

SHORT COMMUNICATION
CONTROL OF DIEBACK (“MORIA”) OF *CORYLUS AVELLANA*
IN CENTRAL ITALY USING COPPER COMPOUNDS

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SUMMARY

Bacterial dieback of hazelnut (*Corylus avellana*), commonly called *moria* is the most serious problem in hazelnut orchards in Central Italy. Various copper compounds (copper oxychloride, tribasic copper sulphate with or without addition of a leaf fertilizer, and copper sulphate pentahydrate) were tested in areas affected by the disease, and all gave effective control. Copper oxychloride and tribasic copper sulphate, in combination with a leaf fertilizer performed best, but copper sulphate pentahydrate containing a very low concentration of copper (5.5 g/l), still gave sufficient protection.

Key words: bacterial disease, hazelnut, copper salts, disease incidence.

In Central Italy, the most dangerous disease of hazelnut (*Corylus avellana* L.) is a bacterial dieback, commonly called *moria*. The causal agent was identified by Varvaro *et al.*, (1990) as a member of the “true *erwiniae*” group (Schroth and Hildebrand, 1980).

The symptoms are similar to those described for the bacterial canker in Greece (Psallidas and Panagopoulos, 1979). Later, another bacterial pathogen, *Pseudomonas avellanae* (Janse *et al.*, 1996), was linked to the same hazelnut disease (Scortichini *et al.*, 1994). In spring, infected plants typically have light-green or yellowish leaves which, in summer, wither within a few days. Infected trunks, twigs and offshoots suddenly die and leaves remain attached, often after the autumn leaf fall (Fig. 1).

Branches typically show dark brown discolorations on the bark and cambium and, in some cases, the xylem appears involved. Infected trees generally die within 2-3 years. New suckers continue to be produced but even they usually die within the third year.

To control the disease, integrated approaches (Cu treatments and good agronomic practices) have been

suggested (Aloj *et al.*, 1994; Varvaro, 1994) but these recommendations are often not followed in time. The present work involved *in vitro* and *in vivo* studies on the effectiveness of known and new Cu compounds (also when combined with a leaf fertilizer), at a range of Cu concentrations, to control the disease in the Latium region. The compounds tested were: copper oxychloride ($3 \text{ CuO} \times \text{CuCl}_2 \times 3 \text{ H}_2\text{O}$), Cu ions (c.i.) 30g/100g, field-dose (f.d.) 300 g hl^{-1} H_2O ; tribasic copper sulphate ($3 \text{ Cu}(\text{OH})_2 \times \text{CuSO}_4 \times 1/2 \text{ H}_2\text{O}$), c.i. 24 g l^{-1} , f.d. 200 ml hl^{-1} , and copper sulphate pentahydrate ($\text{CuSO}_4 \cdot 5 \text{ H}_2\text{O}$) c.i. 5.5 g l^{-1} , f.d. 150 ml hl^{-1}).

Twelve bacterial isolates from the collection of the Department of Plant Protection, University of Viterbo, were tested *in vitro*. They were from 171 isolates obtained from diseased hazelnut plants in the province of Viterbo (Central Italy), and from known strains (*Brenneria nigri-fluens*, *Pseudomonas avellanae*) associated with bacterial dieback (Varvaro *et al.*, 1990; Scortichini *et al.*, 1994). Pathogenicity tests and fatty acid profiling identified these isolates as members of the “true *erwiniae*” group (Varvaro *et al.*, 1990) and *P. avellanae* (Psallidas and Panagopoulos, 1979) (six strains in each group, Table 1).

For determining bactericidal activity, Cu incorporation and broth tests were used (Balestra *et al.*, 1998). For

Table 1. Bacterial strains used for *in vitro* tests.

