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Field efficacy of chitosan to control *Pseudomonas syringae* pv. *actinidiae*, the causal agent of kiwifruit bacterial canker

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Abstract In order to provide an alternative for controlling bacterial canker of kiwifruit, caused by *Pseudomonas syringae* pv. *actinidiae*, in case of the appearance of copper/antibiotic-resistant strains of the pathogen, the field efficacy of a chitosan-based compound was compared with a copper compound on *Actinidia deliciosa* cv. Hayward. The 3-year trials were carried out in central Italy, in an area heavily affected by the disease. Both compounds were sprayed on the same day according to the following schedule: every 14 days, from early April to early June (a total of seven treatments), and once a month, from mid November to mid February (a total of four treatments). Chitosan did not incite any phytotoxic effect on plant and fruit and showed an overall higher level of performance than the copper compound in reducing disease symptoms throughout the 3-year trials. Chitosan significantly reduced also the presence of exudates on trunk and leader recorded at the end of winter.

Keywords *Actinidia deliciosa* · Green-fleshed kiwifruit · Antibiotic resistance · Copper resistance

Pseudomonas syringae pv. *actinidiae* is the causal agent of bacterial canker of both *Actinidia chinensis*, the yellow-fleshed, and *A. deliciosa*, the green-fleshed kiwifruit crops. A pandemic population of the pathogen is currently causing severe economic losses in all major areas of kiwifruit production of the world (Scortichini et al. 2012). Main symptoms are: leaf spotting, twig wilting, flower necrosis, cankers and exudates along the main trunk and leaders. Recently, fundamental knowledge on the origin, population structure, and genomic features jointly with basic information on its pathogenic interaction with the plant and on its cycle of disease has been achieved (Marcelletti et al. 2011; Ferrante et al. 2012; Mazzaglia et al. 2012; Butler et al. 2013; Petriccione et al. 2013, 2014; Ferrante and Scortichini 2014b). However, the effective control of the disease has not yet been achieved. Despite the potential of copper and antibiotic resistance shown by the current pandemic population (Marcelletti et al. 2011), these two categories of agrochemicals were largely suggested in New Zealand for controlling the disease (Cameron and Sarojini 2014). Similarly, in Italy, despite the well-known possibility of emergence of copper resistant *P. s.* pv. *actinidiae* strains, as occurred in the past in Japan (Goto et al. 1994; Nakajima et al. 2002), the control strategy is mainly based on the massive application of copper compounds both during the vegetative and dormant season (Anonymous 2011; Antoniacci et al. 2012).

Chitosan, a natural non-toxic biopolymer, obtained by the N-deacetylation of chitin, the main component of fungal cell wall and crustacean exoskeleton (Miranda Castro and Lizarraga-Paulin 2012), is a promising compound to effectively control phytopathogenic fungi both in field and during the post-harvest condition

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(Romanazzi et al. 2002, 2007; Liu et al. 2007; Faoro et al. 2008; Reglinski et al. 2010; Iriti et al. 2011; Zhang et al. 2011). Concerning plant pathogenic bacteria, there is interesting evidence of a bactericidal activity either in vitro (Ferrante and Scortichini 2010; Li et al. 2011) or in vivo (Li et al. 2011; Mansilla et al. 2013). An effective result in reducing bacterial head rot of broccoli by means of different chitosan-based compounds upon artificial inoculation with *P. fluorescens* was also shown (Li et al. 2010). However, no field trial has been performed so far to test the field efficacy of this compound towards a phytopathogenic bacterium.

In order to provide an alternative for controlling bacterial canker of kiwifruit in case of appearance of copper/antibiotic-resistant strains of the pathogen, the field efficacy of a chitosan-based compound was tested in a 3-year trial carried out in central Italy, in an area heavily affected by the disease. A formulation based on a 60 % solution (99.9 % purity) of chitosan (deacetylation degree ~85 %; molecular weight 20–30 KDa) and obtained from the exoskeleton of crustaceae was used for the trial that was carried out in Cisterna di Latina (Latium region, central Italy), in farms where the presence of the pathogen was previously ascertained (Ferrante et al. 2012). The compound was preliminary tested in vitro for its bactericidal activity towards representative strains of the three populations of the pathogen (Ferrante and Scortichini 2014b), according to procedures described elsewhere (Ferrante and Scortichini 2010).

