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ORIGINAL ARTICLE

Evaluation of a new natural adjuvant obtained from locust bean gum to reduce the amount of copper necessary to control downy mildew of grapevine

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Abstract Two field trials in integrated cultivation (2014 and 2016) were carried out with the aim to evaluate the efficacy and the persistence of copper oxychloride to control downy mildew of grapevine in mixture with a new natural adjuvant (PSS®) derived from locust bean (*Ceratonia siliqua* L.) gum. Copper amount applied in vineyards is the subject of interest for its use in organic farming due to the concern about its ecotoxicological profile in the soil that need to modify its use in rates, strategy and total amount per season. Copper oxychloride was applied every 7 and 14 days mixed or not with locust bean gum. Locust bean gum was also applied alone. To evaluate the distribution of copper on grapevine leaves, observations by scanning electron microscope were made. Locust bean gum alone did not influence the development of disease, while significantly increased efficacy and persistence of copper oxychloride both on leaves and bunches. On bunches, at 14 days interval, efficacy on incidence of copper oxychloride plus locust bean gum was about 60% while efficacy on severity was 60.1 and 65.6% for the year 2014 and 2016, respectively. With no locust bean gum, at the same interval, efficacy was significantly lower. Scanning electron microscopy demonstrated that copper fungicide particles when mixed with locust bean gum were still uniformly distributed on leaves after 14 days from application, but if applied alone already after 7 days became scattered with a lower concentration. To our knowledge, it is the first time that galactomannans-derived compounds have been used as adjuvant in pesticides. The use of locust bean gum reduced the annual amount of copper in integrated cultivation from 7.9 kg ha^{-1} applying copper oxychloride every 7 days to 4.5 kg ha^{-1} when applied every 14 days mixed with the new locust bean gum sticker.

Keywords Biodegradable sticker · Downy mildew · Grapevine · SEM analysis · Galactomannans · Locust bean gum · *Plasmopara viticola* · Adjuvant · Copper fungicides

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Introduction

Copper compounds are largely used in agriculture in the control of a wide range of plant diseases caused by fungi. Copper has been used for the first time as fungicide in 1761; for more than 100 years, it was the only fungicide used to control the oomycetes *Plasmopara viticola* the causal agent of downy mildew. However, its effect is not sufficient to provide protection unless combined with several other methods and even then, if the climatic conditions are too favorable to the disease, organic farmers have little means to combat downy mildew effectively except for copper sprays (Finckh et al. 2015). Copper is the subject of new interest for two opposite reasons: The first is the interest for the use in organic farming and the second the concern about its ecotoxicological profile particularly due to accumulation in the soil (Mackie et al. 2012). This new scenario modified the phytosanitary use of copper in the rate, strategy and created the need to reduce the total amount used.

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Copper does not degrade in the soil, leading to its accumulation into agricultural soils and watercourses, and it is toxic for terrestrial fauna. For this reason, the amounts of copper compounds are limited in organic farming: 6 kg $year^{-1}$ ha^{-1}, according to EU regulation (889/2008). Sustainable methods to reduce toxicological impact of copper pollution and copper residues on plant products for human consumption should be explored.

Number and interval of copper applications depend on plant growth, washout, risk of infection, disease pressure and quality of distribution on leaves. Formulation (Stevens 1993) and adjuvants could play an important role in increasing bioavailability and regulate frequency of application. The addition of an appropriate adjuvant to a foliar fungicide can significantly improve coverage, absorption, efficacy and can reduce the total amount applied in a season. Field studies evaluated coverage, absorption and efficacy of commercial adjuvants with diverse chemistries on multiple hosts–pathogen systems (Finckh et al. 2015; Gent et al. 2003; Grayson et al. 1996a, b). Pesticide adjuvants are additives used for many purposes: to increase persistence on leaves (Steurbaut 1993); regulate absorption, spray retention (Hart et al. 1992) rainfastness (Kudsk et al. 1991) foliar washoff and runoff losses (Reddy and Locke 1996), evaporation and spread (Xu et al. 2010) and pesticide translocation (Maschoff et al. 2000; Orbovic et al. 2007). Moreover, adjuvant can influence the efficacy of pesticides negatively or positively (Grayson et al. 1996a, b; Krogh et al. 2003; Percich and Nickelson 1982; Rogiers et al. 2005; Rowen 1979; Van Zyl et al. 2010a, b). In most part, adjuvants were used to obtain both economic and environmental benefits (Kirkwood 1993; Van Zyl et al. 2010a). Regarding grapevine, Van Zyl et al. (2010a) reported the effect on grapevine leaves and efficacy toward *B. cinerea* of 3 surfactants alone, in mixtures with acid or vegetable oil compared with a sticker reporting the potential as well as the problems that can be encountered using adjuvants to improve spray application in grapevines. Rogiers et al. (2005) reported an increasing in susceptibility to *B. cinerea* due to the influence of adjuvants on epicuticular wax and grape berry microflora. A few studies in the last years were published on the interaction between copper fungicides and adjuvant in particular on citrus fruits (Orbovic et al. 2007; Schutte et al. 2012). Only 3% of publications about adjuvants were referred to fungicides use (Steurbaut 1993); actually, the number of work on this matter decreased further. Definitely, field studies on spray adjuvants with the aim to improve fungicide utility and efficacy have been demonstrated with few chemistries and crop species (Gent et al. 2003).

Among different chemicals, polysaccharides—such as chitosan (Feliziani et al. 2015), carrageenan (Patier et al. 1995), fucans (Lizzi et al. 1998), laminarins (Aziz et al. 2003) and ulvans (Loffaguen et al. 2004)—have been tested on economically relevant crops, prevalently to investigate their interaction with the plants and the eventual ability to induce resistance to fungal pathogens. Galactomannans are used in edible films or coatings for food (Cerqueira et al. 2011) make them very interesting for any potential use in agriculture.