Isolates	Identification
3	<i>Brenneria rubrifaciens</i>
41	<i>B. rubrifaciens</i>
60	<i>B. rubrifaciens</i>
87	<i>B. nigrifluens</i>
39	<i>B. rubrifaciens</i>
60	<i>Pseudomonas avellanae</i>
44	<i>P. avellanae</i>
153	<i>P. avellanae</i>
29	<i>P. avellanae</i>
74	<i>P. avellanae</i>
7 B	<i>P. avellanae</i>
8 B	<i>P. avellanae</i>

each copper compound three different Cu concentrations were tested (copper oxychloride: 3 g l⁻¹ (field-dose), 1.5 g l⁻¹ (1/2 field-dose), 0.75 g l⁻¹ (1/4 field-dose); tribasic copper sulphate: 2 ml l⁻¹ (field-dose), 1 ml l⁻¹ (1/2 field-dose), 0.5 ml l⁻¹ (1/4 field-dose); copper sulphate pentahydrate: 1.5 ml l⁻¹ (field-dose), 0.75 ml l⁻¹ (1/2 field-dose), 0.38 ml l⁻¹ (1/4 field-dose). In the Cu incorporation tests, growth of almost all bacterial isolates was inhibited at all concentrations used. Only four bacterial isolates (3 and 87, *Enterobacteriaceae*, 41 and 44, *Pseudomonadaceae*) were able to grow with copper sulphate pentahydrate at 1/4 field-dose. In broth tests, growth of all isolates was completely inhibited by all Cu compounds.

Since response to Cu compounds was similar in both groups of bacteria, we report only the results obtained with bacterial strains 87 (*Brenneria nigrifluens*) and 44 (*P. avellanae*). Using 1/4 field-dose Cu concentration, growth of strain 87 was inhibited after 3 h with tribasic copper sulphate and copper oxychloride and after 6 h with copper sulphate pentahydrate (Fig. 2). Using 1/2 field-dose, tribasic copper sulphate was effective after 6 h, copper oxychloride after 12 h, and copper sulphate pentahydrate only after more than 24 h (Fig. 2). Using 1/2 field-dose, growth of isolate 44 was inhibited after 3 h by tribasic copper sulphate and by copper oxychloride, while copper sulphate pentahydrate was effective only after 24 h (Fig. 3). Tribasic copper sulphate and copper oxychloride (1/4 field-dose) were effective after 6 h, while pentahydrate copper sulphate inhibited the bacteria after 24 h (Fig. 3).

Field experiments were done over four years (1999-2002) in two different hazelnut areas in the province of Viterbo; one in the Soriano nel Cimino area (1999 and 2000) and the other in the Caprarola area (2001 and 2002). Both sites were characterized by typical *moria*



Fig. 1. Typical dieback (*moria*) symptoms in a hazelnut plant of cv Tonda Gentile Romana.

symptoms on hazelnut plants cv Tonda Gentile Romana. Experimental plots of hazelnuts (30 each) were treated with: A, copper oxychloride (300g l⁻¹); B, tribasic copper sulphate (200ml/hl); C, tribasic copper sulphate (200ml/hl) in combination with a nitrogenous leaf fertilizer (200ml hl⁻¹); D, copper sulphate pentahydrate (150 ml hl⁻¹); E, no treatment. During 1999, at Soriano nel Cimino three applications/plot were made, two during spring and summer and one at leaf fall (autumn).

Cu treatments in spring were aimed at reducing the risk of primary infection and those in summer were aimed at avoiding possible re-infections due to different factors (e.g. insect damage, thunderstorms). All diseased parts were seasonally removed and totally infected trees were uprooted. From the second year (2000) onwards the number of treatments was increased to four per plot (two in spring, one in summer and one at leaf fall).