The trials were performed in three different (i.e., each year a different farm was chosen) but very closely located kiwifruit orchards, during 2011–12, 2012–13 and 2013–14, with adult *A. deliciosa* cv. Hayward plants, spaced at 5×4 m and trained as a "pergola". Green-fleshed kiwifruit was chosen for its widespread utilization worldwide. The trials were set up as a randomized block design with four replications with 15 plants per treatment in each block. Main climatic parameters (i.e., rainfall and average maximum and minimum temperature) were obtained from the meteorological station located in the area (Centro funzionale Regione Lazio) (Fig. 1). The chitosan efficacy was compared with that of a standard copper compound, namely Airone (Isagro, Italy), a mixture of copper hydroxide (10 %) and copper oxychloride (10 %), for a total of 20 % of active copper, commonly used in Italy for controlling *P. s.* pv. *actinidiae* (Anonymous 2011). Chitosan was tested at a dose of 1.0 kg/ha, whereas the copper compound was tested at 1.7 kg/ha, as recommended by the manufacturers. To avoid spray drift to neighbouring plants, spraying was carried out with a spray lance distributing a volume corresponding to 800 l/ha, equal to that commonly used in the area for the spray treatments. Untreated plants were used as negative controls. Previous epidemiological studies carried out in the area allowed us to ascertain the periods when *P. s.* pv. *actinidiae* mainly colonized the plants, namely spring, autumn and winter (Ferrante et al. 2012; Scortichini et al. 2012), so that the spray treatments were applied accordingly. Both compounds were sprayed on the same day according to the following schedule: every 14 days, from early April to early June (a total of seven treatments), and once a month, from mid November to mid February (a total of four treatments). The following disease parameters were assessed: a) disease incidence, as total number of plants showing exudates along the trunk and leaders at the end of winter, before to start the trial; and b) disease severity, evaluated by: i) leaf spots (mean number of leaf spot/leaf, assessed by randomly detaching ten leaves per plant and by counting the total number of spots per each leaf; ii) twig wilting (by counting the mean number of wilted twigs per plant); iii) necrotic flowers (total number of necrotic flowers, assessed by randomly collecting ten flowers per plant); and iv) presence of exudates (by counting the total number of oozing cankers along the trunk and leaders). Data were recorded in spring (May), autumn (before harvest) and end of winter (March) (see also Table 2). Isolations and identification to confirm the presence of *P. s.* pv. *actinidiae* were performed in each orchard, during spring, autumn and winter, according to well-established techniques (Ferrante and Scortichini 2009, 2010). The possible presence of phytotoxic effects was assessed by the presence of necrotic spots and scorched areas on the leaves and by measuring the fruit weight at harvest time. The field efficacies of the tested compounds were expressed as mean ± standard deviation of data. Data were subjected to one-way analysis of variance and comparison among means was determined by Fisher's least significant difference test. Significant differences are revealed by different letters at $P<0.05$.

The chitosan, similarly to the copper compound herein tested, at the dose used for the field trials, was bactericidal towards all three *P. s.* pv. *actinidiae* populations, namely Psa 1, Psa 2 and Psa 3 (Ferrante and Scortichini 2014b), upon the in vitro test. In fact, when loopfuls of the broth medium added with chitosan or copper

