J. Ent. Acarol. Res.
Ser. II, 43 (2): 277-285

30 September 2011

L. MAISTRELLO, A. BERZOLLA, I. MACIAS-PAVON, F. VIGNALI,
G. PREDIERI, E. CHIAPPINI

Wood impregnated with metal chelates dissolved in organic media tested for termite resistance

Abstract - Wood manufactured products are subjected to biological decay due to fungi and insects. The use of copper chelates as biocides was proposed, due to their high stability which minimizes copper leaching into the environment. Considering the remarkable effectiveness showed by copper chelates on brown rot fungi, zinc and copper salicylate complexes were prepared in order to have metal chelates soluble in organic media available. The present study aimed at evaluating these metal chelates complexes as preservative agents for wood treatment against termites. Trials were performed on *Reticulitermes lucifugus* (Rossi) and *Kalotermes flavicollis* (Fabricius). Results showed that in both termite species wood consumption was significantly lower on Cu-chelates treated samples compared to untreated wood, whereas the wood slices impregnated with Zn-chelates and the organic media alone gave an intermediate response. Interestingly, in one case solvent-impregnated wood was significantly more attractive than untreated wood for both species and further investigations are being carried out to clarify this behaviour.

Key words: wood treatment, preservative agents, copper chelates, *Kalotermes flavicollis*, *Reticulitermes lucifugus*.

INTRODUCTION

Wood employed in manufactured products is exposed to physical-chemical degradation and to biological decay due to insects and fungi. Chromate copper arsenate (CCA) has been used for wood preservation for more than 30 years, but in the last decade the use of CCA has been restricted in Europe due to its toxicity against humans and animals. The new generation of copper based preservatives consists of copper complexes with amines; in particular ethanolamine is largely used owing to its copper fixative properties (Humar & Lesar, 2007). Very recently, the use of copper chelates as biocides, in particular aminoacid derivatives such as copper glycinate, was proposed (Palanti *et al*., 2008), taking advantage of the high stability of these compounds, which would minimize copper leaching into the environment. Copper glycinate chelate showed remarkable effectiveness against brown rot *Coniophora puteana* even without any association with other biocides such as boric acid (Palanti *et al*., 2008).

Zinc-based products have also been used as preservatives in the past, among them chromate and zinc chloride formulation and zinc naphtenate are well known; zinc mainly has an antibacterial activity (Perelshtein, 2009) but it is also known to inhibit fungal growth at adequate concentration (Schultz, 2008). Zinc has also been employed against mould fungi, decay fungi, and Eastern subterranean termites (Kartal, 2009).

The aim of the present study was to evaluate copper and zinc salicylates with a biocide action against fungi as preservative agents for wood treatment against termites, using different types of solvents.

MATERIALS AND METHODS

The efficacy of the selected treatments as wood preservatives was tested on the termites *Kalotermes flavicollis* (Fabricius) and *Reticulitermes lucifugus* (Rossi), taken from naturally infested wood branches fallen on the ground in San Rossore forest (Pisa) in Tuscany. *Pinus sylvestris* L. wood slices, previously impregnated in the treatment solutions, were used. Each pine slice (40 x 30 x 3 mm^3 in size) was numbered, oven dried at 103 ± 2°C for 18 hours and weighed, than it was soaked for 8 h in one of the treatment solutions listed below, left 24 h to air dry at room temperature, oven dried at 103 ± 2°C for 18 hours and then re-weighed. Wood slices impregnated with chelated copper (Cu) or chelated zinc (Zn) in different media were compared with slices left untreated or treated with solvent alone. In test 1, the following treatments were performed:

- wood not impregnated (control),
- wood impregnated with oily solvent (linseed oil),
- wood impregnated with Cu-salicylate in oily solvent,
- wood impregnated with Zn-salicylate in oily solvent,

In test 2 the oily solvent was substituted with a very polar solvent (ethylene glycol) in order to increase chelates concentrations in the solutions and a treatment with copper glycinate, that proved to be effective against fungi, was added. Therefore, the following treatments were performed:

- wood impregnated with water,
- wood impregnated with ethylene glycol,
- wood impregnated with Cu-glycinate in water,
- wood impregnated with Cu-salicylate in ethylene glycol,
- wood impregnated with Zn-salicylate in ethylene glycol.