All field-results obtained are reported in Table 2. Overall, the Soriano nel Cimino and Caprarola plots showed that *moria* could be reduced by Cu treatments. In particular, in 1999, at Soriano nel Cimino at the beginning of the season, *moria* incidence was 11.4% in control plots and, in the same plot, was 5.7% at the end of the 1999 and 28.6% at end of 2000. The copper oxychloride plots initially showed a disease incidence of 8.7% and it was 4.3% at the end of 2000. Using tribasic copper sulphate, *moria* incidence was 16.2% at the beginning of 1999, 2.7% at the end of 1999 and 38.1% at the

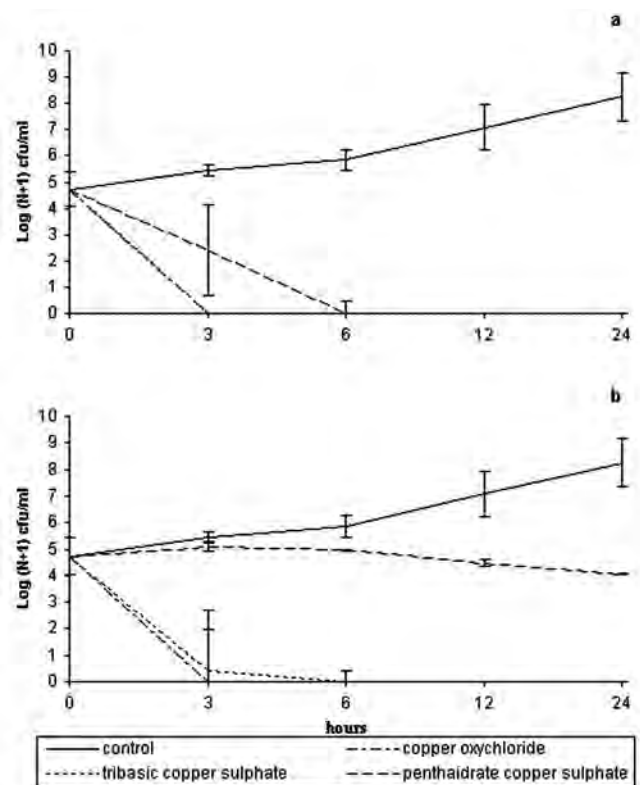


Fig. 2. *In vitro* experiments with bacterial strain 87 using 1/2 (a) and 1/4 (b) field-doses of different copper compounds. All results were significant ($P < 0.0001$).

Table 2. Incidence of *moria* (%) in experimental field plots in two different districts (Caprarola and Soriano nel Cimino) in the province of Viterbo, treated with different copper formulations.

Treatments	Soriano				Caprarola			
	1999		2000		2001		2002	
	b.s.	e.s.	b.s.	e.s.	b.s.	e.s.	b.s.	e.s.
Control	11,4	5,7 a	0	28,6 a	0	22,2 a	0	11,1 a
3CuO × CuCl ₂ × 3H ₂ O	8,7	0 a	0	4,3 b	0	0 c	0	0 b
3Cu(OH) ₂ × CuSO ₄ × 1/2H ₂ O	16,2	2,7 a	0	38,1 a	0	5,5 a	0	5,5 b
3Cu(OH) ₂ × CuSO ₄ × 1/2H ₂ O + l.f.	2,7	0 a	0	11,1 b	0	0 c	0	0 b
CuSO ₄ × 5 H ₂ O	0	0 a	0	0 b	0	5,5 a	0	2,7 a

l.f.: leaf fertilizer; b.s.: beginning of hazelnut season; e.s.: end of hazelnut season.

Values in the same column followed by the same letter do not differ significantly at $P < 0.05$ according to Fisher's exact test.

end of 2000. Using the tribasic copper sulphate combined with leaf fertilizer, disease incidence in 1999 was initially 2.7% and 11.1% at the end of 2000 (Table 2).

In Caprarola, in the control plots, *moria* incidence was 22.2% and 11.1% at the end of 2001 and 2002 seasons, respectively; no *moria* was observed in plots treated with copper oxychloride. Using tribasic copper sulphate, the same disease incidence (5.5%) was recorded at the end of 2001 and 2002. In plots treated with tribasic copper sulphate combined with leaf fertilizer no disease was found in 2001 and 2002; incidence, using copper sulphate pentahydrate, was 5.5% and 2.7% at the end of the 2001 and 2002 seasons, respectively (Table 2).

