Durability improvement of wood by treatment with Methyl Alkenoate Succinic Anhydrides (M-ASA) of vegetable origin

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Abstract

Methyl Alkenoate Succinic Anhydride (M-ASA) is the product of the reaction between methyl esters of fatty acids and maleic anhydride. Crude M-ASA was synthesized from rapeseed oil methyl esters. The main compounds in this adduct are methyl oleate succinic anhydride (30%), methyl linoleate succinic anhydride (24%), unreacted methyl esters (41%) and unreacted maleic anhydride (4%). The treatment of wood at high temperature with crude M-ASA conferred protection against fungal decay and insects. Biological tests were carried out on Scots pine (*Pinus sylvestris*) sapwood and beech (*Fagus sylvatica*) according to European standards. M-ASA treatment was efficient against mould fungi (BS 3900), blue staining (EN 152), white and brown rot fungi (EN 113), longhorn beetle larvae (EN 46 and 47) and termites (EN 117). This treatment delayed the degradation of wood by soft rot (ENV 807) but it did not prevent it. Therefore, M-ASA combines all the necessary conditions to fulfil the requirements of the biological use classes 2 and 3, but not for class 4.

Keywords: Wood chemical modification; Biological durability; ASA; Alkenyl succinic anhydride; Biological tests

1. Introduction

The microbiological degradation of lignocellulosic material is one of the most important processes in nature. Fungal activity in timber limits its use by reducing its density, strength and aesthetic properties. Protection of wood by conventional methods has long been established to prevent and eradicate wood-decay fungi. Conventional wood-preservation methods were based primarily on the use of chemicals such as creosotes, chlorinated phenols and a variety of inorganic salt preservatives. Restriction on use were imposed in recent years (European "Biocide" directive, 98/8/CE) due to environmental concerns, stability and longevity of its protective action, which results in strong limitations on the utilisation of conventional wood-protection treatments.

Chemical modification as an innovative strategy for the environmental-friendly protection of wood is accomplished by reaction between selected chemicals and the major wood macromolecules without leaving toxic residues within the wood.

Anhydrides have a high reactivity to hydroxyl groups and the grafting of the substituent molecule has been shown to occur using relatively simple reaction systems (Goldstein et al., 1961; Rowell et al., 1988; Hill and Jones, 1996; Ramsdam et al., 1997). The most common anhydride still used today is acetic anhydride, which improves the technical properties of Scots pine sapwood as the resistance to brown and white rot fungi, as well as the resistance to insects (Imamura and Nishimoto, 1986; Rowell and Plackett, 1988; Hill and Jones, 1996; Larsson-Brelind et al., 1997).

A new anhydride recently synthesized (Quesada et al., 2003): Methyl Alkenoate Succinic Anhydride (M-ASA), was prepared by reaction of rapeseed oil methyl esters (RME) with maleic anhydride (MAH).

Our main objective in this work was to assess, with European biological standard tests, the protection against various wood-decaying agents. It is known that

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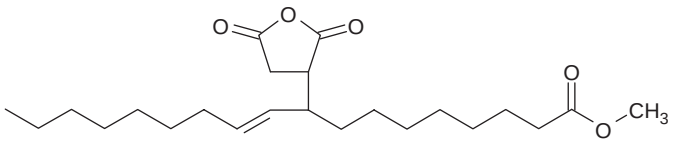

Fig. 1. Structure of one of the isomers of methyl oleate succinic anhydride.

"crude M-ASA", prepared as described by Quesada and co-workers is a brown liquid having a viscosity of 188 cPoise, a Gardner's colour index of 11 and a specific gravity of 0.97. Its composition is:

- 54% of M-ASAs: 30% methyl oleate succinic anhydride (Fig. 1) and 24% methyl linoleate succinic anhydride. A mixture of these molecules was isolated by vacuum distillation of crude M-ASA and will be called in the rest of this study "pure M-ASA".
- 41% of unreacted RME.
- 4% of unreacted MAH and 1% of other secondary products.

A second scientific objective of this study was to evaluate the individual efficiency of the major M-ASA components in order to identify the main active compound in crude M-ASA. Thus, pure M-ASA, RME and MAH efficiencies were assessed on their resistance to brown rot and white rot fungi using an accelerated decay test.

2. Materials and methods

2.1. Treatments and conditioning of wood

2.1.1. Treatment of wood blocks

Vacuum/grafting protocol: Initially, small wood specimens (dimensions are detailed below) were dried during 24 h at 103 °C. They were placed in a cylindrical stainless-steel vessel (inner diameter: 5 cm and height: 20 cm) and then immersed in one of the following: crude M-ASA, pure M-ASA or rapeseed methyl esters (RME). The treatment consisted in two steps: impregnation at 30 °C under reduced pressure (10 kPa) during 30 min and then, without taking out the samples from the liquid, heating the whole system at 140 °C at atmospheric pressure (100 kPa) during 2 h.

2.1.2. Artificial ageing

Ageing by water soaking: The specimens were immersed in water during three days with a water change after 2 h, then every 24 h.