The preservative formulations were prepared as follows. Zinc and copper salicylates were directly synthesized starting from a salicylic acid and a metal oxide or salt according to a patent method (Leonardi, 2011). In a typical experiment, the carboxylic acid was dissolved in oil at 50°C under stirring. When complete solution was achieved, zinc basic carbonate or copper oxide was added. The system was maintained under vigorous stirring at 60°C for about 30 minutes. A pale pink (Zn-salicylate) or green (Cu-salicylate) solution was obtained, which was then transferred in a large glass container in order to facilitate evaporation and subsequent formation of crystals. Copper glycinate was prepared by reaction of copper acetate and glycine in water.

In test 1, oily solutions were obtained by solving the powdered metal complexes in oil at 60°C under stirring for 1 hour. A Zn-salicylate 0.065M solution (pale orange) and a Cu-salicylate 0.043M solution (dark green) in linseed oil were prepared under stirring at room temperature. The same solutions were also obtained by solving the carboxylic acid in oil (at 50°C for about 60 minutes) and allowing it to react with zinc or copper added in the form of oxides or salts directly in the oily solution.

For test 2, copper chelates were directly solved in water or ethylene glycol at room temperature in order to obtain solutions with the following concentrations: 0.04M copper glycinate in water, 0.11M copper salicylate in ethylene glycol, 0.2M zinc salicylate in ethylene glycol.

In both tests, wood treatment procedure consisted in the immersion of the conditioned wood slices into a volume of solution corresponding to 10/1 of the sum of wood sample volumes. In all cases wood samples were maintained bathed by means of tweezers.

Treated samples were employed in the tests of effectiveness against termites in the number of 5 replicates for each treatment. Test chambers and procedures were different for the two species.

Considering *R. lucifugus*, for each replicate 50 workers plus 1 soldier were introduced in a sterilized cylindrical plastic container (3.5 cm height, 6 cm diameter) whose lid had a hole with an externally applied metallic mesh, together with 20 g of sterile sand, 2 ml micro-filtered water and a wood slice. All containers were kept inside a plastic box (30 x 19 x 7 cm) closed with a hermetic lid whose bottom had layers of absorbing paper completely soaked in water, so that inside each box R.H. = 95%. During the experiment the boxes and containers were regularly opened to add water in order to maintain proper moisture. Both tests lasted 21 days.

For *K. flavicollis*, sterilized unventilated glass Petri dishes (10 cm diameter), with 35 g of sterile sand, 1 ml of micro-filtered water and the pine sample laying on the sand, were used. In each capsule, one soldier plus 28 workers in the first tests and 18 in the second one were introduced. Tests 1 and 2 lasted respectively 60 and 95 days.

For both species, the boxes were kept in a thermostatic chamber in the dark at t = 25 ± 2°C; during the trial period, test chambers were checked every 10 days to assess mortality. At the end of the experiments, live and dead specimens were counted. Wood slices were removed, carefully brushed clean from debris and faeces, then oven-dried at 103 ± 2°C for 18 hours and weighed to assess wood consumption. Statistical analyses were performed on angular transformations of the percentage data of wood consumption and mortality, however, untransformed data are shown in the figures. Data were processed using parametric statistical analysis (ANOVA) and Tukey test to separate the media ($p \leq 0.05$). When error variances were not homogenous at the Levene test for homogeneity of variance, non parametric Kruskal-Wallis analyses of variance was performed.

