf)	24+108	600	2, 3, 4, 5, 6, 7, 8, 9, 10	1, 2, 3, 4, 5, 6, 7, 8, 9, 10
Gluco humates - NP fertilizer (leaf)	(20+90)	500 +	2, 3, 4, 5, 6, 7, 8, 9, 10	1, 2, 3, 4, 5, 6, 7, 8, 9, 10
Gluco humates - NP fertilizer (root)	(20+90)	500	2, 4, 6, 8, 10	1, 3, 5, 8, 10
Potassium silicate (root)	10	10	2, 3, 4, 5, 6, 7, 8, 9, 10	1, 2, 3, 4, 5, 6, 7, 8, 9, 10
Potassium phosphite	12	40	3, 4, 5, 6, 7, 8, 9, 10	2, 3, 4, 5, 6, 7, 8, 9, 10
Synthetic zeolites + Copper oxychloride	950+60	1,000+300	3, 4, 5, 6, 7, 8, 9, 10	2, 3, 4, 5, 6, 7, 8, 9, 10
Compost 1 (Maturation 6 months)	20 %		1	1
Compost 2 (Maturation 4 months)	20 %		1	1

*Trial 1–1: 04/03; 2: 05/03; 3: 12/03; 4: 19/03; 5: 26/03; 6: 09/04; 7: 23/04; 8: 09/05; 9; 22/05; 10: 06/06

**Trial 2: 1: 16/04; 2: 23/04; 3: 03/05; 4: 09/05; 5: 13/05; 6: 22/05; 7: 30/05; 8: 06/06; 9: 18/06; 10: 02/07

were performed, in order to evaluate the potential induction of resistance by compost.

At the end of June, when the plants had reached 2 m in height and started to show symptoms of water and nutritional stress due to the high ambient temperatures of this time of the season in Italy, not favourable to the growth of the bacterium, the trial was interrupted. In July, plants were pruned, transferred into larger pots (7 l), fertilized and left to rest in order to repeat the trial in the following year.

The second group of plants were kept in a growth chamber at 12 °C to slow down development and transferred to a greenhouse on March 29, 2012. The first treatment with resistance inducers was applied on April 4, 2012, while the second treatments with all the products were applied 7 days later.

The first inoculation with Psa was conducted on April 12, 2012, as described previously, but due to the absence of symptoms, a second inoculation was conducted on April 24, just after the treatment on April 23. The other treatments were applied according to the schedule in Table 1. In May, the pots were moved outside, under a shade cloth (50 % shade). At the end of June, the trial was suspended as for the first group of plants. In July, the plants were pruned, repotted in larger pots (7 l), fertilized and left to rest in order to repeat the trial in the following year.

Greenhouse trials in 2013

At the end of January 2013, the first group of pots was placed in the greenhouse at approximately 20 °C. The treatments conducted were similar to the trial in 2012, with the introduction of new products and with new dosage levels for already used products (Table 2). Plants were treated by spraying a 25 ml suspension of product per plant; first with the resistance inducers on March 5, 2013, and then with all the products 7 days later. On the following day, plants were spray-inoculated, as described previously. The plants were inoculated with Psa a second time on March 19, 2013, due to the lack of symptoms. When the first leaf spots occurred, the treatments were applied according to the schedule in Table 2.

On April 20, 2013, plants were moved outside, but the low temperatures registered during the month of May induced symptoms of phytotoxicity, particularly on plants treated with copper products. Such damages and a severe hail on May 17, progressively worsened the plant health, therefore the trial was concluded on June 18.

The second group of plants was placed in a greenhouse in February and the first treatments were performed on April 16, 2013. The second and third treatments were performed on May 3 and 13, while Psa was inoculated on May 4 and 14. After the first occurrence of leaf spots, treatments continued according to the schedule in Table 2. At the end of May, pots were moved outside under a shade cloth (50 % shade). On July 15, 2013, when the plants started to show symptoms of water and nutritional stress, as temperatures increased, the trial was concluded.

Orchard trials

To test the efficacy of different products, a two-year trial was established in a young orchard of *A. deliciosa* cv. Hayward, planted during spring 2011 in Costigliole Saluzzo (Piedmont, Northern Italy). The treatments included the use of single products, alternating two products, and mixtures of products (Table 3). A randomized block design with four replicates, each one of five plants, was used for the trials. Treatments were applied by using a motor knapsack sprayer. Chemicals were applied with 500 to 800 l/ha, depending on the growth phase of the plants.