In this study, we consider a new polysaccharide gel used as a natural adjuvant to reduce the amount of copper need to control *Plasmopara viticola*. The use of natural polysaccharides, extracted from locust bean gum (Svenssons and Svensson 2010) as adjuvant of copper fungicides has never been reported.

In this work, two field trials were carried out with the aim to: (i) evaluate the effect of copper oxychloride associated with locust bean gum on the efficacy to control *P. viticola*; (ii) reduce the total amount of copper used; (iii) evaluate the persistence of copper associated with locust bean gum on grapes and leaf surfaces.

Materials and methods

PSS preparation and phytotoxicity

For experimental activity, a polysaccharide gel that contains galactomannans from locust bean gums was used (PSS®). PSS consisted of 2% w/v of locust bean gum. In this gel, there is also 0.2% w/v of agar used for its physical reticulation. In order to inhibit the agglomeration of locust bean gum, it was introduced ethanol (1% w/v). Finally, the mixture contains 1% w/v of glycerol. This composition is stable to water. The gel has a capacity of hydration and expansion equal to four times of its initial volume without breaking. This is the composition of the adjuvant before adding copper. Degradation time is equal to 4 weeks. Assessments of phytotoxicity were performed according to OEPP/EPPO guideline (OEPP, EPPO Bullettin 2014) using an empirical scale ranging from 1 (no symptoms) to 9 (leaves or bunches completely covered by necrotic spots).

Field trials

Two trials were carried out in 2014 and 2016 to compare the following treatments: (i) untreated control (UC); (ii) locust bean gum adjuvant applied alone (PSS); (iii) copper oxychloride and PSS adjuvant every 7 days (CuPSS7); (iv) copper oxychloride and PSS adjuvant every 14 days (CuPSS14); (v) copper oxychloride alone every 7 days (Cu7); vi) copper oxychloride alone every 14 days (Cu14).

The trials were carried out in two commercial integrated vineyards at Tufo (41°0′48.96″N. 14° 49′7.32″E), Avellino province in 2014 and Torrecuso (41°11′25″44 N.

14°40′48″72E) Benevento province in 2016, both located in South of Italy. The vine was Aglianico at Tufo and Falanghina at Torrecuso. Aglianico is utilized to produce a very famous DOCG wine (Taurasi) while F Trials dimensions were 2000 m^2 (45 m^2 each treatments) using 10 plants per plot. The two trials were planned using a randomized complete block design (RCBD) with four replicates. Rate of commercial product Cuproram (Arysta Lifescience) was 3000 g ha^{-1} (containing 37.5% of copper oxychloride); the water volume used for each application was 1000 l ha^{-1}. Formulated locust bean gum, where necessary, was added at the rate of 3000 g ha^{-1}. Dates of applications, for both trials, are reported in Table 1. The number of applications in the treatment PSS, Cu7 and CuPSS7 was 7, and only 4 in the remaining treatments. After 21 days from the last application, on bunches were performed residues analyses by a private company (Laboratoria, Naples, Italy). In Italy, the time from the last application and harvesting reported on the label for Cuproram is 20 days while the maximum residues allowed is 50 mg kg^{-1}.

Meteorological data

Meteorological data were collected during the test using a data logger for daily recording of temperatures (EL-USB-2-LCD, Lascar Electronics, UK), and a rain gauge (HD2013, DELTA OHM) was used to measure the rainfalls that occurred during the trials.

Efficacy assessment

Observations were made out weekly, and the data of the last two (2014) or three (2016) assessments were used to evaluate efficacy. Efficacy has been assessed according to guideline OEPP/EPPO (2001) for *Plasmopara viticola.* Incidence was assessed as diseased leaves/total leaves observed × 100. Severity was assessed by evaluating % of leaf surface affected by symptoms on 100 leaves per plot as with incidence. At the end of trial periods, one assessment was performed on bunches using the same method as for leaves for incidence and to assess severity percent of affected grapes per bunch was evaluated. Efficacy data were measured as Abbott index. Severity data were not normally distributed so they needed to be transformed in angular values, following the formula arcsen$\sqrt{}$relative value. All data were submitted to analysis of variance and mean were separated according to Tukey test ($p \leq 0.05$). All statistical calculations were made using the STATISTICA, software package (StatSoft Inc., Tulsa, OK, USA).

Table 1 Dates of applications for the 2 years of field trial

Treatment/initials	Date of applications	
	2014	2016
Untreated control (UC)	–	–
Treatments carried out every 7 days	June 03	May 12
PSS	June 10	May 19
CuPSS7	June 17	May 26
Cu7	June 24	June 02
	July 01	June 09
	July 08	June 16
	July 15	June 23
Treatments carried out every 14 days	June 03	May 12
CuPSS14	June 17	May 26
Cu14	July 01	June 09
	July 15	June 23

Scanning electron microscopy (SEM) observations

In order to investigate the distribution of sprayed materials on plants after 8 and 14 days after the last treatments, we performed a SEM morphological analysis on leaves picked at different times. SEM allowed us: (i) to investigate the physical interaction PSS-plant at interface on leaves; (ii) to evaluate if PSS film covers leave surfaces in a homogeneous way; (iii) to analyze the layout of copper in the film after spraying and (iv) to assess copper and PSS persistance on vine leaves. Micrographs on grapevine leaf surfaces were taken by using a scanning electron microscope FEI Quanta 200 FEG. Before observation, the samples were coated with Au–Pd alloy by means of a sputtering device (MED 020. Bal-Tec AG). The coating provided the entire sample surfaces with a homogeneous layer of metal alloy of 18 ± 0.2 nm. Energy-dispersive X-ray spectrometry was performed by using an Oxford INCA Energy system 250 equipped with INCAX-act detector. Three samples from leaf surfaces for each treatment applied were collected from the field and then observed.