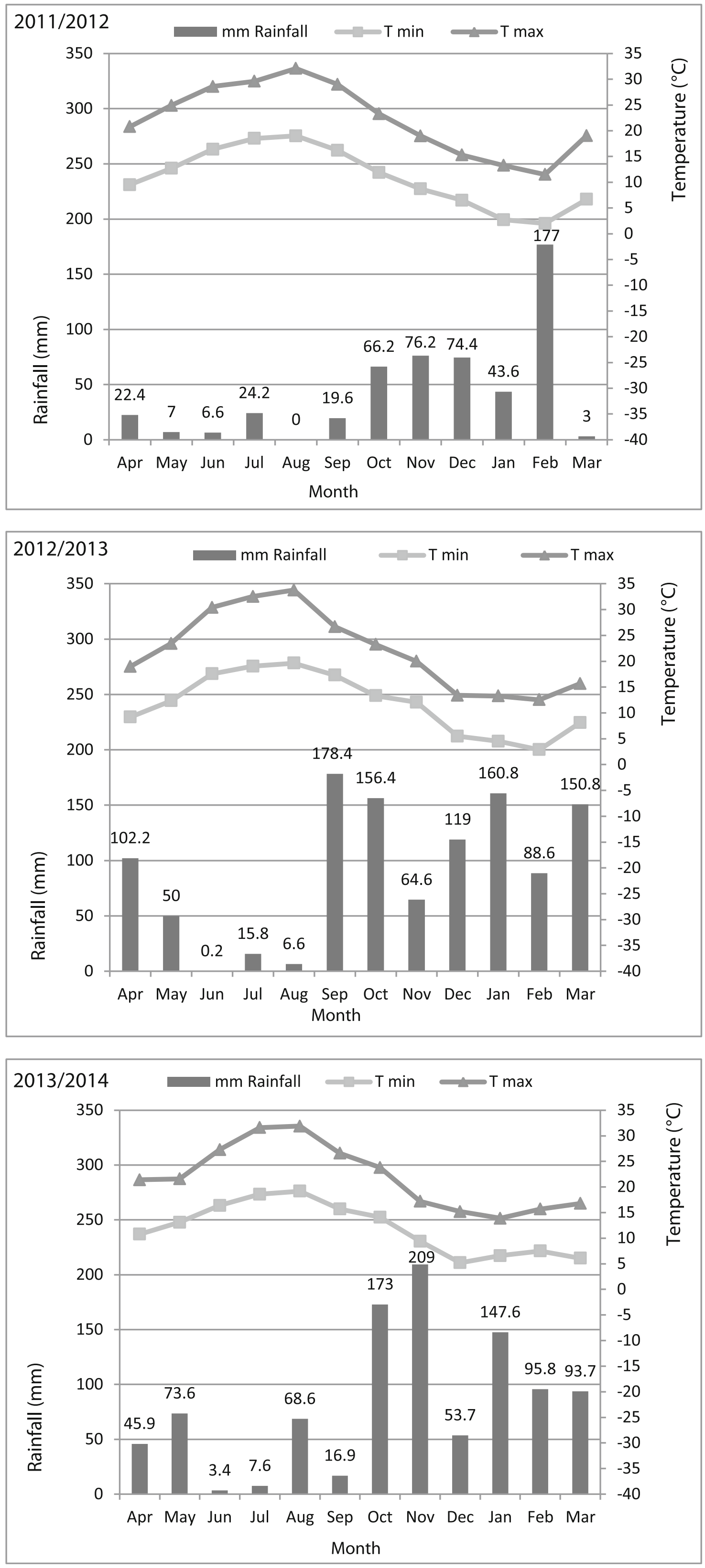

Fig. 1 Rainfall and average minimum and maximum temperatures recorded in Cisterna di Latina (Latium region, central Italy) during the trials (2011–12; 2012–13; 2013–14). The number on the bars indicated the total amount of precipitation recorded in the month

Table 1 Incidence of bacterial canker caused by *Pseudomonas syringae* pv. *actinidiae* on *Actinidia deliciosa* cv. Hayward in the orchards hosting the trials. The parameter is based on the total number of exudates scored on the main trunk and leaders, and counted at the end of winter, before starting the trial. Significant differences are shown by different letters at $P<0.05$, according to Fisher's least significant difference test

	2011–2012	2012–2013	2013–2014
Chitosan	38.6±2.4a	39.8±1.7a	34.3±0.9a
Copper (Hydroxide + Oxychloride)	37.9±1.7a	40.1±1.6a	36.5±1.2a
Non treated control	36.4±1.6a	38.0±1.3a	37.5±1.6a

compound were spread, after 1 and 3 days of incubation at 24±1 °C, onto nutrient agar supplemented with 3 % of sucrose (NSA), no bacterial growth was observed. *P. s.* pv. *actinidiae* was always isolated from the three experimental plots during spring, autumn and winter, including the plots treated with chitosan and copper. The incidence of the disease, measured as the presence of exudates oozing out from trunk and leaders at the end of winter, was quite high in each farm hosting the trial (Table 1). This parameter revealed that each orchard was affected by bacterial canker and showed a similar incidence of the disease at the beginning of the trial. The results of field efficacy of the two compounds are shown in Table 2. Neither compound caused any sign of phytotoxicity (i.e., leaf spotting and or scorching) to the tested plants. The fruit weight, at the end of the trial, was similar to that of the untreated control plants. In the trial 2013–14, the fruits collected from the plots treated with chitosan and copper showed a statistically significant weight increase (Table 2). The efficacy of chitosan and copper towards *P. s.* pv. *actinidiae* was evident especially as far as leaf spot in spring, twig wilting and presence of exudate concerns. In fact, when compared to the untreated plants, the reduction of the disease measured by these parameters was statistically significant during the 3-year trial (Table 2). Interestingly, chitosan