RESULTS

R. lucifugus

In test 1, considering wood consumption (Fig. 1), significant differences were detected among treatments (H= 12.51, p= 0.006, Kruskal-Wallis test) and, in particular, between untreated wood (highest weight loss, about 1-2%) and the Cu-salicylate impregnated wood (almost no consumption) (z= 2.47, p= 0.003, multiple comparisons after Kruskal-Wallis test). Considering mortality, observations performed during the periodical water additions inside the containers allowed to detect a high number (no exact counts were performed to avoid disturbance) of dead termites in the groups with Cu-

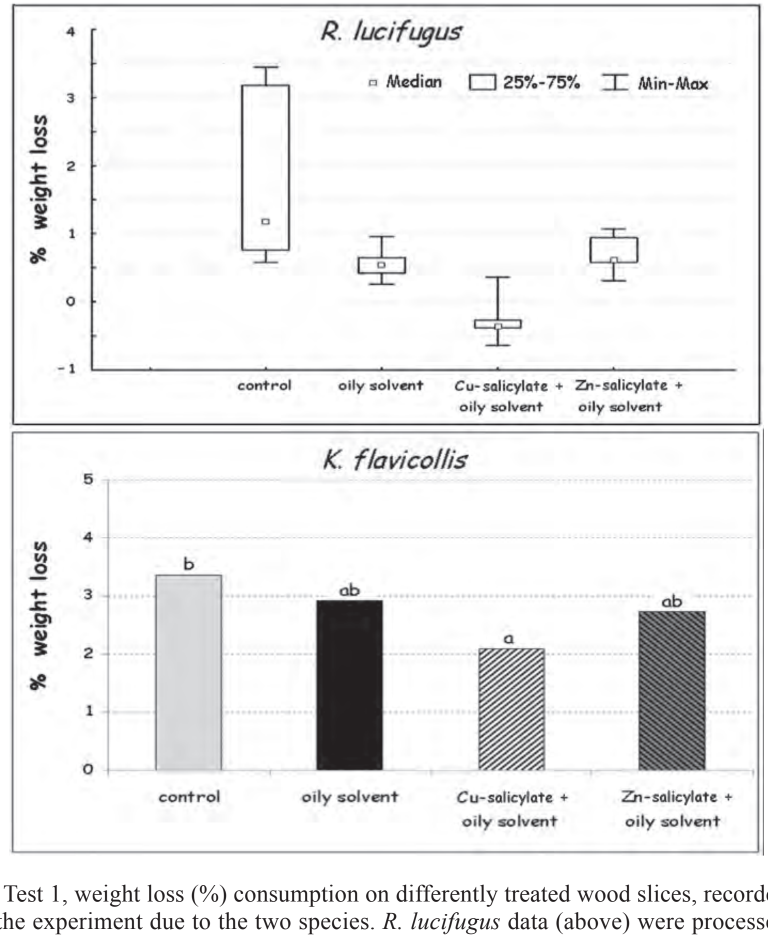

Fig. 1 - Test 1, weight loss (%) consumption on differently treated wood slices, recorded at the end of the experiment due to the two species. *R. lucifugus* data (above) were processed using non parametric Kruskal-Wallis analyses of variance. *K. flavicollis* data (below) were processed using Tukey test to separate the media ($p \leq 0.05$). Columns indicated by different letters show significant differences.