Results

PSS preparation and phytotoxicity

The formulation of PSS used did not show any phytoxicity symptoms when applied alone or when applied in combination with copper oxychloride in both years on the two different cultivars tested.

Field trials

Meteorological data

In 2014, the rainfall of early May were not able to induce disease because of the low temperature (below 10 °C) registered in the same period. Disease favorable rains occurred probably between 25 and 31 May and then the infection was maintained by a succession of rainy events that characterized the entire season the longer period of incubation should be explained with the level of temperature in that period lower than the optimal one (24 °C) (Fig. 1). During the trial in 2016, disease favorable rains occurred between 11 and 19 May (Fig. 2) and then the infection has been maintained by the rains that occurred in the second half of June and the heavy rainfall of July 14.

Trial 2014

In 2014, the outbreak of symptoms was at June 18 after three applications in the plots of PSS, Cu7 and CuPSS7 and two applications in the plots of treatments Cu14 and CuPSS14 (Table 1). At the end of the trial, the disease incidence in untreated control plots was 88.3%.

The treatments with only locust bean gum (PSS) showed a value equal to 71.4% as disease incidence and 50.1% for severity. In the plots Cu7 and Cu14, the values of incidence were 44 and 54%, respectively, with a similar severity of 38.5 and 38.7%. Better results were obtained when PSS was added to copper in plots CuPSS7 and CuPSS14 with values of incidence equal to 37 and 33%, respectively (Fig. 3).

The severity was 24.8 and 24% for CuPSS7 and CuPSS14, respectively (Fig. 4). The efficacy on incidence was higher for CuPSS7 and CuPSS14 with Abbott's values of 58.1 and 62.6%, while Cu7 and Cu14 gave efficacy of 50.2 and 38.8%, respectively. CuPSS7 and CuPSS14 showed also higher efficacy in reducing the severity. At the end of the trial, we observed an efficacy above 53% with no statistical significant differences between CuPSS7 and CuPSS14 (Tables 2, 3). Table 4 shows the assessment on bunches, incidence in UC and PSS were 73.2 and 70.3, respectively, and severity was 35.2 for UC and 33.2 for PSS. PSS did not influence the incidence and severity when applied alone. Efficacy was above 60% for CuPSS7 and CuPSS14 both for incidence and severity of symptoms.

Trial 2016

In 2016, the outbreak of symptoms was recorded at June 3 after four applications of copper and PSS in the plots PSS, Cu7 and CuPSS7 while two applications were made at that time in the plots Cu14 and CuPSS14 (Table 1). The incidence of disease in UC plots at the end of trial was 100%, and severity was 70.7%. PSS alone gave similar statistical results of UC confirming that it has no direct effect on *P. viticola*. Cu14 gave higher significant value of incidence (68%) than CuPSS7 and CuPSS14 (43 and 47%,

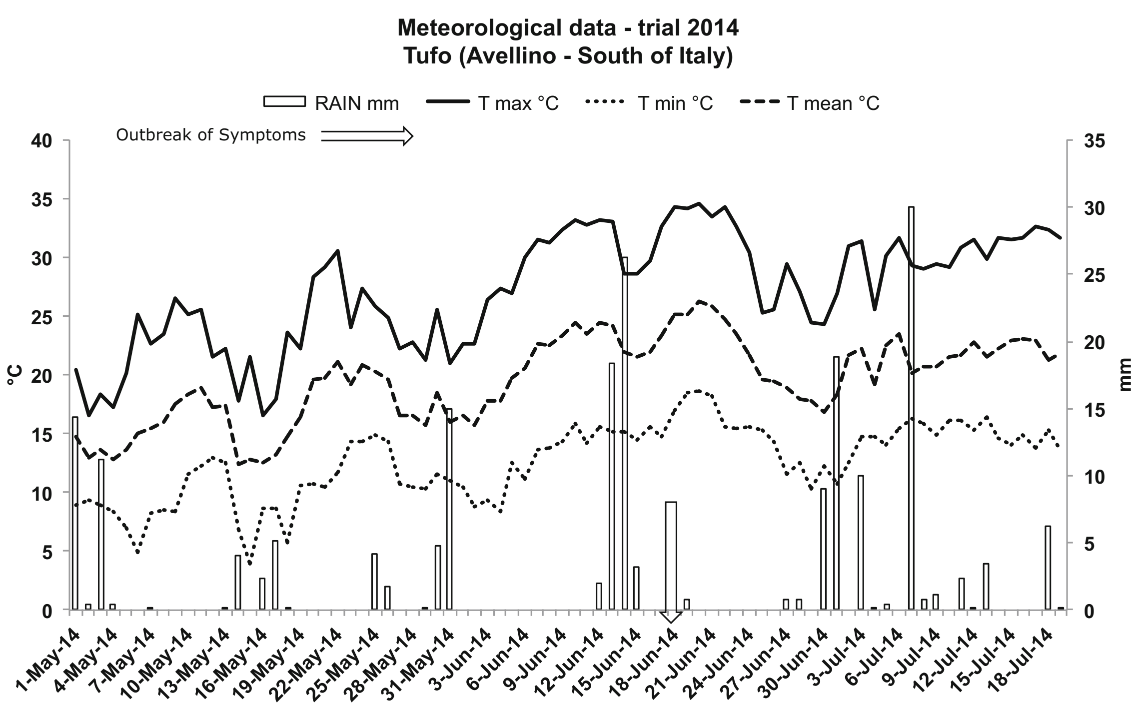

Fig. 1 Environmental parameters; mean temperatures (°C) and rainfall (mm) of the area where the experimental trial was carried out in 2014

