

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

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

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/157911> since 2016-07-04T22:28:07Z

Published version:

DOI:10.1007/s13313-014-0328-1

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

1 **Short title: Control of *Pseudomonas syringae* pv. *actinidiae* on kiwifruit**

2

3 **Title:**

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

6

7 Matteo Monchiero¹, Maria Lodovica Gullino^{1,2}, Massimo Pugliese^{1,2}, Davide Spadaro^{1,2*}, Angelo
8 Garibaldi¹

9

10 ¹ Centre of Competence for the Innovation in the Agro-environmental Sector (AGROINNOVA),
11 University of Turin, Grugliasco (TO), Italy.

12

13 ² DISAFA, Dept. Agricultural, Forestry and Food Sciences, University of Turin, Grugliasco (TO),
14 Italy.

15

16 **Correspondence*:**

17 Davide Spadaro, University of Turin, Via L. da Vinci 44, I-10095 Grugliasco (TO), Italy.

18

19 Telephone: +39-011-6708942

20 Fax: +39-011-6709307

21 E-mail: davide.spadaro@unito.it

22

23 **Keywords**

24 *Actinidia deliciosa*, bacterial canker, chemical control, control strategies, Italy.

25 **Abstract**

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

43

44 **Introduction**

45 Kiwifruit originated in China, but it was New Zealand that introduced its cultivation, which has been
46 gradually adopted by other countries, becoming of primary importance in global fruit production.
47 Kiwifruit is characterized by a high adaptability of the two species *Actinidia deliciosa* and *A.*
48 *chinensis* (Ferguson and Huang, 2007). The global production was over 1.4 million tons in 2012, and
49 Italy produced around 384,000 tons (FAOSTAT, 2014). Italy produces more kiwifruit than any other
50 country apart, possibly, from China (Testolin and Ferguson, 2009). Piedmont (northwest Italy), where

51 6,050 hectares of kiwifruit are cultivated (Piedmont Region - AGRISTAT, 2011), is the largest Italian
52 region responsible for kiwifruit storage and export, and the second largest producer of the fruit
53 (ISTAT, 2011).

54 *Pseudomonas syringae* pv. *actinidiae* (Psa) was first isolated and described in Japan in 1984
55 (Takikawa et al., 1989), then in Italy and South Korea (Koh et al., 1994; Scortichini, 1994) on cv.
56 Hayward (*A. deliciosa*). For more than 15 years this bacterial disease was considered to be of low
57 importance on kiwifruit produced in Italy. However, since 2008, the first outbreaks of bacterial canker
58 on kiwifruit cultivated in Central Italy, led to the removal of most orchards of *A. chinensis* in the
59 region (Balestra et al., 2009; Ferrante and Scortichini, 2009, 2010). During 2009-2011, *P. syringae*
60 pv. *actinidiae* started to severely infect *A. deliciosa* cv. Hayward in the main fruit producing regions
61 of Italy. During spring 2010, the first outbreaks were identified in Piedmont (northern Italy), where
62 the pathogen was most probably introduced by infected propagation material (Armentano, 2010;
63 Spadaro et al., 2010). Despite the strict application of preventative measures to avoid the spread of
64 the disease, in 2011 the pathogen was present throughout the kiwifruit production area in northern
65 Italy, with considerable yield losses and the removal of hundreds of hectares (Spadaro et al., 2011).
66 In 2010, the pathogen was also officially reported in New Zealand, where severe damage was caused
67 to *A. chinensis* and *A. deliciosa* crops (Everett et al., 2011). The disease has reached pandemic
68 proportions by spreading to France, Spain, Portugal, Switzerland, Chile, Turkey, South Korea and
69 Japan (Vanneste et al., 2011; Renzi et al., 2012; EPPO, 2012).

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

75 Damages caused by *P. syringae* pv. *actinidiae* are worsening the possibility of cultivating kiwifruit,
76 particularly in areas such as northern Italy, which are closer to the thermal limits for *Actinidia* spp.