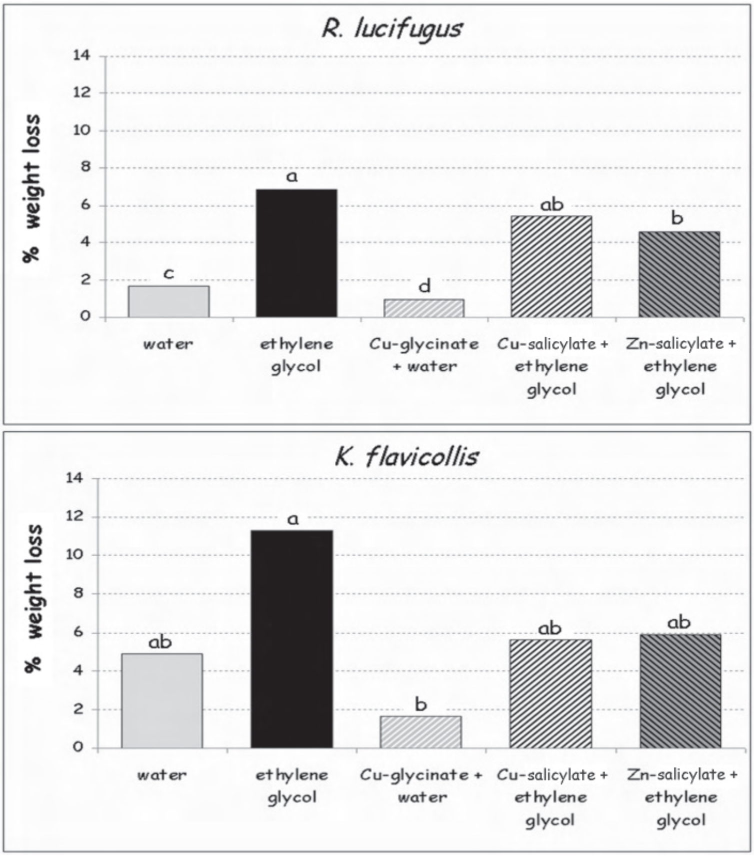

Fig. 2 - Test 2, weight loss (%) due to *R. lucifugus* (above) and *K. flavicollis* (below) consumption on differently treated wood slices, recorded at the end of the experiments. Columns indicated by different letters show significant differences at the Tukey test ($p \leq 0.05$).

and Zn-salicylate impregnated wood even after the first 5-10 days, whereas in the control termites appeared very active and performed galleries inside the sand. At the end of the experiment, which lasted 21 d, no significant differences were detected among treatments with the lowest mortality found in the control group (78.40 ± 19.60%) and the highest in the Cu-chelate treated wood (100%).

In test 2, considering wood consumption (Fig. 2), highly significant differences were detected among treatments ($F= 91.73$, $p< 0.0001$): lowest consumption was detected for the wood treated with Cu-chelate + water, whereas in presence of the solvent (with or without the metal chelates), wood consumption was always significantly high-

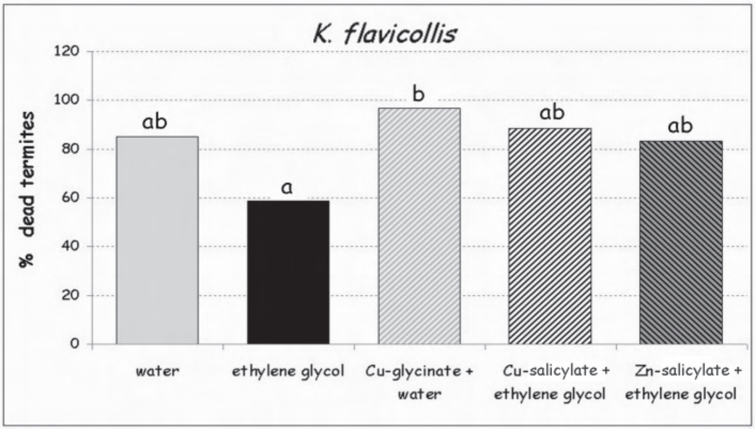

Fig. - Test 2, *K. flavicollis* average percentage of mortality in differently treated wood slices, recorded at the end of the experiment (90 d). Columns indicated by different letters show significant differences at the Tukey test ($p \leq 0.05$).

er than in the control group. Regarding mortality, similar observations as those reported for test 1 were reported for copper glycinate in water and metal chelates treated wood.

At the end of the experiment, after 21 d, no significant differences were detected among groups: all termites were dead in all groups except of those with the solvent treated wood (where mortality was 80.80 ± 11.83%).

K. flavicollis

In test 1, no mortality was observed during the trial period of sixty days. Therefore, only wood consumption percentage was analyzed. The samples' weight loss was significantly lower ($F= 4.011$, $p= 0.015$) in those impregnated with Cu-chelates, especially compared to non treated ones, while those impregnated with Zn-chelates and the oily solvent alone gave an intermediate response (Fig. 1).

In test 2, wood consumption was significantly lower ($F= 6.297$, $p= 0.02$) in the samples impregnated with Cu-chelates in water and significantly higher in wood impregnated with ethylene glycol, while the other treatments gave an intermediate response (Fig. 2).