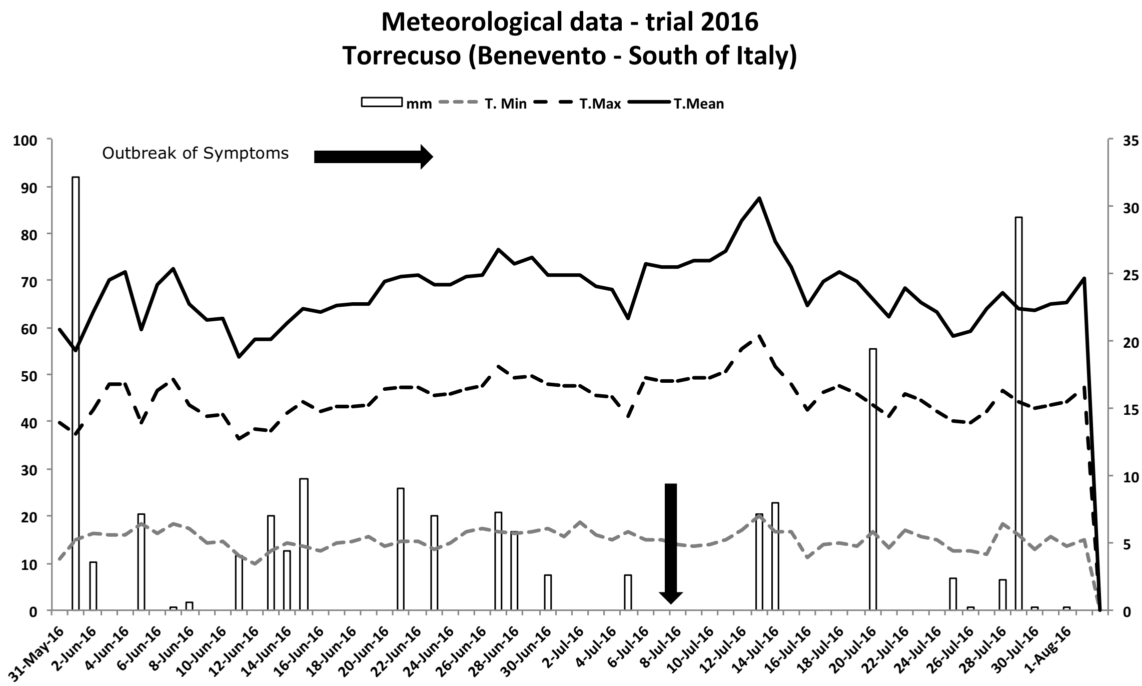

Fig. 2 Environmental parameters; mean temperatures (°C) and rainfall (mm) of the area where the experimental trial was carried out in 2016

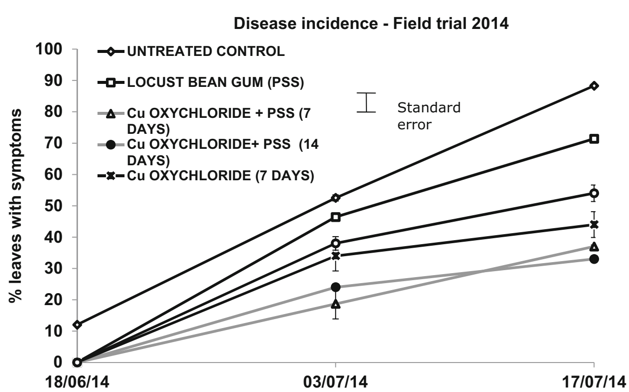

Fig. 3 Time course of disease incidence on leaves during field trial 2014 at Tufo (Avellino, South of Italy)

respectively) and gave the same results of Cu7 (49%), (Fig. 5). Severity was significantly lower for CuPSS7 and CuPSS14 (11.9 and 11.1%, respectively) than Cu7 and Cu14 (21.6 and 29.9%), (Fig. 6).

Efficacy on incidence was statistically the same for CuPSS7 (57%), CuPSS14 (53%) and Cu7 (51%), while was significantly lower for Cu14 (32%). Efficacy on symptoms severity was statistically the same for CuPSS7 (83.2%) and CuPSS14 (84.3%) while was significantly lower for Cu14 and Cu7 (Tables 2, 3).

In the trial 2016 in UC plots, bunches had a disease incidence of 68.2 and a severity of 36.6. PSS had the same statistically level of disease of UC, confirming the results of the trial performed in 2014. Both CuPSS7 and CuPSS14 showed lower significant disease incidence and severity than Cu7 and Cu14. Cu14 gave results not significantly different from UC. CU14 were completely ineffective on bunches. Efficacy greater than 60 and 64% was obtained, respectively, for CuPSS7 and CuPSS14 (Table 5).

The level of copper residues on bunches 21 days after the last application in both CuPSS7 and CuPSS14 was less than 2.5 mg kg^{-1}.

Scanning electron microscopy (SEM) observations

Figure 7 shows the presence of copper oxychloride particles on leaf surfaces. Energy-dispersive X-ray spectrometry analysis confirmed that they were copper particles. Table 6 reports the data of element analysis in three different areas with different apparent concentrations of copper. Analysis confirmed that where particles were more evident (spectrum 1) the presence of copper was 7.66% while was below the detection level where very low particles were apparently present (spectrum 2). Copper concentration was 2.93% in the spectrum 3 that represents an intermediate situation. Figure 8 shows that 8 days after treatment, on Cu7 leaves, the copper particles are scattered or localized only in some areas. Figure 9 shows that copper is uniformly distributed on leaves surface of CuPSS7. After 14 days (Fig. 10), copper still remained uniformly distributed on leaves surface of CuPSS14 treatment.

