Short Communication

FIELD EFFICACY OF A ZINC-COPPER-HYDRACID OF CITRIC ACID BIOCOMPLEX COMPOUND TO REDUCE OOZING FROM WINTER CANKERS CAUSED BY *PSEUDOMONAS SYRINGAE* pv. *ACTINIDIAE* TO *ACTINIDIA* spp.

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SUMMARY

Pseudomonas syringae pv. *actinidiae* (Psa) is the causal agent of bacterial canker of green-fleshed (*Actinidia deliciosa*) and yellow-fleshed (*A. chinensis*) kiwifruit and is causing severe economic losses worldwide. In Italy, a combined strategy that takes into consideration timely spray treatments to the plant canopy, a correct pruning management including the disinfection of the tools and the protection of the pruning cuts, the avoidance of excessive nitrogen fertilization and water stress, and training systems favoring a better air circulation, are giving satisfactory results in controlling the pathogen severity in the field. However, despite these efforts, the dormant phase of the plant still represents a main problem, especially in the areas where winter frosts regularly occur. During winter 2013-14 and 2014-15 in four sites, spanning from Northern to Southern Italy, the field efficacy of a zinc (4.7%)-copper (2.6%)-hydracid of citric acid (21.4%) biocomplex compound has been tested on *Actinidia deliciosa* and *A. chinensis* orchards. The biocomplex compound was applied three times, at a dose of 1.0%, to reduce the occurrence of bacterial exudates oozing out from the main trunk and leaders in early spring. During the trial, winter frosts were recorded in all sites. The biocomplex compound was more effective than a 1% Bordeaux mixture used under the same conditions. This biocomplex formulation could be used to effectively reduce the amount of exudates caused by Psa in areas where winter frost occurs regularly.

Keywords: kiwifruit bacterial canker, control, copper, zinc, cycle of disease, winter frost.

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Pseudomonas syringae pv. *actinidiae* (Psa) is the causal agent of bacterial canker of green-fleshed (*Actinidia deliciosa*) and yellow-fleshed (*A. chinensis*) kiwifruit and is causing severe economic losses worldwide (Scortichini *et al*., 2012; Donati *et al*., 2014; Tanner, 2015). In the recent years, many aspects related to this pandemic disease have been elucidated. In particular, in-depth studies have been performed to ascertain the geographic origin of the new Psa lineage (i.e., the so called Psa 3) of kiwifruit bacterial canker (Butler *et al*., 2013; Mc Cann *et al*., 2013), its population structure (Ferrante and Scortichini, 2010; Mazzaglia *et al*., 2012; Ferrante *et al*., 2015; Ciarroni *et al*., 2015), main virulence factors (Marcelletti *et al*., 2011; Mc Cann *et al*., 2013) and some basic features related to the interaction with the host plants (Petriccione *et al*., 2013, 2014, 2015; Patel *et al*., 2014). In addition, it is now possible to reliably detect Psa in both symptomatic and asymptomatic plant tissues by applying several detection techniques (Gallelli *et al*., 2011, 2014; Balestra *et al*., 2013; Ruinelli *et al*., 2016). Many aspects of the cycle of disease and the relationships between some agronomic techniques and the severity of symptoms have also been elucidated (Minardi *et al*., 2011; Stefani and Giovanardi, 2011; Vanneste *et al*., 2011; Ferrante *et al*., 2012; Tyson *et al*., 2012, 2015; Gao *et al*., 2016; Mauri *et al*., 2016). Copper-based compounds are widely used during both the vegetative season and the dormant period in all major areas of kiwifruit cultivation (Vanneste, 2013; Donati *et al*., 2014). Alternative treatments reduce the severity and the incidence of bacterial canker include chitosan (Scortichini, 2014) and acibenzolar-S-methyl (Cellini *et al*., 2014) as well as bacterial biocontrol agents (Tontou *et al*., 2016). In Italy, a combined strategy that takes into consideration timely spray treatments to the plant canopy, a correct pruning management including the disinfection of the tools and the protection of the pruning cuts, the avoidance of excessive nitrogen fertilization and water stress, and training systems favoring a better air circulation, are giving satisfactory results in controlling the pathogen severity in the field (Scortichini *et al*., 2014; Mauri *et al*., 2016).

Table 1. Number of days recorded during winter 2013-2014 and 2014-2015, with minimum temperature below °C, in the four sites hosting the field trials.