77 cultivation (EPPO, 2012). The climate pattern of northern Italy, characterized by cold winters with
78 minimum temperatures lower than -15°C , and fresh and humid spring and autumn seasons, creates
79 favourable conditions for the pathogen spread.

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

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

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

102 Moreover, other interesting products are resistance inducers, such as fosetyl-AI, potassium
103 phosphites, and acibenzolar-S-methyl, registered and used on other crops, and antagonistic
104 microorganisms, such as *Bacillus subtilis* and *B. amyloliquefaciens*. Natural products, such as
105 essential oils, could be used to control different diseases on fruit crops in the orchard (Lopez-Reyes
106 et al. 2010; 2013). Together with commercial products, many other formulations can be found on the
107 market, generally described as leaf fertilizers or plant strengtheners, to avoid the strict European
108 regulations for registering and commercialising new agrochemicals. Another preventative strategy,
109 which has previously been shown to have disease suppression characteristics, is to use soil amended
110 with compost obtained from digested organic matrices of municipal solid waste (Pugliese et al., 2008;
111 2011).

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

117

118 **Materials and methods**

119

120 **Plants and microorganisms**

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

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

133

134 **Greenhouse trials in 2012**

135 To test the efficacy of different products, trials were performed in two subsequent years, 2012 and
136 2013. In each trial, 25 treatments were compared, with each treatment being tested on 10 plants (Table
137 1 and 2).

138 A set of 500 potted plants of *Actinidia deliciosa* cv. Hayward 30-40 cm high were divided into two
139 250 plant groups. In January 2012, the first group of plants was moved to the greenhouse where the
140 temperature was kept at 20°C.

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

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

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

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

167

168 **Greenhouse trials in 2013**

169 At the end of January 2013, the first group of pots was placed in the greenhouse at approximately
170 20°C. The treatments conducted were similar to the trial in 2012, with the introduction of new
171 products and with new dosage levels for already used products (Table 2). Plants were treated by
172 spraying a 25 ml suspension of product per plant; first with the resistance inducers on March 5, 2013,
173 and then with all the products seven days later. On the following day, plants were spray-inoculated,
174 as described previously. The plants were inoculated with Psa a second time on March 19, 2013, due
175 to the lack of symptoms. When the first leaf spots occurred, the treatments were applied according to
176 the schedule in Table 2.

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

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

187

188 **Orchard trials**

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

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

203 **Assessments**

204 In the greenhouse conditions tested, only leaf spots with a chlorotic halo were observed, while large
205 necrotic areas never occurred. Also in orchard experiments, leaf spots with a chlorotic halo were
206 observed during the vegetative season. Exudates were not observed during winter of 2011-12 and
207 2012-13. The first exudates were observed in February 2014, at the end of the experiments. In all the
208 trials, surveys were carried out every 14 days to evaluate the percentage of symptomatic plants, the
209 percentage of infected leaves, the percentage of infected leaf surface, and any symptoms of
210 phytotoxicity. The percentage of infected leaf surface was assessed by adopting a standardized leaf
211 infection index. The same specialized technician did all the assessments. Data were analysed by
212 performing an analysis of variance and Tukey's test. Phytotoxicity was evaluated using the scale
213 reported in Table 4.

214

215 **Results**

216

217 **Greenhouse trials**

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

221 In the two trials carried out in 2012, the best results were obtained by using formulations of copper
222 and acibenzolar-S-methyl. Particularly, in the first trial, the copper formulate IRF 155, composed of
223 copper oxychloride and copper hydroxide, and acibenzolar-S-methyl at 20 g/hl a.i., significantly
224 controlled the disease compared with all the other treatments tested. Copper hydroxide, the mixtures
225 of copper hydroxide and oxychloride, fosetyl-Al and the two fertilizers, one composed of copper
226 oxychloride, manganese oxide and zinc oxide (Kendal TE, Valagro S.p.A.), and the other one of
227 glucohumates (Inductor kiwi, Fertirev s.r.l.), all significantly reduced disease symptoms compared
228 with the control, but to a lesser extent. The results obtained with copper oxychloride, sodium silicate,

229 zinc sulphate, *Bacillus subtilis*, *Bacillus amyloliquefaciens* and zeolites were not significantly
230 different from the inoculated control (Table 5).