Figure 9 shows that copper is uniformly distributed on leaves surface of CuPSS7. After 14 days (Fig. 10), copper

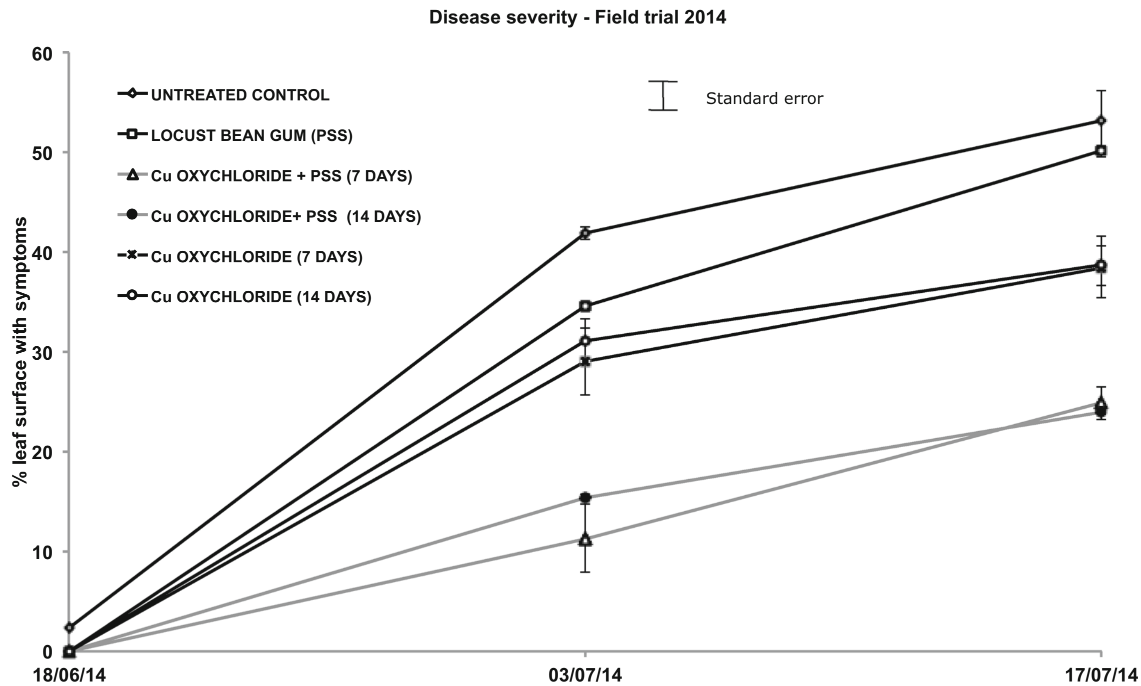

Fig. 4 Time course of severity on leaves during field trial 2014 at Tufo (Avellino, South of Italy)

Table 2 Efficacy on disease incidence (Abbott's) on leaves

	2014		2016		
	July 03	July 17	June 03	June 08	June 22
PSS	11.6c	19.1c	8.1b	6.5b	13.6c
CuPSS 7	64.4a	58.1a	100a	100a	57.0a
CuPSS14	54.3a	62.6a	91.9a	90.7a	53.0a
Cu7	35.2b	50.2a	100a	99.1a	51.0a
Cu14	27.6b	38.8b	98.2a	99.1a	32.0b

Locust bean gum (PSS); Cu Oxychloride + PSS every 7 days (CuPSS7); Cu Oxychloride + PSS every 14 days (CuPSS14); Cu Oxychloride every 7 days (Cu7); Cu Oxychloride every 14 days (CuPSS14). Means with the same letters are not significant different at Tukey's test ($p = 0.05$)

Table 3 Efficacy on disease severity on leaves (Abbott's)

	2014		2016		
	July 03	July 17	June 03	June 08	June 22
PSS	17.4c	5.8b	11.8b	14.1b	8.3d
CuPSS 7	73.0a	53.3a	100a	100a	83.2a
CuPSS14	63.1a	54.9a	96.5a	97.6a	84.3a
Cu7	30.6b	46.4a	100a	99.8a	69.4b
Cu14	25.9b	46.1a	99.5a	99.8a	57.7c

Locust bean gum (PSS); Cu Oxychloride + PSS every 7 days (CuPSS7); Cu Oxychloride + PSS every 14 days (CuPSS14); Cu Oxychloride every 7 days (Cu7); Cu Oxychloride every 14 days (CuPSS14). Means with the same letters are not significant different at Tukey's test ($p = 0.05$)

still remained uniformly distributed on CuPSS14 leaves surface.

Discussion and conclusion

The aim of this study was to consider a new polysaccharide gel used as a natural adjuvant to reduce the annual amount of copper need to control downy mildew of grapevine. Downy mildew occurred in both years of trials at high level of incidence and severity of symptoms; this allowed evaluating the role of PSS adjuvant in the control of disease. The similar results obtained in the 2 years (2014 and 2016) demonstrated that meteorological conditions during the trials were favorable to the disease development and leaching of copper fungicides from leaves when applied alone. The addition of PSS in both years prolonged the efficacy of copper fungicide with no significant interactions between the quantity and distribution of rainfall in the 2 years and PSS performance.