For statistical analysis of the *in vitro* tests, analysis of variance (ANOVA) and the Tukey-Kramer multiple comparisons test were used; the results were highly significant ($P < 0.0001$). The *in vivo* results were compared by contingency tests, based on the Fisher exact test (Table 2).

In this study, all copper treatments controlled the disease. These chemicals, very active in *in vitro* tests, also showed *in vivo* their capacity to kill the bacteria on aerial plant surfaces, reducing infection risk. Considering each season's trend, three copper treatments during spring and summer appeared to provide a good level of protection of initially healthy trees. Their use after leaf fall also appeared to provide prolonged benefit.

Copper oxychloride was most appropriate if sprayed at leaf fall, while tribasic copper sulphate combined with leaf fertilizer, and also copper pentahydrate, if sprayed in spring and summer, gave the best performance.

All copper treatments, except tribasic copper sulphate at Soriano in 2000 reduced the mean values of disease incidence (2.5% and 8% in Caprarola and in Soriano nel Cimino, respectively). These results demonstrate the importance of Cu salts for control of *moria* when they are used in a proper manner.

Copper sulphate pentahydrate treatments gave interesting results since the Cu concentration used was about 10 times lower than for the other copper compounds used (9 g hl⁻¹, as opposed to 90 g hl⁻¹ of copper

oxychloride). This aspect is important because it will allow easier compliance with the new European Union (EU) regulations restricting the use of copper compounds in organic crops. At present use of copper compounds in association with good agronomic practices, provides the most effective control of dieback of hazelnut in the province of Viterbo.

Epidemiological, molecular and nutritional studies are in progress in order to improve protection of hazelnut trees against the disease (Balestra *et al.*, 2004; Fabi *et al.*, 2004).

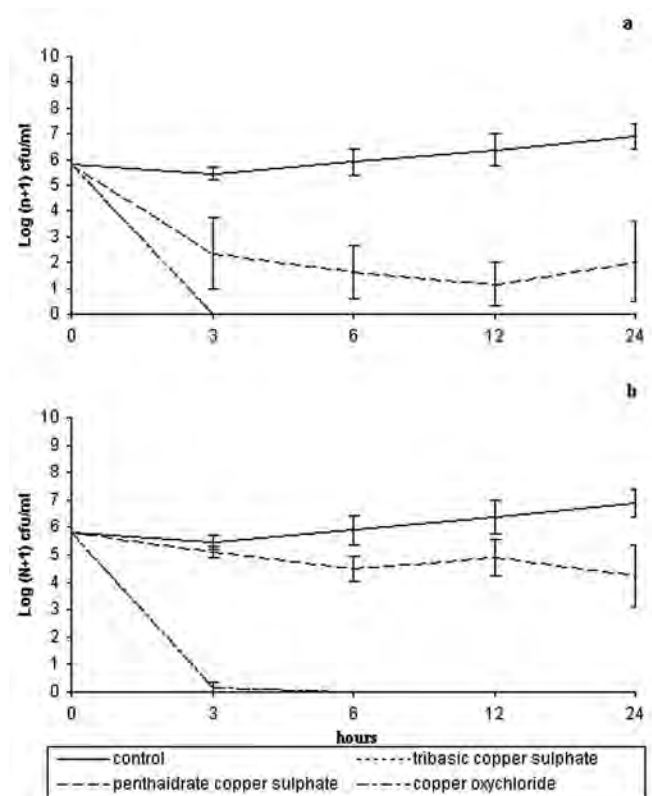


Fig. 3. *In vitro* experiments with bacterial strain 44 using 1/2 (a) and 1/4 (b) field-doses of different copper compounds. All results were significant ($P < 0.0001$).

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