	Lagnasco (CN)	Buttapietra (VR)	Cisterna (LT)	Taurianova (RC)
2013-2014	13	13	0	1
2014-2015	18	47	2	14

However, despite these efforts, the dormant phase of the plant still remains the main problem, especially in areas where winter frosts regularly occur. It has been verified that short periods of temperatures below 0°C promote rapid Psa multiplication in both *A. deliciosa* and *A. chinensis* young plants (Ferrante and Scortichini, 2013). In addition, a 4°C temperature is very conducive for the systemic migration of Psa in *A. deliciosa* with movements within the twig of 14 cm over a five day period (Gao *et al*., 2016). Geographical areas of kiwifruit cultivation where winter temperatures range from just below to just over 0°C are present worldwide. In these areas, it has been observed that orchards showing very light symptoms of bacterial canker from spring to autumn (i.e., leaf spotting) often exhibit, at the end of winter, extensive bacterial exudates oozing out from small cankers along the main trunk and leaders. In many cases, this phenomenon takes place after the occurrenc, of days with temperatures varying around 0°C during winter. In addition, it is well known that drops of such exudates, carried by the rain and wind, can effectively spread the pathogen within and between the orchards (Serizawa *et al*., 1989; Ferrante *et al*., 2012). Consequently, a more stringent control programme should be applied in areas where winter frosts occur. This issue is further complicated by the fact that the plant is in a dormant stage (i.e., it cannot absorb and translocate active compounds) and that copper-based compounds cannot penetrate into the plant to exert their bactericidal effect.

The aim of this investigation was to determine the field efficacy of a zinc (4.7%)-copper (2.6%)-hydracid of citric acid (21.4%) biocomplex compound, patented in Israel, that would effectively reduce the amount of bacterial exudates oozing out of cankers during early spring following a previous multiplication of the pathogen during winter. The compound was preliminary tested *in vitro*, at a dose of 1.0%, for its bactericidal activity towards representative Psa 3 strains obtained from different continents, according to procedures described elsewhere (Ferrante and Scortichini, 2009, 2010).

The trials were performed during winter 2013-14 and 2014-15 at four sites from Northern to Southern Italy. These included mature orchards of *A. deliciosa* cv. Hayward in Lagnasco (Cuneo province, Northern Italy), Buttapietra (Verona province, Northern Italy), and Cisterna di Latina (Latina province, Central Italy) and of *A. chinensis* cv. Jin Tao in Taurianova (Reggio Calabria province, Southern Italy) trained as "pergola" (i.e., main trunk and two leaders). The trials were set up as a block design with 15 plants per treatment, per each block. Within the block, each treatment was separated from the other with one row of untreated plants. The main climatic parameters (i.e., rainfall and maximum and minimum daily temperature) were obtained from the local meteorological stations of the regional extension services. For each site, the number of days with minimum temperature below 0°C is reported in Table 1. The orchards were selected because they all had shown in the years preceding the trials symptoms of kiwifruit bacterial canker judged as medium-high. In each orchard, during the vegetative season the occurrence of leaf spotting, twig wilting and the reddening of the lenticels were observed. Moreover, in the area of the farm hosting the trial, any difference among the rows in the term of incidence and severity of the disease were recorded. During winter, before starting the trials, Psa 3 was isolated in all orchards from the main trunk and the leaders according to procedures described elsewhere (Ferrante *et al*., 2012). However, at the time of the first spray treatment (i.e., mid-December), exudates were not observed in all orchards. In all sites, the presence of Psa in exudates oozing from the trunk and leaders during early spring was confirmed by plating some drops of the exudates on plates of nutrient agar containing sucrose (3.0%) and incubating at 23-25°C for two days. Representative colonies from all orchards were identified as described by Ferrante and Scortichini (2009, 2010).

The efficacy of the zinc-copper-hydracid of citric acid biocomplex compound was compared to a standard copper compound (Bordeaux mixture, containing 25% of active copper). Both compounds were sprayed three times (mid-December, mid-January and mid-February), directly to the main trunk and leaders using the farm atomizer, at a dose of 1.0% in 300 l of water per hectare. Untreated plants were used as negative controls. The efficacy of the treatments were determined in early spring by counting the total number of exudates per each plant observed along the main trunk and the leaders. The field efficacy of the tested compounds was expressed as mean standard deviation of data. Data were subjected to one-way analysis of variance and comparison among the means was determined by least significant difference (LSD) test. Significant differences are revealed by different letters at $P < 0.05$.

The results of the efficacy of the tested compounds are shown in Fig. 1. The spraying of bactericides significantly reduced the amount of bacterial exudates in comparison with the untreated plants. The zinc-copper-hydracid of citric acid biocomplex compound was more effective than the Bordeaux mixture in both years and in all orchards reducing the mean number of exudates per plant to < 1.0 (except Taurianova in winter 2013-2014). In contrast, the mean number of exudates was closer to 2.0 per plant after treatment with Bordeaux mixture. The identification of the isolates collected from the exudates confirmed the presence of Psa in all orchards. It should be noted that in