Spray adjuvants are added to a spray tank to improve the performance of the treatment, but in order to develop new adjuvants it is necessary taken into account several aspects. Many agrochemical products are formulated with adjuvants and may not need an additional one. An appropriate adjuvant should improve fungicide performances by

Table 4 Trial 2014. Data of disease incidence, severity of symptoms and efficacy (Abbott's) on bunches

	July 17, 2014			
	Incidence	Efficacy	Severity	Efficacy
UC	73.2a	–	35.2a	–
PSS	70.3a	4.3d	33.2a	5.7c
CuPSS 7	29.0d	61.2a	13.8b	60.1a
CuPSS14	28.4d	60.3a	13.9b	60.1a
Cu7	43.7c	40.5b	29.3a	16.8b
Cu14	60.7b	17.1c	35.2a	0c

Untreated control (UC); Locust bean gum (PSS); Cu Oxychloride + PSS every 7 days (CuPSS7); Cu Oxychloride + PSS every 14 days (CuPSS14); Cu Oxychloride every 7 days (Cu7); Cu Oxychloride every 14 days (CuPSS14). Means with the same letters are not significant different at Tukey's test ($p = 0.05$)

reducing the amount of active ingredient per hectare and prolonging spray intervals (Orbovic et al. 2007; Patier et al. 1995). Improper use of an adjuvant may result in poorer performance and possible phytotoxicity as well as increasing the cost of treatment. On grapevine, some spray adjuvants—such as ethoxylated phenol, alcoxylated alcohols, emulsifiable vegetable oil and polyether—may cause an alteration of epicuticular wax morphology and natural microflora, rendering the grapevine more susceptible to *B. cinerea* (Reddy and Locke 1996). The role of adjuvants in pesticide performances varies depending on their chemical properties (Green and Beestman 2007). Xu et al. (2010) reported that 4 oil-based adjuvants improved spread and droplets evaporation rates, while Gent et al. (2003) reported that some adjuvants increased the efficacy of a fungicide even if no variations in coverage were recorded.

Moreover, adjuvants are reported to improve bioefficacy of the fungicide fenhexamide on grapevine leaf surfaces, by improving spray deposition quantity and quality (Svenssons and Svensson 2010; Van Zyl et al. 2010a). Furthermore, 35-day interval applications of copper fungicides, using adjuvants and three different formulations, were effective against citrus black spot in South Africa (Rowen 1979).

Some adjuvants (urea and petroleum spray oil) did not improve penetration of copper through leaf and fruit cuticles, while organosilicone surfactant enhances the penetration of Cu into citrus leaves and fruit thereby leading to phytotoxicity, also producing some concerns about residue levels (Orbovic et al. 2007).

Our results pointed out that no interaction existed between the PSS applied alone and downy mildew epidemiology; PSS applied alone gave the same incidence and severity of untreated control, not influencing grapevine susceptibility to *P. viticola*. SEM observations performed right after the application (2 h) showed no difference in spreading and covering of copper alone, while copper particles remained longer on leaf surfaces when PSS was added. Many studies have shown the benefits of adjuvants in laboratory and in controlled conditions (Steurbaut 1993), but almost all of them gave negative results in the field. In the present study, PSS was tested on grapevine in the field

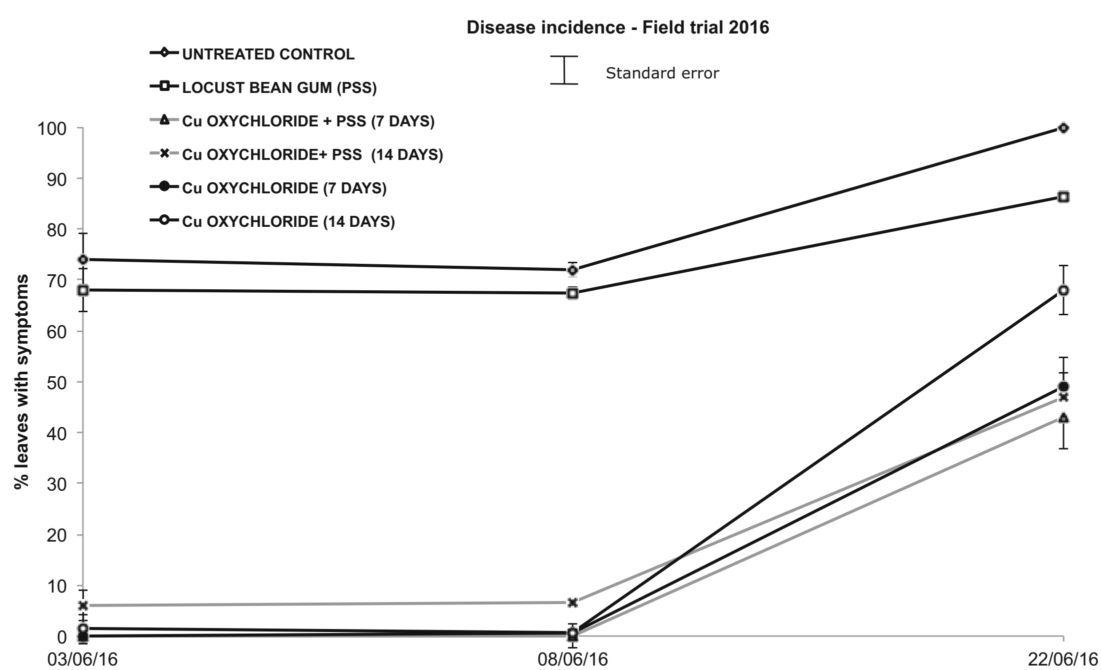

Fig. 5 Time course of disease incidence during field trial 2016 at Torrecuso (Benevento, South of Italy)