Also for mortality significant difference were detected ($F= 3.381$, $p= 0.029$) and the situation was exactly the opposite (Fig. 3).

DISCUSSION AND CONCLUSIONS

Results of the present study show that wood impregnated with Cu-chelates provides a better protection against both *R. lucifugus* and *K. flavicollis* than untreated wood. Results from the bioassays, despite being performed with different protocols in different laboratories, showed that wood consumption by both species of termites was significantly lower in presence of copper salicylate in oily solvent or copper glycinate in water. Efficacy of these products is confirmed by the higher mortality observed in *K. flavicollis* on the same treatments. This is in agreement with bibliographic data. The efficacy of copper-based wood preservatives has been proved in formulations such as copper soaps (Pizzi, 1993), with amine solvents such as ACQ (alkaline copper quat) and MCQ (micronized copper quat) (Cookson *et al*., 2010; Tascioglu & Tsunoda, 2010; Lin *et al.*, 2009), in association with tannins (Yamaguchi *et al*., 2002) or with metaborates (Furuno *et al*., 2006), as nanoparticles (Kartal *et al.*, 2009) and in combination with boron and hydroxylamine (Köse *et al*., 2009). Nevertheless, in our experiments, efficacy of the same Cu-chelate in a different solvent (ethylene glycol) was not demonstrated. Wood impregnated with copper chelate in ethylene glycol was not protected by the treatment. In addition, from this study it emerged that for both termite species ethylene glycol alone elicited a significantly higher wood consumption and resulted in lower mortality for *K. flavicollis*. Therefore it seems that the use of ethylene glycol as solvent elicits a phago-stimulant effect which is able to contrast the negative effects of copper for both species.

Zinc action is not so clear. In test 1, when only oily solvent was used, wood treated with Zn-chelate gave exactly the same results with both termites species. Wood consumption was intermediate between control and Cu-chelate, being slightly lower, although not significantly different than the control and higher than Cu-chelate. In test 2, the results for Zn-chelate in ethylene glycol are different for the two species. Mortality and wood consumption in *K. flavicollis* were the same as those on wood treated with Cu-chelate or water. In this case, the result could be due to the solvent, as hypothesized for Cu-chelate. On the opposite, in *R. lucifugus* wood consumption in Zn-chelate treatment was similar to Cu-chelate treatment but significantly lower than in wood impregnated with ethylene glycol alone, showing that the attractive effect of the solvent is reduced by the presence of Zn-chelate.

The attractive action of ethylene glycol is recognized for some insect species, especially for fruit flies (Diptera, Tephritidae) (Thomas *et al*., 2001; Uchida *et al*., 2007), where it has been used in baited traps. Use of this substance is known also for other insect species in pit-fall traps (Koivula *et al*., 2003; Greenslade & Greenslade, 1971), acting as a preservative although it poses serious environmental hazards (Ash & Ash, 2004; Beasley, 1985; Barceloux *et al.*, 1999; Hall, 1991).
Further investigations are needed and will be carried out to clarify this behavior.

REFERENCES

Ash M., Ash I., 2004 - Handbook of preservatives. Synapse Information Resources Endicott. New York. U.S.A. 850 pp.

Barceloux D. G., Krenzalik E. P., Olson K., Watson W., 1999 - Guidelines on the treatment of ethylene glycol poisoning. Clinical Toxicology, 37: 537-560.

Beasley V. R., 1985 - Diagnosis and management of ethylene glycol (antifreeze) poisoning. Feline Practice, 15: 41-46.

Cookson L.J., Creffield J.W., Mccarthy K.J., Scown D.K., 2010 - Trials on the efficacy of micronized copper in Australia. Forest Products Journal, 60 (1): 6-12.

Furuno T., Wada F., Yusuf S., 2006 – Biological resistance of wood treated with zinc and copper metaborates. Holzforschung, 60 (1): 104-109.

Greenslade P., Greenslade P. J., 1971 - The use of baits and preservatives in pitfall traps. Journal of the Australian Entomological Society, 10: 253-260.

Hall D., 1991 - The environmental hazard of ethylene glycol in insect pitfall traps. Coleopterists Bulletin, 45: 193-194.