In 2011, treatments started after spring budding and continued every 14 days up to complete leaf fall (Table 3). The winter of 2012, particularly the month of February, was characterized by a prolonged cold period, with temperatures below −20 °C for long periods. For this reason, plants were severely damaged and it was necessary to cut them back to the crown to allow growth from a basal bud. This caused a budding delay in 2012, so that treatments started at the end of May, and were performed every 14 days up to complete leaf fall (Table 3). In 2012, the copper product IRF 096 was substituted by the newer formulation IRF 155, including the same active ingredients but in a different formulation: emulsifiable, instead of wettable powder.

Assessments

In the greenhouse conditions tested, only leaf spots with a chlorotic halo were observed, while large necrotic areas never occurred. Also in orchard experiments, leaf spots with a chlorotic halo were observed during the vegetative season. Exudates were not observed during winter of 2011–12 and 2012–13. The first exudates were observed in February 2014, at the end of the experiments. In all the trials, surveys were carried out every 14 days to evaluate the percentage of

Table 3 Treatments and dates of trials performed in 2011 and 2012 to test the efficacy of strategies to control *P. syringae* pv. *actinidiae* on kiwifruit in an orchard environment

Active ingredient	Commercial product	Dose a.i. (g/ha)	Dose c.p. (g/ha)	Dates of application Trial 2011*	Dates of application Trial 2012
Untreated	–	–	–	–	–
Copper oxychloride + Copper hydroxide	AIRONE	300.8	1,100	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16
Copper oxychloride + Copper hydroxide	IRF 120	300	1,000	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16
Copper oxychloride + Copper hydroxide	IRF 096 (2011) IRF 155 (2012)	300.8	2,200	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16
Mancozeb	Dithane 45WP	1575	3,500	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16
Acibenzolar-S-methyl	Bion	100	200	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16
(Organic + ureic N) + K oxide alternated with	Kendal	105 + 600	3,000	1,3,5,7,9,11,13,15	1,3,5,7,9,11,13,15
Cu oxychloride + Mn oxide + Zn oxide	Kendal TE	1,035+22.5+22.5	3,000	2,4,6,8,10,12,14,16	2,4,6,8,10,12,14,16
Fosetyl- Al	Aliette	1,600	2,000	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16
(Cu oxychloride + Cu hydroxide) +mancozeb	Airone + Dithane	300+1,125	1,100+2,500	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16
Acibenzolar-S-methyl + (Cu oxychloride + Cu hydroxide) alternated with	Bion+Airone	50+300	100+1,100	1,3,5,7,9,11,13,15	1,3,5,7,9,11,13,15
Acibenzolar-S-methyl	Bion	50	100	2,4,6,8,10,12,14,16	2,4,6,8,10,12,14,16

*Trial 2011: 1: 26/5; 2: 9/6; 3: 24/6; 4: 7/7; 5: 22/7; 6: 4/8; 7: 18/8; 8: 2/9; 9: 16/9; 10: 30/9; 11: 14/10; 12: 27/10; 13: 10/11; 14: 24/11; 15: 9/12; 16: 21/12. ** Trial 2012: 1: 29/5; 2: 12/6; 3: 27/6; 4: 11/7; 5: 25/7; 6: 8/8; 7: 22/8; 8: 6/9; 9: 20/9; 10: 4/10; 11: 17/10; 12: 30/10; 13: 14/11; 14: 29/11; 15: 13/12; 16: 27/12

symptomatic plants, the percentage of infected leaves, the percentage of infected leaf surface, and any symptoms of phytotoxicity. The percentage of infected leaf surface was assessed by adopting a standardized leaf infection index. The same specialized technician did all the assessments. Data were analysed by performing an analysis of variance and Tukey's test. Phytotoxicity was evaluated using the scale reported in Table 4.

Results

Greenhouse trials

In the greenhouse trials, no one product tested was able to totally control the development of the bacterial disease, with all plants showing leaf symptoms 1 week after spray-inoculation. There was, however, significant control of disease symptoms by some treatments.