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

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

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

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

257 In the four trials carried out in 2012 and 2013 (Table 5 and 6), a partial but significant symptom
258 reduction occurred when the plants were grown in a mixture of a steamed peat mixture substrate
259 (80%) and compost obtained from digested organic matrices of municipal solid waste (20%).

260

261 **Orchard trials**

262 In the 2011 trial, though the experimental site was a new kiwifruit orchard, the first foliar symptoms
263 occurred during that summer, and by October 19, 2011, all the plants of the untreated control were
264 characterized by leaf spots typical of Psa. The reason for this high infection level could be explained
265 by the close proximity (50 m distance) of infected orchards. The symptoms appeared in plants tested
266 with all the treatments except for acibenzolar-S-methyl (0% infected plants) and the mixture copper
267 hydroxide + copper oxychloride (IRF 096), where the symptoms occurred in just two plants of one
268 replicate. The other products and strategies partially controlled disease development, however when
269 mancozeb was used, the percentage of infected plants was not statistically different from the untreated
270 control (Table 7).

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

279 the mixture copper hydroxide + copper oxychloride was statistically similar to the treatment with the
280 copper product alone.

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

284

285 **Discussion**

286 In this study, four trials in greenhouse conditions and two trials in an orchard were conducted to
287 assess the level of control of Psa on kiwifruit cv. Hayward, by using chemical and biological products.

288 Chemical control of bacterial diseases, and particularly of Psa, is highly dependent on spraying
289 antibiotics, such as streptomycin, or copper formulations (Cameron and Sarojini, 2014). In Europe
290 the use of streptomycin for control of plant pathogens is not legal, so copper formulations remain the
291 main management strategy for crop protection against bacterial diseases (Gullino and Brunelli, 2012).

292 Copper fungicides should be used carefully, particularly on young plants and during spring, because
293 low temperatures can induce phytotoxicity. In addition, copper bactericides have other possible
294 disadvantages after long-term use, including resistance to copper in bacterial populations (Rinaldi
295 and Leite, 2000) and the accumulation of copper metal in soils with potential environmental effects
296 (Alva et al., 1995). Repeated spraying with copper bactericides resulted in Japanese Psa strains
297 developing maximal copper resistance (Marcelletti et al., 2011). However, the strains of Psa from
298 the recent Italian outbreak were sensitive to copper compounds (Ferrante and Scortichini, 2010).

299 Traditional copper formulations, including Cu-hydroxide, Cu-oxychloride or their mixture, were
300 tested during the experiments, together with new experimental formulations of copper products,
301 which should have high biological efficacy achieved with much lower hectare rates. In the new
302 experimental formulations tested (IRF 120 and IRF 155) copper is complexed with organic
303 molecules, but the exact formulation is covered by trade secret. However, traditional copper
304 fungicides are preventive, acting by contact compounds, while copper complexes show also partially

305 systemic and curative properties. In the greenhouse and orchard experiments, copper hydroxide and
306 the mixtures of copper hydroxide and copper oxychloride, significantly reduced foliar symptoms of
307 the disease, and showed low phytotoxicity levels. In all the trials, the new formulate IRF 155 (copper
308 oxychloride + copper hydroxide) was one of the most promising products for bacterial disease control,
309 able to reduce the leaf disease symptoms by 70-80%, compared with the control.

310 Acibenzolar-S-methyl, a functional analogue of salicylic acid, has demonstrated good efficacy against
311 bacterial diseases, including bacterial spot and bacterial speck on tomato (Louws et al., 2011) and fire
312 blight in apples (Bastas and Maden, 2007). Similar efficacy was provided by acibenzolar-S-methyl
313 against Psa on kiwifruit. The product showed a high level of phytotoxicity in 2012, on one-year old
314 plants in pots, but it was much less phytotoxic on the same plants the following year suggesting that
315 younger plants are more susceptible. The use of acibenzolar-S-methyl has been temporarily extended
316 to kiwifruit in Italy, with a maximum of four treatments per year.