Table 2 Field efficacy of chitosan and a copper compound (hydroxide (10 %) plus oxychloride (10 %)) towards *Pseudomonas syringae* pv. *actinidiae* during 2011–12; 2012–13 and 2013–14 on *Actinidia deliciosa* cv. Hayward. Leaf spot, mean number of spot/leaf; twig wilting, mean number of wilted twigs/plant; necrotic flowers, total number of necrotic flowers/150 flowers randomly collected; exudates, total number of trunk and leader exudates. Significant differences are revealed by different letters at $P<0.05$, according to Fisher's least significant difference test

	Spring			Autumn	Winter	Fruit weight (g)
	Leaf spot	Twig wilting	Necrotic flowers	Leaf spot	Exudates	
2011–2012						
Chitosan	2.4±0.4a	3.7±0.5a	10.9±0.4a	0.8±0.2a	24.1±0.5a	135.1±5.2a
Copper (Hydroxide + Oxychloride)	3.6±0.7b	3.8±0.5a	11.4±0.5a	0.9±0.4a	28.4±0.5b	128.7±4.2a
Non treated control	8.8±1.2c	6.4±1.3b	12.6±0.7ab	1.8±0.5ab	49.4±1.0c	134.5±4.4a
2012–2013						
Chitosan	2.7±0.3a	1.8±0.8a	10.2±0.5a	0.1±0.1a	23.8±0.5a	158.7±4.3a
Copper (Hydroxide + Oxychloride)	3.9±0.4b	7.5±0.9b	10.3±0.7a	0.1±0.1a	29.6±0.6b	165.1±5.1a
Non treated control	9.4±0.8c	9.3±0.6c	11.4±0.6a	0.4±0.3a	44.5±1.1c	152.6±6.4a
2013–2014						
Chitosan	0.7±0.3a	0.5±0.2a	11.0±0.5a	0.1±0.1a	18.5±0.1a	115.5±5.1b
Copper (Hydroxide + Oxychloride)	0.7±0.4a	0.5±0.3a	11.9±0.6a	0.2±0.1a	21.5±0.2a	117.3±6.4b
Non treated control	4.4±1.2b	2.3±0.8b	15.3±1.2b	0.6±0.2b	43.0±0.1b	104.2±4.7a

always showed an overall higher performance than Airone in reducing the disease symptom throughout the 3-year trial. The significant reduction in the number of exudates is particularly important. In fact, this data has been recorded at the end of winter, after the occurrence of frost events that incite the aggressiveness of the pathogen (Ferrante and Scortichini 2014a). During winter 2011–12, in the area hosting the trial, 16 days of frost were recorded, with two consecutive days of a minimum temperature of −4 °C (i.e., 17 and 18 January), whereas during 2012–13 and 2013–14 7 and 2 days of frost, respectively, were recorded with temperature never lower than −2 °C. In any case, chitosan significantly reduced the number of exudates compared to Airone. Such a performance could be explained by the protection of the natural openings of the plant (i.e., lenticels) and wounds played by the film-forming property of chitosan (Samicho 2011), thus reducing the possibility of *P. s.* pv. *actinidiae* colonization during a period of the year very conducive for the disease progression. In fact, the progression of the disease for the untreated control plants when compared to the data recorded before the starting of the trial (Table 1), showed a significant increase in the number of exudates (Table 2).

The effect of the spray treatments with both chitosan and Airone on the occurrence of necrotic flowers and leaf spot in autumn was less evident. In fact, the reduction in this symptom, when compared to the untreated plants, was not always statistically significant. Remarkably, the autumn record on leaf spot revealed, for the 3 years of the investigation, a very low presence of damage, This, according to previous studies on the disease cycle of the pathogen (Ferrante et al. 2012), strongly confirms that during summer, in Mediterranean climate, *P. s.* pv. *actinidiae* does not spread effectively within and between the orchard(s) to colonize the plant as in the other seasons. So, this investigation confirms that scheduled timing of spray treatments based on the cycle of disease of the pathogen is effective in reducing the severity of the disease in a heavily contaminated area. Finally, these trials also indicate this formulation of chitosan as a very promising compound for the field control of *P. s.* pv. *actinidiae* throughout the season.

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