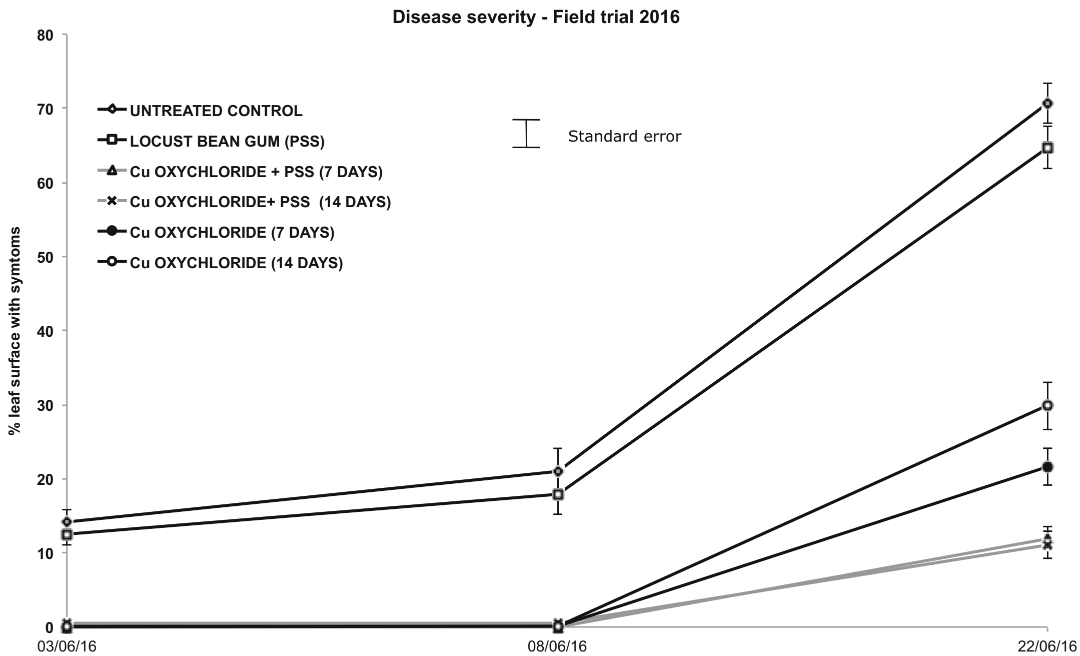

Fig. 6 Time course of severity during field trial 2016 at Torrecuso (Benevento, South of Italy)

Table 5 Trial 2016. Data of disease incidence, severity and efficacy (Abbott's) on bunches

	June 22, 2016			
	Incidence	Efficacy	Severity	Efficacy
UC	68.2a	–	36.6a	–
PSS	69.8a	0.0d	38.3a	0.0c
CuPSS 7	26.8d	61.9a	16.9b	64.6a
CuPSS14	27.9d	60.3a	16.6b	65.6a
Cu7	38.4c	44.9b	30.7a	19.3b
Cu14	58.3a	15.7c	38.4a	0.0c

Untreated control (UC); Locust bean gum (PSS); Cu Oxychloride + PSS every 7 days (CuPSS7); Cu Oxychloride + PSS every 14 days (CuPSS14); Cu Oxychloride every 7 days (Cu7); Cu Oxychloride every 14 days (CuPSS14). Means with the same letters are not significant different at Tukey's test ($p = 0.05$)

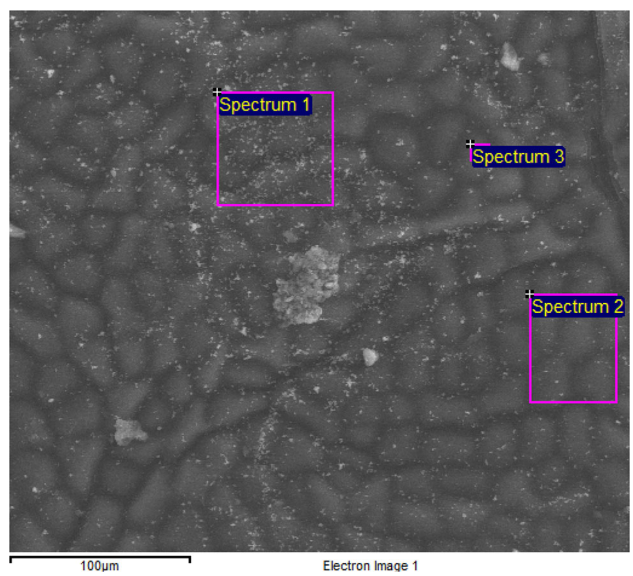

Fig. 7 Upper leaf surface treated with copper oxychloride alone after 2 days from application and small areas (Spectrum) in which analyses were performed

and resulted in a longer persistence rendering copper oxychloride more effective. We demonstrated that PSS is an appropriate adjuvant that could improve disease control and may allow longer fungicide application intervals. In this case, PSS reduced the annual amount of copper from 7.9 kg ha^{-1} applying copper oxychloride every 7 days (7 applications per year) to 4.5 kg ha^{-1} when applied every 14 days mixed to PSS. In years like 2014 and 2016 in which *P. viticola* infection resulted in huge product loss, especially in organic cultivations (> 70%), the use of PSS as adjuvant gave the possibility to use copper fungicides having longer persistence, contemporary maintaining the permissible total amount of copper per year and a satisfactory effectiveness, maintaining the allowed minimum residue level of copper on grape berries. On the other hand, our field studies demonstrated that copper oxychloride did not bind strongly within spray droplets containing PSS, allowing copper oxychloride to control fungal disease. This property is confirmed by the increased persistence and efficacy of copper oxychloride in mixture with PSS. PSS seemed acting as stickers enhancing the adherence of

Table 6 Data of analysis performed in three small areas (Spectrum), as reported by SEM equipment, with different level of presence of copper particles

Processing option: all elements analyzed (normalized)