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

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

328 The antagonistic microorganisms tested, *Bacillus subtilis* and *B. amyloliquefaciens*, commercialized
329 mainly to control soilborne pathogens (Spadaro and Gullino, 2005) or for flowering treatments
330 (Oancea et al., 2009), showed a low efficacy in the protection of leaves and buds. *B.*

331 *amyloliquefaciens*, in particular, was effective against *Psa in vitro*, and was able to survive on
332 kiwifruit female flowers, reducing the *Psa* population on flowers (Biondi et al., 2012), by producing
333 iturins which disrupt pathogen cell wall production (Highland et al., 2012).
334 Even in absence of other treatments, a reduction of the disease was reported when using the mixture
335 of a steamed peat mixture substrate with compost obtained from digested organic matrices of
336 municipal solid waste. Composts could become an effective low cost tool to be included in the crop
337 protection strategy. Compost-amended soils offer the potential to manage soilborne diseases, but also
338 to reduce foliar diseases, by improving plant health and inducing systemic resistance (Hahn et al.,
339 2000). Infected radish and tomato grown in compost-amended substrates harbored significantly lower
340 populations of the bacterial pathogens *Xanthomonas campestris* pv. *armoraciae* and *Xanthomonas*
341 *campestris* pv. *vesicatoria*, respectively (Aldahmani et al., 2005).
342 In conclusion, no one product tested here could be considered as a suitable solution for the control of
343 *Psa* on kiwifruit, but in the framework of integrated control strategies, copper compounds alternated
344 with resistance inducers could be used in combination with compost, to develop new strategies to
345 reduce the disease development and spread. New strategies should be tested under natural conditions,
346 by considering the different climate conditions of the kiwifruit production areas, as it is not possible
347 to design one unique strategy to control *Psa* on kiwifruit for every region.

348

349 **Acknowledgements**

350 The authors wish to thank the Piedmont Region and the European Union in the framework of the
351 projects SAFEFOODCONTROL (European Fund for Development POR FESR 2007/2013, Axis I –
352 I.1.1 Innovative Platforms and PSR FEASR 2007/2013, European Fund for Rural Development,
353 Measure 124, Action 1), “Agrobiocat” and “ECOMOL”, Sustainable Chemistry Pole (European Fund
354 for Development POR FESR 2007/2013, Axis I – I.1.3 Innovation and SMEs) for the financial
355 support. A particular thank to Dr. Graziano Vittone (CReSO S.C. a R.L.) for his technical support
356 and to Dr. Kathryn Webb for her accurate English revision.

357

358 **References**

359

360 Alva AK, Graham JH, Anderson CA (1995). Soil pH and copper effects on young ‘Hamlin’ orange
361 trees. *Soil Science Society of America Journal* **59**, 481–487.

362 Armentano G (2010) La mappa del cancro batterico in Italia. *L’Informatore Agrario* **66 (25)**, 54.

363 Balestra GM, Mazzaglia A, Quattrucci A, Renzi M, Rossetti A (2009) Occurrence of *Pseudomonas*
364 *syringae* pv. *actinidiae* in Jin Tao kiwi plants in Italy. *Phytopathologia Mediterranea* **48**, 299-301.

365 Bastas KK, Maden S (2007). Evaluation of host resistance inducers and conventional products for
366 fire blight management in loquat and quince. *Phytoprotection* **88**, 93–101.

367 Biondi E, Kuzmanovic N, Galeone A, Ladurner E, Benuzzi M, Minardi P, Bertaccini A (2012).
368 Potential of *Bacillus amyloliquefaciens* strain D747 as control agent against *Pseudomonas syringae*
369 pv. *actinidiae*. *Journal of Plant Pathology* 94 (Supplement 4): S4.58.