Ageing using a weather-o-meter: An ATLAS device equipped with a xenon lamp was used. This accelerated ageing consisted in an alternation of a wet cycle (6 days of high moisture followed by 1 day of cold) and a dry cycle (6 days of low moisture followed by 1 day of freezing). The duration of ageing varied from 1 week (a single wet cycle) to 8 weeks (four wet cycles and four dry cycles) according to the desired ageing extent.

Ageing by leaching (*European standard* EN 84, 1996): The treated wood specimens were placed in water (100 ml for 18.75 cm^3 of wood) during 14 days with nine water changes. Then they were dried at atmospheric pressure and room temperature during 3 days.

Ageing by evaporation (*European standard* EN 73, 1988/1992): The wood specimens were placed during 12 days in an evaporation tunnel in which circulates a hot air flow (40±1 °C; 1 m s^{-1}; free from dust). They were regularly moved so that all the treated faces have undergone the same ageing.

2.1.3. Assay of M-ASA components against basidiomycetes (mini-block tests)

In order to carry out accelerated tests to assay the efficiency of M-ASA components against basidiomycetes, mini-blocks of Scots pine sapwood and beech (dimensions: 30 × 10 × 5 mm) were treated according to one of the following protocols.

To determine the efficiency of pure M-ASA, wood specimens were treated with the vacuum-grafting protocol with a solution containing 90% oleic M-ASA and 10% linoleic M-ASA (the higher volatility of oleic M-ASA led to a natural enrichment of this molecule content during the distillation process).

To determine the efficiency of RME, wood specimens were treated with the vacuum-grafting protocol with commercial methyl esters of rapeseed oil, the same as that used in the preparation of M-ASA (provided by NOVANCE, Compiègne, France).

To determine the role of the MAH in wood protection, specimens were treated with the vacuum-grafting protocol with M-ASA having different contents of MAH (0%, i.e. crude M-ASA from which MAH was distiled off; 4%, i.e. crude M-ASA; and 8%, i.e. crude M-ASA enriched with 4% MAH).

In all these cases, the wood specimens were tested with fungi responsible of white and brown rot fungi: *Coniophora puteana* on Scots pine sapwood and *Coriolus versicolor* on beech. Accelerated mini-block tests were performed according to the principles of the European standard EN 113 (1996).

2.2. Study of resistance against fungi

In all the following tests, crude M-ASA (and not pure M-ASA) was used as treating agent.

2.2.1. Mould fungi

The M-ASA efficiency as wood preservative against the mould fungi (ascomycetes and Fungi imperfecti) was assessed in agreement with British standard BS 3900 (1989).

Screening: Scots pine sapwood specimens (dimensions: 40 × 30 × 5 mm) were treated with the vacuum-grafting protocol using either crude M-ASA or RME. Some of them underwent ageing by leaching. Specimens were inoculated by one of the following fungi: *Cladosporium cladosporioides*, *Penicillium purpurogenum*, *Alternaria alternata*, *Aspergillus versicolor* or *Trichoderma chiarcianu*.

Standard test: Scots pine sapwood specimens (dimensions: 110 × 40 × 10 mm) were treated with the vacuum-grafting protocol with crude M-ASA or RME. Some of them underwent ageing using a weather-o-meter. Specimens (dimensions reduced to 90 × 40 × 10 mm) were inoculated with a mould fungi cocktail containing *Penicillium purpurogenum*, *Alternaria alternata*, *Aspergillus versicolor*, *Stachybotrys chartarum*, *Ulocladium atrum*, *Sporobolomyces roseus* and *Phoma violacea*.

At the end of the tests, the evaluated parameter was the covering of the specimens by the organisms. A rating scale is given in the British standard BS 3900 (1989) (from 1: no mould fungi on the sample to 5: more than 70% of coverage).

2.2.2. Blue stain fungi

The efficiency of M-ASA as wood preservative against blue stain discolouration due to *Aureobasidium pullulans* and *Sclerophoma pithyophila* was evaluated in agreement with the European standard EN 152 (1988/1989).

Wood treatment: Scots pine sapwood specimens were treated by vacuum grafting with crude M-ASA or RME.

Screening: wood specimens (dimensions: 40 × 30 × 5 mm) were treated and inoculated with fungi responsible of blue staining during 4 weeks. They did not undergo ageing.

Standard test: Wood specimens (dimensions: $110 \times 40 \times 30$ mm) were treated and inoculated with fungi responsible of blue staining. Two-thirds of these specimens underwent weather-o-meter artificial ageing (1 and 2 weeks).

The evaluated parameters were the index of blue staining (from index 0: no blue staining to index 5: maximum blue staining) on the wood surface.

2.2.3. Brown and white rot fungi

The efficiency of M-ASA as wood preservative against brown and white rot fungi (basidiomycetes) was evaluated in agreement with the European standard EN 113 (1996). This method corresponds with the requirements of biological use classes 2 and 3. Scots pine sapwood and beech treated specimens were inoculated with *Coniophora puteana* and *Coriolus versicolor*, respectively.