Spectrum	In stats.	O	P	Cl	K	Ca	Cu	Br	Total
Spectrum 1	Yes	80.32		1.89	1.02	7.12	7.66	1.99	100.00
Spectrum 2	Yes	91.78	1.00		1.16	7.06			100.00
Spectrum 3	Yes	87.82		1.01	1.49	5.75	2.93		100.00
Max.		91.78	1.00	1.89	1.49	7.12			
Min.		80.32	1.00	1.01	1.02	5.75	2.93	1.99	

Spectrum 1 high presence; *spectrum 2* no presence; *spectrum 3* intermediate presence

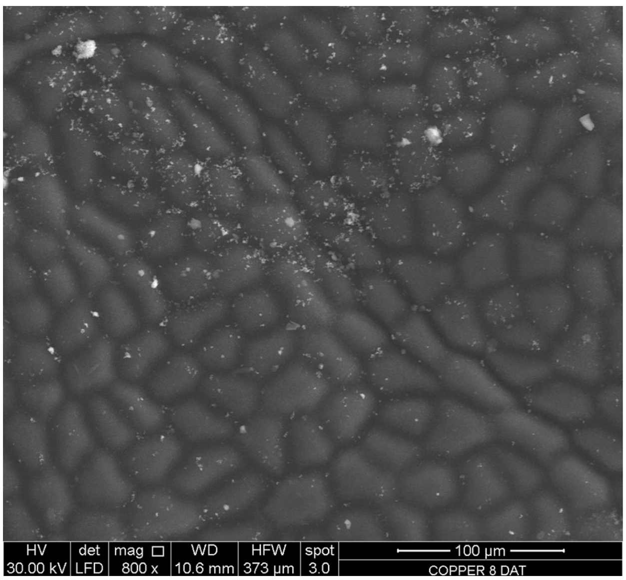

Fig. 8 Leaf surface treated with copper oxychloride alone after 8 days from application. Copper is scattered with low presence of particles

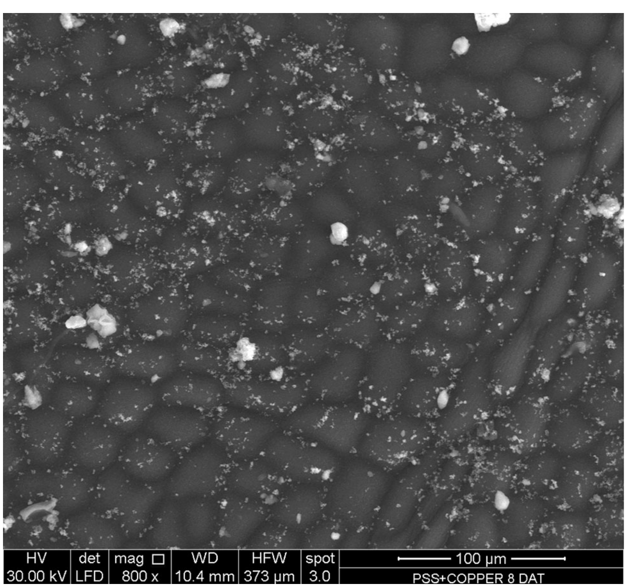

Fig. 9 Leaf surface treated with copper oxychloride mixed with locust bean gum preparation (PSS) observed by SEM after 8 days from application. Copper particles are uniformly distributed on leaf

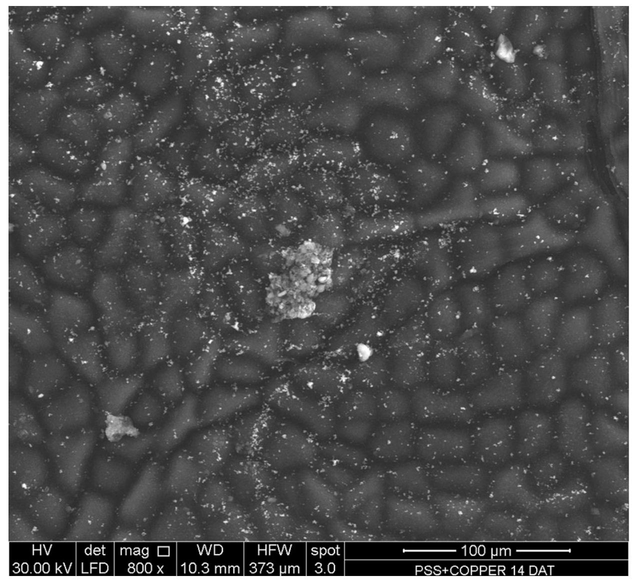

Fig. 10 Leaf surface treated with copper oxychloride mixed with locust bean gum preparation (PSS) observed by SEM after 14 days from application. Copper particles are still uniformly distributed on leaf after 14 days

agrochemicals to the target surface. Sticker activity is generally better for non-systemic fungicides because of their trend to be leached precociously. Like-latex and terpene-based stickers usually need to dry on plant surface or need sunlight to set the film. PSS increased the efficacy of copper applications probably, as reported above, acting as a sticker prolonging the persistence of the copper on the leaves with no other specific requirements. Some adjuvants such as alcohol ethoxylates and alkylamine ethoxylates can have an environmental impact, due to ability of these compounds to enhance the mobility of pollutants in the soil (Krogh et al. 2003). Ferreira et al. (2016) described a wide range of different polysaccharides, their properties, and their state of the art in research and commercial fields as additive. Galactomannans prevalently used as edible films or coatings for food (Cerqueira et al. 2011) for their biological, chemical and physical properties make them easily usable as fungicides spray adjuvant.

The proposed adjuvant is biodegradable, with any environmental impact, and will be easy to transfer its use in practice in crop protection. To our knowledge, this is the first time that galactomannans-derived compound have been used as adjuvant in fungicides.

Further studies are in progress to confirm the results on other host–pathogen systems and to better understand the mechanisms underlying the interaction between copper-based fungicides with locust bean gum adjuvant.

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