370 Brown S, Koike ST, Ochoa OE, Laemmlen F, Michelmore RW (2004). Insensitivity to the
371 Fungicide Fosetyl-Aluminum in California Isolates of the Lettuce Downy Mildew Pathogen, *Bremia*
372 *lactucae*. *Plant Disease* 88, 502-508.

373 Butler MI, Stockwell PA, Black MA, Day RC, Lamont IL (2013) *Pseudomonas syringae* pv.
374 *actinidiae* from recent outbreaks of Kiwifruit Bacterial Canker belong to different clones that
375 originated in China. *PLoS ONE* **8 (2)**, e57464.

376 Cameron A, Sarojini V (2014). *Pseudomonas syringae* pv. *actinidiae*: chemical control, resistance
377 mechanisms and possible alternatives. *Plant Pathology* **63**, 1-11.

378 EPPO (2012) Pest Risk Analysis for *Pseudomonas syringae* pv. *syringae*. September 2012.
379 Available at: <http://www.eppo.org> . Accessed on September 20, 2013.

380 Everett KR, Pushparajah IPS, Vergara MJ (2012) *Pseudomonas syringae* pv. *actinidiae* on surfaces
381 in the orchard. *New Zealand Plant Protection* **65**, 19-24.

382 Everett KR, Taylor RK, Romberg MK, Rees-George J, Fullerton RA, Vanneste JL, Manning MA

383 (2011) First report of *Pseudomonas syringae* pv. *actinidiae* causing kiwifruit bacterial canker in New
384 Zealand. *Australasian Plant Disease Notes* **1**, 67–71.

385 FAOSTAT (2014) Available at: <http://faostat3.fao.org/faostat-gateway/go/to/browse/Q/QC/E>
386 Accessed on September 26, 2014.

387 Ferguson AR, Huang H (2007) Genetic resources of kiwifruit: domestication and breeding. In:
388 Janick J (ed) Horticultural reviews, vol 33. Wiley, Hoboken. doi:[10.1002/9780470168011.ch1](https://doi.org/10.1002/9780470168011.ch1)

389 Ferrante P, Scortichini M (2009) Identification of *Pseudomonas syringae* pv. *actinidiae* as causal
390 agent of bacterial canker of yellow kiwifruit (*Actinidia chinensis* Planchon) in central Italy. *Journal*
391 *of Phytopathology* **157**, 768–70.

392 Ferrante P, Scortichini M (2010) Molecular and phenotypic features of *Pseudomonas syringae* pv.
393 *actinidiae* isolated during recent epidemics of bacterial canker on yellow kiwifruit (*Actinidia*
394 *chinensis*) in central Italy. *Plant Pathology* **59**, 954-96.

395 Fratarcangeli L, Rossetti A, Mazzaglia A, Balestra GM (2010) Il ruolo del rame nella lotta al cancro
396 batterico del kiwi. *L'Informatore Agrario* **66 (8)**, 52-55.

397 Gent DK, Schwartz HF (2005) Management of *Xanthomonas* Leaf Blight of Onion with a Plant
398 Activator, Biological Control Agents, and Copper Bactericides. *Plant Disease* **89**, 631-639.

399 Gullino ML, Brunelli A, (2012) Prevenzione e difesa del kiwi dalla batteriosi da PSA. *Rivista di*
400 *Frutticoltura* **74 (S9)**, 20-24.

401 Hadar Y (2011) Suppressive compost: when plant pathology met microbial ecology.
402 *Phytoparasitica* **39**, 311-314.

403 Han DY, Coplin DL, Bauer WD, Hoitink HAJ (2000). A rapid bioassay for screening rhizosphere
404 microorganisms for their ability to induce systemic resistance. *Phytopathology* **90**, 327–332.

405 Highland H, Ockey S, Dimock M (2012). A new generation of bacterial biofungicides based on the
406 bacterium *Bacillus amyloliquefaciens* (strain D747) from Certis USA for use in vegetable and fruit
407 disease control. *Phytopathology* **102 (8)**, 53.