In the two trials carried out in 2012, the best results were obtained by using formulations of copper and acibenzolar-S-methyl. Particularly, in the first trial, the copper formulate IRF 155, composed of copper oxychloride and copper hydroxide, and acibenzolar-S-methyl at 20 g/hl a.i., significantly controlled the disease compared with all the other treatments tested. Copper hydroxide, the mixtures of copper hydroxide and oxychloride, fosetyl-Al and the two fertilizers, one composed of copper oxychloride, manganese oxide and zinc oxide (Kendal TE, Valagro S.p.A.), and the other one of glucohumates (Inductor kiwi, Fertirev s.r.l.), all significantly reduced disease symptoms compared with the control, but to a lesser extent. The results obtained with copper oxychloride, sodium silicate, zinc sulphate, *Bacillus subtilis*, *Bacillus amyloliquefaciens* and zeolites were not significantly different from the inoculated control (Table 5).

Table 4 Phytotoxicity scale used throughout the greenhouse and orchard trials

% Rating	Effects / Symptoms
0	No evident effects/symptoms.
10	Negligible effects/symptoms.
20	Slight discolouration, distortion and / or stunting.
30	Moderate twisting and stunting. No burning.
40	Severe twisting and up to 40 % leaf burning
50	50 % leaf burnt/lost.
60	60 % leaf burnt/lost.
70	70 % burnt/lost.
80	80 % burnt/lost.
90	90 % burnt/lost.
100	Dead plant.

In the second trial of 2012, a temperature increase slowed down the pathogen spread. All the products were significantly different from the inoculated control. The best results were obtained by using the copper formulate IRF 155, acibenzolar-S-methyl, copper hydroxide, the mixtures of copper hydroxide and copper oxychloride, fosetyl-Al and the two fertilizers, one composed of copper oxychloride, manganese oxide and zinc oxide (Kendal TE, Valagro S.p.A.), and the other one of glucohumates (Inductor kiwi, Fertirev s.r.l.), copper chelate and propolis (Table 5). Some formulates, such as some copper formulates, acibenzolar-S-methyl, the fertilizer composed of copper oxychloride, manganese oxide and zinc oxide, and copper chelate, showed phytotoxicity by causing foliar lesions and reduction of plant growth (Table 5). In particular, acibenzolar-S-methyl strongly reduced plant growth and development.

Also in the two trials performed in 2013, the best results were obtained by using the copper formulate IRF 155 and acibenzolar-S-methyl, together with potassium phosphite, introduced in the second year of experimentation. In particular, in the first trial, only these three commercial formulations significantly reduced the percentage of infected leaf surface (0.9, 1.2, and 0.8 % respectively) and the foliar disease incidence (26.5, 29.4, and 26.8 % respectively) more than the other products. Most of the other products, though different from the inoculated control, only partially controlled disease development. The results obtained by using copper oxychloride, potassium silicate and thyme essential oil were not significantly different from the control (Table 6).

In the second trial, all the products significantly reduced the disease severity compared to the inoculated control. The best results were obtained by using the copper formulate IRF 155, acibenzolar-S-methyl and potassium phosphite. Copper hydroxide, the mixture copper hydroxide + copper oxychloride, and fosetyl-Al reduced the percentage of infected leaves by 60 %, while the other treatments only partially reduced disease symptoms on the leaves (Table 6).

Due to the temperatures in May 2013 being below the average for that time of year, when plants were moved outside, symptoms of phytotoxicity became evident in the plants treated with copper products, as in 2012, and with acibenzolar-S-methyl.

In the four trials carried out in 2012 and 2013 (Tables 5 and 6), a partial but significant symptom reduction occurred when the plants were grown in a mixture of a steamed peat mixture substrate (80 %) and compost obtained from digested organic matrices of municipal solid waste (20 %).