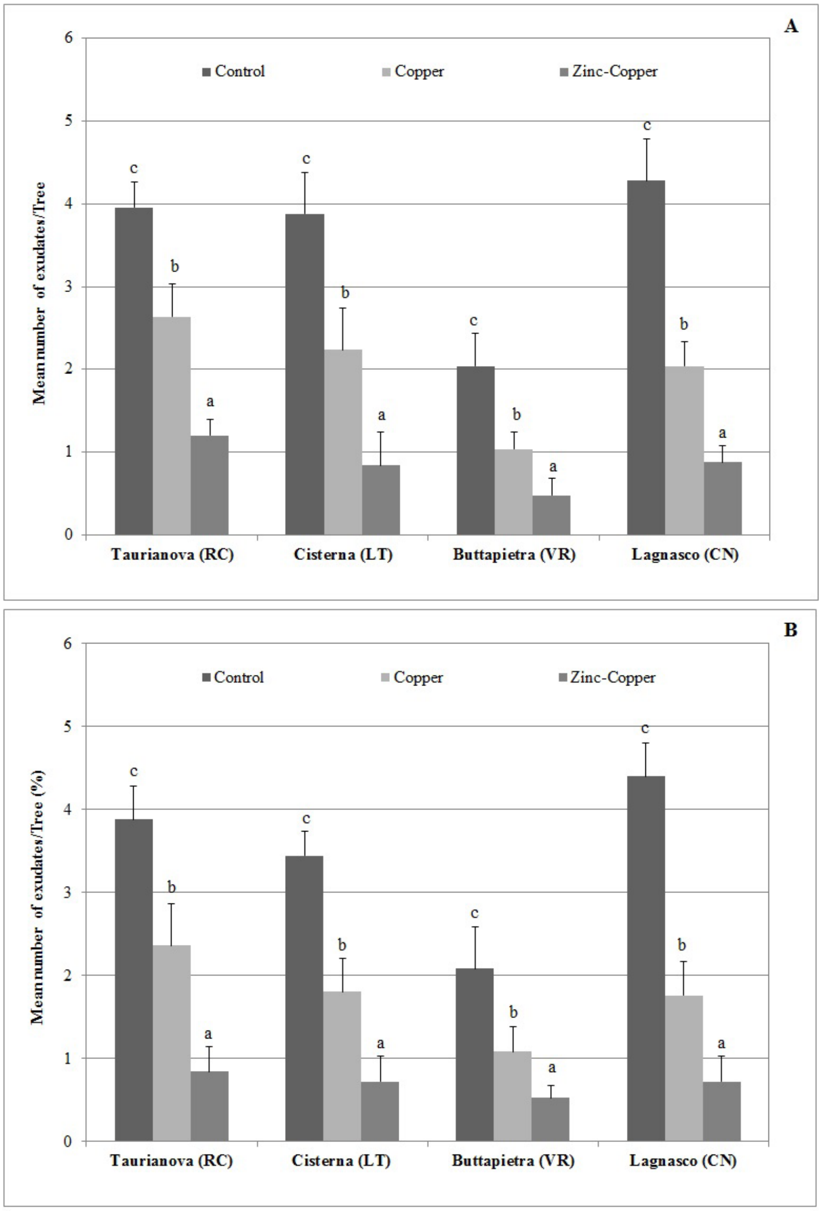

Fig. 1. Field efficacy of a zinc (4%)-copper (2%)-citric acid complex compound (1.0%) and Bordeaux mixture (1.0%) towards *Pseudomonas syringae* pv. *actinidiae* during winter 2013-2014 (A) and 2014-2015 (B), recorded as the mean number of bacterial exudates per tree. Control plants did not receive any treatment. Significant differences are revealed by different letters at P<0.05, according to the least significant difference (LSD).

three of the four sites, namely Lagnasco, Buttapietra and Taurianova, during both winter 2013-2014 and 2014-2015 were recorded some days with temperatures below 0°C, whereas in Cisterna di Latina temperatures below 0°C were recorded only during winter 2014-2015. In particular, in Buttapietra (Verona province) during both winter seasons were recorded long periods (i.e., about two weeks) with minimum temperature continuously lower than 0°C, with peaks of –6.0°C (i.e., December 17, 2013 and December 31, 2014). In Taurianova (Southern Italy), during winter 2014-2015, the minimum temperature was below 0°C for four days (i.e., from December 30, 2014 to January 2, 2015), with a peak of –5°C (i.e., January 2, 2015). These records strongly confirm low temperatures increase the rapid multiplication of Psa (Ferrante and Scortichini, 2013; Gao *et al*., 2016).

This study demonstrates that a patented zinc-copper-hydracid of citric acid biocomplex compound can significantly reduce the occurrence of bacterial oozing from trunk and leaders of both *A. deliciosa* and *A. chinensis* plant infected by Psa. The application of zinc plus copper as single compound is not a novel treatment. Due to the wide occurrence of copper-resistant strains, walnut (*Juglans regia*) blight, caused by *Xanthomonas arboricola* pv. *juglandis*, is successfully controlled in California (U.S.A.) by using zinc-copper Bordeaux (Adaskaveg *et al*., 2015). The biocomplex compound tested here may effectively penetrate into the kiwifruit epidermis and reach the tissues (i.e., parenchyma, phloem and xylem) where Psa survives during winter (Gao *et al*., 2016) due to the very high purity (i.e., 98.0%) of the hydracids of citric acid biocomplex. This formulation provides a promising compound to effectively reduce the amount of Psa exudates for the areas where winter frost occurs regularly.

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