408 ISTAT (2011) Available at: <http://www.istat.it> Accessed on September 20, 2013.

409 Koh JK, Cha BJ, Chung HJ, Lee DH (1994) Outbreak and spread of bacterial canker in kiwifruit.
410 *Korean Journal of Plant Pathology* **10**, 68–72.

411 Lopez-Reyes JG, Spadaro D, Garibaldi A, Gullino ML (2010) Efficacy of plant essential oils on
412 postharvest control of rot caused by fungi on four cultivars of apples *in vivo*. *Flavour and Fragrance*
413 *Journal* **25**, 171-177.

414 Lopez-Reyes JG, Spadaro D, Prella A, Garibaldi A, Gullino ML (2013) Efficacy of plant essential
415 oils on post-harvest control of rots caused by fungi on different stone fruits *in vivo*. *Journal of Food*
416 *Protection* **76**, 631-639.

417 Louws FJ, Wilson M, Campbell HL, Cuppels DA, Jones JB, Shoemaker PB, Sahin F, Miller SA
418 (2001). Field control of bacterial spot and bacterial speck of tomato using a plant activator. *Plant*
419 *Disease* **85**, 481–488.

420 Marcelletti S, Ferrante P, Petriccione M, Firrao G, Scortichini M, 2011. *Pseudomonas syringae* pv.
421 *actinidiae* draft genomes comparison reveal strain-specific features involved in adaptation and
422 virulence to *Actinidia* species. *PLoS ONE* **6**, e27297.

423 Mazzaglia A, Studholme DJ, Taratufolo MC, Cai R, Almeida NF, Goodman T, Guttman DS,
424 Vinatzer BA, Balestra GM (2012) *Pseudomonas syringae* pv. *actinidiae* (PSA) isolates from recent
425 bacterial canker of kiwifruit outbreaks belong to the same genetic lineage. *PLoS ONE* **7** (5), e36518.

426 McCann HC, Rikkerink EHA, Bertels F, Fiers M, Lu A, Rees-George J, Andersen MT, Gleave AP,
427 Haubold B, Wohlers MW, Guttman DS, Wang PW, Straub C, Vanneste J, Rainey PB, Templeton
428 MD (2013) Genomic analysis of the kiwifruit pathogen *Pseudomonas syringae* pv. *actinidiae*
429 provides insight into the origins of an emergent plant disease. *PLoS Pathogens* **9** (7), e1003503.

430 Noble R, Coventry E (2005) Suppression of soil-borne plant diseases with composts: a review.
431 *Biocontrol Science and Technology* **15**, 3-20.

432 Oancea F, Cornea P, Popa G, Siciua O, Draganoiu M, Dinu S, Elad Y, Maurhofer M, Keel C, Gessler
433 C, Duffy B (2009). *Bacillus amyloliquefaciens* strain antagonistic to *Erwinia amylovora* and elicitor of
434 tomato defense response. *IOBC/WPRS Bulletin* **43**, 172.

435 Piedmont Region – AGRISTAT (2011) Available at:
436 <http://www.sistemapiemonte.it/anau/elenco.jsp> Accessed on September 20, 2013.

437 Pugliese M, Liu BP, Gullino ML, Garibaldi A (2011) Microbial enrichment of compost with
438 biological control agents to enhance suppressiveness to four soil-borne diseases in greenhouse.
439 *Journal of Plant Disease and Protection* **118**, 45-50.

440 Pugliese M, Liu BP, Gullino ML, Garibaldi A (2008) Selection of antagonists from compost to
441 control soil-borne pathogens. *Journal of Plant Disease and Protection* **115**, 220-228.

442 Quattrucci A, Renzi M, Rossetti A, Ricci L, Taratufolo C, Mazzaglia A, Balestra GM (2010) Cancro
443 batterico del kiwi verde: nuove strategie di controllo. *L'Informatore Agrario* **66 (16)**, 53-57.