Orchard trials

In the 2011 trial, though the experimental site was a new kiwifruit orchard, the first foliar symptoms occurred during that summer, and by October 19, 2011, all the plants of the untreated control were characterized by leaf spots typical of

Table 5 Efficacy of different strategies to control *P. syringae* pv. *actinidiae* on kiwifruit in controlled conditions. Results of trials performed in 2012

Active ingredients	Trial 1								Trial 2							
	% infected leaf surface		% infected leaves		Phytotoxicity (0–100)		Height (cm)		% infected leaf surface		% infected leaves		Phytotoxicity (0–100)		Height (cm)	
Untreated	4.1	Ilm*	70.4	L*	0	A*	231.5	a*	1.9	g*	43.4	j*	0	a*	186.0	abcde*
Copper oxychloride	3.3	ghil	66.0	hil	10	b	150.0	ghi	0.5	abcde	25.0	fghi	0	a	182.0	abcde
Copper hydroxide	1.2	abcd	39.2	bc	10	b	186.0	cdef	0.3	ab	14.2	abcd	10	b	167.0	bcdef
Copper oxychloride + Copper hydroxide	1.9	def	49.5	de	0	a	194.5	bcdef	0.5	abcde	19.9	bcdefgh	0	a	195.0	abcd
C. oxychloride + C. hydroxide (IRF120)	1.2	abcd	40.0	bc	10	b	170.5	fghi	0.5	abcde	18.9	abcdefg	10	b	152.0	defgh
C. oxychloride + C. hydroxide (IRF155)	0.4	a	19.6	a	10	b	189.0	bcdef	0.2	a	11.6	a	10	b	180.5	abcde
Acibenzolar-S-methyl	1.3	abcd	35.7	b	20	c	64.5	m	0.3	ab	13.7	abc	30	d	60.0	h
Acibenzolar-S-methyl	0.5	ab	20.9	a	20	c	54.5	m	0.3	ab	15.9	abcde	30	d	50.5	h
$Cu_2(OH)_3Cl + MnO_2 + ZnO$ (Kendal TE)	0.8	abc	36.4	b	30	d	103.0	l	0.4	abcd	19.2	abcdefg	20	c	140.0	efg
Fosetyl-Al	1.4	bcd	38.4	b	0	a	207.0	abcde	0.3	ab	12.6	ab	0	a	204.4	abc
Oil of *Satureja*	3.2	ghi	60.9	fghi	30	d	183.0	defg	0.6	abcde	22.9	efgh	0	a	165.0	bcdef
Oil of thyme	4.3	lmn	58.8	fgh	0	a	174.0	efgh	0.9	e	26.7	ghi	0	a	160.5	cdef
Propolis	4.3	ilm	57.5	efg	10	b	178.5	defgh	0.4	abcd	19.0	abcdefg	0	a	189.0	abcd
Sodium silicate	5.3	n	68.2	il	0	a	140.0	i	0.5	abcde	21.2	cdefgh	0	a	141.5	efg
ZnSO4 + CH3CO3H + H2O2 (BIOBACTER+)	4.1	ilm	66.0	hil	0	a	179.5	defgh	0.7	bcde	25.0	fghi	0	a	111.0	g
Bacillus subtilis	4.7	mn	69.7	l	0	a	182.5	defg	0.5	abcde	24.0	fghi	0	a	167.6	bcdef
Bacillus amyloliquefaciens	3.9	hilm	67.0	hil	0	a	170.5	fghi	0.7	bcde	27.2	ghi	0	a	132.0	fg
Gluco humates - NP fertilizer (leaf)	1.6	cde	39.4	bc	0	a	211.5	abcd	0.4	abcd	17.9	abcdefg	0	a	203.5	abc
Gluco humates - NP fertilizer (root)	2.9	fgh	52.4	def	0	a	219.5	abc	0.8	de	24.4	fghi	0	a	211.5	ab
Gluco humates - NP fertilizer (leaf + root)	3.2	ghi	57.2	efg	0	a	221.5	ab	0.7	bcde	22.6	efgh	0	a	215.5	a
Potassium silicate	2.7	efg	56.2	ef	0	a	148.0	hi	0.9	e	25.6	fghi	0	a	180.0	abcde
Copper chelate	1.7	cde	47.0	cd	30	d	189.0	bcdef	0.4	abcd	15.8	abcde	20	c	154.5	defg
Synthetic zeolites	4.3	lmn	65.0	ghil	10	b	177.0	efgh	1.4	f	31.3	i	0	a	165.0	bcdef
Compost 1 (Maturation 6 months)	2.1	def	52.4	def	10	b	205.4	abcde	0.7	bcde	23.3	efgh	10	b	154.0	defg
Compost 2 (Maturation 4 months)	2.1	def	52.5	def	20	c	177.5	efgh	0.6	abcde	21.7	defgh	20	c	152.5	defg