Humar M., Lesar B., 2007 - Fungicidal properties of individual components of copper ethanolamine-based wood preservatives. International Biodeterioration & Biodegradation, 62: 46-50.

Kartal S.N., Green F., Clausen C.A., 2009 - Do the unique properties of nanometals affect leachability or efficacy against fungi and termites?. International Biodeterioration & Biodegradation, 63: 490-495.

Koivula M., Kotze D. J., Hiisivuori L., Rita H., 2003 - Pitfall trap efficacy: do trap size, collecting fluid and vegetation structure matter. Entomologica Fennica, 14: 1-14.

Köse C., Terzi E., Kartal S.N., 2009 – Evaluation of decay and termite resistance of wood treated with copper in combination with boron and N’-N-(1,8-naphthalyl) hydroxylamine (NHA-Na). International Biodeterioration & Biodegradation, 63: 727-731.

Leonardi G., 2011 - Italian Patent Application n° MI2011A001033.

Lin L.D., Chen Y.F., Wang S.Y., Tsai M.J., 2009 - Leachability, metal corrosion, and termite resistance of wood treated with copper-based preservative. International Biodeterioration & Biodegradation, 63: 533-538.

Palanti S., Predieri G., Casoli A., Vignali F., Feci E., 2008 - New preservatives based on copper chelates and copper complexes grafted to functionalized silica gel. Proceedings of COST Action E37 Final Conference, Bordeaux (France), 29-30 September 2008: 23-29.

Pizzi A., 1993 - A New Approach to Non-Toxic, Wide-Spectrum, Ground-Contact Wood Preservatives. Part II. Accelerated and Long-Term Field Tests. Holzforschung, 47 (4): 343-348.

Tascioglu C., Tsunoda K., 2010 - Laboratory evaluation of wood-based composites treated with alkaline copper quat against fungal and termite attacks. International Biodeterioration & Biodegradation, 64: 683-687.

Thomas D. B., Holler T. C., Heath R. R., Salinas E. J., Moses A. L., 2001 - Trap-lure combinations for surveillance of *Anastrepha* fruit flies (Diptera: Tephritidae). Florida Entomologist, 84: 344-351.

Uchida G.K., Mackey B.E., Mcinnis D.O., Vargas R.I., 2007 - Attraction of *Bactrocera dorsalis* (Diptera: Tephritidae) and nontarget insects to methyl eugenol bucket traps with different preservative fluids on Oahu Island, Hawaiian Islands. J. Econ. Entomol., 100 (3): 723-729.

Yamaguchi H., Yoshino K., Kido A., 2002 - Termite resistance and wood-penetrability of chemically modified tannin and tannin-copper complexes as wood preservatives. J. Wood Sci., 48: 331-337.

Lara Maistrello, Dip. Scienze Agrarie e degli Alimenti, Univ. di Modena e Reggio Emilia, Via G. Amendola 2, I-42122 Reggio Emilia, Italy.
E-mail: lara.maistrello@unimore.it
Alessia Berzolla, CPBC, Università Cattolica del Sacro Cuore, via Emilia Parmense 84, I-29122 Piacenza, Italy.
E-mail: alessia.berzolla@unicatt.it
Irene Macias-Pavon, Dip. Scienze Agrarie e degli Alimenti, Univ. di Modena e Reggio Emilia, Via G. Amendola 2, I-42122 Reggio Emilia, Italy.
E-mail: irene_pasiempre@hotmail.com
Francesca Vignali, Dipartimento di Chimica GIAF, Università di Parma, Viale G.P. Usberti 17/A, I-43100 Parma, Italy.
E-mail: francescavignali82@gmail.com
Giovanni Predieri, Dipartimento di Chimica GIAF, Università di Parma, Viale G.P. Usberti 17/A , I-43100 Parma, Italy.
E-mail: giovanni.predieri@unipr.it
Elisabetta Chiappini, CPBC, Università Cattolica del Sacro Cuore, via Emilia Parmense 84, I-29122 Piacenza, Italy.
E-mail: elisabetta.chiappini@unicatt.it