444 Rees-George J, Vanneste, JL, Cornish DAS, Pushparajah IPS, Yu J, Templeton MD, Everett KR
445 (2010) Detection of *Pseudomonas syringae* pv. *actinidiae* using polymerase chain reaction (PCR)
446 primers based on the 16S–23S rDNA intertranscribed spacer region and comparison with PCR
447 primers based on other gene regions. *Plant Pathology* **59**, 453-464.

448 Renzi M, Mazzaglia A, Balestra GM (2012) Widespread distribution of kiwifruit bacterial canker
449 caused by the European *Pseudomonas syringae* pv. *actinidiae* genotype in the main production areas
450 of Portugal. *Phytopathologia Mediterranea* **51**, 402–409.

451 Rinaldi DAMF, Leite RP Jr (2000). Adaptation of *Xanthomonas axonopodis* pv. *citri* population
452 to the presence of copper compounds in nature. *Proceedings of the International Society of*
453 *Citriculture* **2**, 1064.

454 Scortichini M (1994) Occurrence of *Pseudomonas syringae* pv. *actinidiae* on kiwifruit in Italy.
455 *Plant Pathology* **43**, 1035-1038.

456 Scortichini M, Marcelletti S, Ferrante P, Petriccione M, Firrao G (2012) *Pseudomonas syringae* pv.
457 *actinidiae*: a re-emerging, multi-faceted, pandemic pathogen. *Molecular Plant Pathology* **13**, 631-
458 640.

459 Spadaro D, Amatulli MT, Garibaldi A, Gullino ML, Vittone G, Nari L, Pellegrino S, Morone C,
460 Mason G, Ortalda E, Grosso S (2010). È arrivato in Piemonte il cancro batterico del kiwi.

461 *L'Informatore agrario* **66** (27), 58-59.

462 Spadaro D, Gullino ML (2005). Improving the efficacy of biocontrol agents against soilborne
463 pathogens. *Crop Protection* **24**, 601-613.

464 Spadaro D, Nari L, Vittone G, Morone C (2011) La batteriosi del kiwi in Piemonte: diagnosi e
465 prevenzione. *Protezione delle colture* **4** (2), 58-61.

466 Takikawa Y, Serizawa S, Ichikawa T, Tsuyumu S, Goto M (1989) *Pseudomonas syringae* pv.
467 *actinidiae* pv. nov.: the causal bacterium of canker of kiwifruit in Japan. *Annals of Phytopathological*
468 *Society of Japan* **55**, 437-444.

469 Testolin R, Ferguson AR (2009). Kiwifruit (*Actinidia* spp.) production and marketing in Italy. *New*
470 *Zealand Journal of Crop and Horticultural Science* **37**, 1-32.

471 Tyson JL, Rees-George J, Curtis CL, Manning MA, Fullerton RA (2012) Survival of *Pseudomonas*
472 *syringae* pv. *actinidiae* on the orchard floor over winter. *New Zealand Plant Protection* **65**, 25-28.

473 Vanneste JL (2013) Recent progress on detecting, understanding and controlling *Pseudomonas*
474 *syringae* pv. *actinidiae*: a short review. *New Zealand Plant Protection* **66**, 170-177.

475 Vanneste JL, Poliakoff F, Audusseau C, Cornish DA, Paillard S, Rivoal C, Yu J (2011a) First report
476 of *Pseudomonas syringae* pv. *actinidiae*, the causal agent of bacterial canker of kiwifruit in France.
477 *Plant Disease* **95**, 1311.

478 Vanneste JL, Yu J, Cornish DA, Maxi S, Clarck G (2011b) Presence of *Pseudomonas syringae* pv.
479 *actinidiae*, the causal agent of bacterial canker of kiwifruit, on symptomatic and asymptomatic tissues
480 of kiwifruit. *New Zealand Plant Protection* **64**, 241-245

481 Vittone G, Nari L, Morone C (2011) Come affrontare in campo la batteriosi del kiwi. *L'Informatore*
482 *Agrario* **67** (18), 46-47.