*Values in the same column followed by the same letter are not statistically different by Duncan's Multiple Range Test ($P<0,05$)

Psa. The reason for this high infection level could be explained by the close proximity (50 m distance) of infected orchards. The symptoms appeared in plants tested with all the treatments except for acibenzolar-S-methyl (0 % infected plants) and the mixture copper hydroxide + copper oxychloride (IRF 096), where the symptoms occurred in just two plants of one replicate. The other products and strategies partially controlled disease development, however when mancozeb was used, the percentage of infected plants was not statistically different from the untreated control (Table 7).

In 2012 the best results were provided by the new copper formulate IRF 155 and by acibenzolar-S-methyl, which partially controlled disease progression (Table 7). It should be considered that from July, 1 month after the start of the treatments, all the plants showed foliar symptoms, though the percentage of infected leaves was low. During the summer, the pathogen spread was slowed down, but not stopped, so that on October 4, 2012, over 40 % leaves were infected in the untreated control, and just the treatments with acibenzolar-S-methyl, the mixture copper hydroxide + copper oxychloride (IRF 155) and fosetyl-Al were significantly different from the control (Table 7). The treatment with mancozeb alone was not significantly different from the control, while mancozeb with the mixture copper hydroxide + copper oxychloride was statistically similar to the treatment with the copper product alone.

Table 6 Efficacy of different strategies to control *P. syringae* pv. *actinidiae* on kiwifruit in controlled conditions. Results of trials performed in 2013

Active ingredients	Dosage a.i. (ml/g/hl)	Trial 1			Trial 2		
		% infected leaf surface	% infected leaves	Phytotoxicity (0–100)	% infected leaf surface	% infected leaves	Phytotoxicity (0–100)
Untreated	–	4.9 h*	71.2 l*	0 a*	4.8 l*	72.3 o*	0 a*
Copper oxychloride	60	2.6 ef	64.8 hil	10 b	2.6 hi	51.0 hil	10 b
Copper hydroxide	30	1.1 ab	39.0 bc	10 b	1.2 bcde	31.1 cd	0 a
Copper oxychloride + Copper hydroxide	30.8	2.2 cde	48.4 cdef	10 b	0.8 abc	29.3 c	10 b
C. oxychloride + C. hydroxide (IRF 155)	45	0.9 a	26.5 a	10 b	1.0 abcd	12.2 ab	20 c
C. oxychloride + C. hydroxide (IRF 155)	30	1.1 ab	30.3 ab	10 b	0.6 ab	9.0 a	20 d
Acibenzolar-S-methyl	10	1.2 abc	29.5 ab	0 a	0.6 a	16.7 b	30 c
Acibenzolar-S-methyl	5	1.3 abcd	29.4 ab	0 a	0.6 ab	14.8 ab	30 d
(Organic + ureic N) + K oxide (KENDAL)	10,5+46,5	2.6 ef	50.8 defg	0 a	1.6 efg	44.0 fgh	0 a
Fosetyl-Al	16	1.8 abcde	46.8 cdef	0 a	1.0 abcd	30.3 cd	0 a
Oil of *Satureja*	100	2.6 ef	51.5 defg	0 a	2.5 hi	65.7 no	0 a
Oil of thyme	100	3.6 fg	69.6 l	0 a	2.6 hi	60.4 mn	0 a
Propolis	200	2.0 bcde	49.3 def	0 a	1.4 cde	36.6 de	0 a
Potassium silicate (leaf treatment)	10	4.0 gh	68.6 il	0 a	2.5 hi	52.5 il	0 a
Ureic N + P_2O_5 (Bio PROTEK)	25+25	2.7 ef	56.3 fgh	0 a	2.6 hi	55.9 ilm	0 a
Bacillus subtilis	62.68	2.6 ef	55.6 fgh	0 a	2.6 hi	61.1 mn	0 a
Bacillus amyloliquefaciens	37,5	2.2 cde	54.7 efg	0 a	2.8 i	60.0 mn	0 a
Gluco humates-NP fertilizer (leaf)	24+108	2.3 de	52.0 defg	0 a	1.5 def	41.5 ef	10 b
Gluco humates - NP fertilizer (leaf + root)	(20+90)+(20+90)	3.4 fg	59.3 ghi	0 a	1.2 cde	42.1 efg	10 b
Potassium silicate (root)	10	3.6 fg	64.7 hil	0 a	2.1 fgh	55.0 ilm	10 b
Potassium phosphite	12	0.8 a	26.8 a	0 a	0.6 ab	16.2 ab	0 a
Synthetic zeolites + Copper oxychloride	950+60	2.8 ef	59.3 ghi	10 b	2.2 hi	48.8 ghi	0 a
Compost 1 (maturation 6 months)	20 %	2.3 de	45.8 cde	0 a	1.5 def	43.0 efg	0 a
Compost 2 (maturation 4 months)	20 %	1.7 bcde	45.0 cd	0 a	2.2 ghi	52.2 il	0 a

*See Table 5

Both in 2011 and 2012, weak phytotoxicity symptoms were visible in the plants treated with copper products. In spring 2013, the bacterial ooze was evaluated on the surface of vines and trunks. Exudates were only visible on the main vines of two plants of the untreated control.

Discussion

In this study, four trials in greenhouse conditions and two trials in an orchard were conducted to assess the level of control of Psa on kiwifruit cv. Hayward, by using chemical and biological products. Chemical control of bacterial diseases, and particularly of Psa, is highly dependent on spraying antibiotics, such as streptomycin, or copper formulations (Cameron and Sarojini 2014). In Europe the use of streptomycin for control of plant pathogens is not legal, so copper formulations remain the main management strategy for crop protection against bacterial diseases (Gullino and Brunelli 2012). Copper fungicides should be used carefully, particularly on young plants and during spring, because low temperatures can induce phytotoxicity. In addition, copper bactericides have other possible disadvantages after long-term use, including resistance to copper in bacterial populations (Rinaldi and Leite 2000) and the accumulation of copper metal in soils with potential environmental effects (Alva et al. 1995). Repeated spraying with copper bactericides resulted in Japanese Psa strains developing maximal copper resistance (Marcelletti et al. 2011). However, the strains of Psa from the recent Italian outbreak were sensitive to copper compounds (Ferrante and Scortichini 2010).

Traditional copper formulations, including Cu-hydroxide, Cu-oxychloride or their mixture, were tested during the experiments, together with new experimental formulations of copper products, which should have high biological efficacy achieved with much lower hectare rates. In the new experimental formulations tested (IRF 120 and IRF 155) copper is

Table 7 Efficacy of different strategies to control *P. syringae* pv. *actinidiae* on kiwifruit in an orchard environment. Treatments and dates of trials performed in 2011 and 2012

Active ingredients	Dosage a.i. (g/ha)	Trial 2011 (survey: 19/10/2011)								Trial 2012 (survey: 04/10/2012)							
		Infected plants (%)		Infected leaf area (%)		Infected leaves (%)		Phytotoxicity		Infected plants (%)		Infected leaf area (%)		Infected leaves (%)		Phytotoxicity	
Untreated	–	100.0	d*	1.5	b*	17.5	b*	0.0	a*	100	a *	4.1	b*	42	b*	0	a *
Copper oxychloride + Copper hydroxide	300.8	40.0	c	0.2	a	4.0	a	10.0	b	100	a	2.7	ab	30.8	ab	5	ab
Copper oxychloride + Copper hydroxide	300	40.0	c	0.2	a	4.3	a	10.0	b	100	a	1.4	a	23.5	ab	10	b
Copper oxychloride + Copper hydroxide	300.8	10.0	b	0.0	a	1.0	a	10.0	b	100	a	0.6	a	13.5	a	10	b
Mancozeb	1,575	80.0	cd	0.5	a	8.8	ab	0.0	a	100	a	4.4	b	43.5	b	0	a
Acibenzolar-S-methyl	100	0.0	a	0.0	a	0.0	a	0.0	a	100	a	1.5	a	17.8	a	0	a
(Organic + ureic N) + K oxide alternated with Cu oxychloride + Mn oxide + Zn oxide	105+600 1,035+22.5+22.5	50.0	c	0.5	a	7.5	ab	0.0	a	100	a	2.4	ab	29.8	ab	10	b
Fosetyl- Al	1,600	50.0	c	0.4	a	6.8	a	0.0	a	100	a	2.3	ab	18.5	a	0	a
(Cu oxychloride + Cu hydroxide) + mancozeb	300+1,125	80.0	cd	0.4	a	7.2	ab	0.0	a	100	a	2.9	ab	31	ab	10	b
Acibenzolar-S-methyl + (Cu oxychloride + Cu hydroxide) alternated with Acibenzolar-S-methyl	50+300 50	0.0	a	0.0	a	0.0	a	0.0	a	100	a	1.4	a	19.3	a	10	b

*See Table 5

complexed with organic molecules, but the exact formulation is covered by trade secret. However, traditional copper fungicides are preventive, acting by contact compounds, while copper complexes show also partially systemic and curative properties. In the greenhouse and orchard experiments, copper hydroxide and the mixtures of copper hydroxide and copper oxychloride, significantly reduced foliar symptoms of the disease, and showed low phytotoxicity levels. In all the trials, the new formulate IRF 155 (copper oxychloride + copper hydroxide) was one of the most promising products for bacterial disease control, able to reduce the leaf disease symptoms by 70–80 %, compared with the control.

Acibenzolar-S-methyl, a functional analogue of salicylic acid, has demonstrated good efficacy against bacterial diseases, including bacterial spot and bacterial speck on tomato (Louws et al. 2001) and fire blight in apples (Bastas and Maden 2007). Similar efficacy was provided by acibenzolar-S-methyl against Psa on kiwifruit. The product showed a high level of phytotoxicity in 2012, on one-year old plants in pots, but it was much less phytotoxic on the same plants the following year suggesting that younger plants are more susceptible. The use of acibenzolar-S-methyl has been temporarily extended to kiwifruit in Italy, with a maximum of four treatments per year.

The efficacy of fosetyl-Al was lower in the first trials of 2012 and 2013, when the disease severity was higher. Vice versa, the product was more effective in the second trials of 2012 and 2013, performed later in the growing season, with higher average temperatures and lower disease severity. Fosetyl-Al shows better efficacy in presence of lower disease pressure, as already demonstrated for other pathogens (Brown et al. 2004).

The efficacy of the other products tested in greenhouse conditions never exceeded 30–40 %, and some products were not significantly different from the control. Similarly, in the orchard trials, the other products tested were less effective, in particular, mancozeb did not show significant preventative action against Psa either alone, or when used together with copper products. Previously mancozeb showed a synergistic effect with copper compounds against bacterial diseases on other crops (Gent and Schwartz 2005).

The antagonistic microorganisms tested, *Bacillus subtilis* and *B. amyloliquefaciens,* commercialized mainly to control soilborne pathogens (Spadaro and Gullino 2005) or for flowering treatments (Oancea et al. 2009), showed a low efficacy in the protection of leaves and buds. *B. amyloliquefaciens*, in particular, was effective against *Psa in vitro*, and was able to survive on kiwifruit female flowers, reducing the *Psa* population on flowers (Biondi et al. 2012),

by producing iturins which disrupt pathogen cell wall production (Highland et al. 2012).

Even in absence of other treatments, a reduction of the disease was reported when using the mixture of a steamed peat mixture substrate with compost obtained from digested organic matrices of municipal solid waste. Composts could become an effective low cost tool to be included in the crop protection strategy. Compost-amended soils offer the potential to manage soilborne diseases, but also to reduce foliar diseases, by improving plant health and inducing systemic resistance (Han et al. 2000). Infected radish and tomato grown in compost-amended substrates harbored significantly lower populations of the bacterial pathogens *Xanthomonas campestris* pv. *armoraciae* and *Xanthomonas campestris* pv. *vesicatoria,* respectively (Aldahmani et al. 2005).

In conclusion, no one product tested here could be considered as a suitable solution for the control of Psa on kiwifruit, but in the framework of integrated control strategies, copper compounds alternated with resistance inducers could be used in combination with compost, to develop new strategies to reduce the disease development and spread. New strategies should be tested under natural conditions, by considering the different climate conditions of the kiwifruit production areas, as it is not possible to design one unique strategy to control Psa on kiwifruit for every region.

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