Mini-block test: the specimens (dimensions: $30 \times 10 \times 5$ mm) were treated by the vacuum-grafting protocol and exposed to fungi. They did not undergo ageing. (see Section 2.1.3).

Standard tests were preformed using Scots pine sapwood specimens with *Coniophora puteana, Poria placenta* and *Gloeophyllum trabeum* and beech specimens with *Coriolus versicolor* (dimensions for all: $50 \times 25 \times 15$ mm, treatment by the vacuum-grafting protocol). Two-thirds of the specimens underwent ageing according to European standards EN 84 (1996) (leaching) and EN 73 (1988/1992) (evaporation in a tunnel by exposure during 12 weeks). The results were evaluated by the calculation of the corrected mass loss.

2.2.4. Soft rot fungi

The efficiency of the M-ASA as wood preservative against soft rot (ascomycetes and Fungi imperfecti) and other soil inhabiting micro-organisms was evaluated in agreement with European standard ENV 807 (1993). This method defines the requirements of the biological use class 4 (wood in ground or fresh water contact). Specimens of beech and Scots pine sapwood (dimensions: $100 \times 10 \times 5$ mm) were treated by the vacuum-grafting protocol with either crude M-ASA or RME. Two-thirds of these wood blocks underwent ageing according to the EN 84 (1996) standard. The results were evaluated by the calculation of the corrected mass loss.

2.3. Study of resistance against insects

Scots pine sapwood specimens were treated according to the vacuum-grafting protocol with either crude M-ASA or RME. Two-thirds of these specimens underwent tests of ageing according to EN 84 (1996) and EN 73 (1988/1992) standards.

2.3.1. Longhorn beetle (*Hylotrupes bajulus*) larvae

Resistance tests of treated wood against longhorn beetle larvae were carried out on Scots pine sapwood blocks (dimensions: $50 \times 25 \times 15$ mm) according to European standards EN 46 (1992) and EN 47 (1992). The test was validated if at least 70% of the larvae stayed alive and drilled holes in the control blocks after 4 weeks. It is then necessary to count the number of larvae still alive in the control blocks. Should the larvae have drilled holes in the treated blocks after 4 weeks, the test must be continued for eight additional weeks.

2.3.2. Termites (*Reticulitermes santonensis*)

Resistance tests of treated wood against termites were carried out on Scots pine sapwood specimens (dimensions: $200 \times 40 \times 10$ mm) according to European standard EN 117 (1981). At the end of the tests, living insects are counted and wood specimens examined in order to calculate the death rate and to determine if attack occurred. The rating system is as follows: 0: no attack; 1: attempts to attack; 2: slight attack; 3: medium attack; 4: strong attack.

3. Results and discussion

3.1. Efficacy of M-ASA components

The efficacy of crude M-ASA main compounds (pure M-ASA, RME, and MAH was tested by accelerated tests based on European standard EN 113 (1996).

According to the results presented in Table 1, pure M-ASA is a promising agent that permits the protection of Scots pine sapwood and beech against basidiomycetes. Ageing by leaching did not affect this performance. However, pure M-ASA-treated specimens present a "sticking to touch" aspect. In crude M-ASA, these compounds are accompanied by RME and this sticky aspect disappears. These results are original and in contrast to those found by Suttie et al. (1999). Indeed, according to the authors, mineral ASA (Fig. 2) does not provide protection against basidiomycetes. It is likely that the properties of M-ASA are enhanced by the ester function at the end of the chain and the position of the anhydride cycle in the centre of the aliphatic chain.

It is interesting to note that the mini-blocks treated by RME showed mass loss values as high as 15.1%, which clearly overpasses the maximum threshold authorised by the EN 599 (1996) standard. This result proves that the mechanism of protection of crude M-ASA does not depend only on the hydrophobic character brought about by an oily product. Besides, wood specimens were loaded with a large amount of RME, indicating that the hydrophobic character of fatty esters do not limit the M-ASA penetration and they might even play the role of carrier.

In order to test the effect of MAH on wood protection, it was necessary to dissolve it in crude M-ASA. Its physical properties prevent to treat wood at high temperature without causing excessive degradation of the wood sample. The content of MAH in crude M-ASA was varied from 0% (complete distillation of MAH) to 8% by controlled addition of MAH.

In the absence of MAH, the mass loss remained below the 3% threshold. Thus, pure M-ASA associated with RME seems to be necessary and sufficient to protect hardwoods (beech) and softwoods (Scots pine sapwood) from rot decay. When the concentration of MAH in M-ASA increases, MAH appears to play a negative role. In the case of beech, the mass loss exceeds 5% when the M-ASA content is higher or equal to 4%. Thus, it seems that the presence of MAH inhibits the role of protection of M-ASA.

As a conclusion, a weak concentration of MAH is desirable in the crude M-ASA and the RME has a positive effect that may be in synergy with the chemical modification by M-ASA molecules. Therefore, for the rest of this work we have used crude M-ASA as defined above (raw adduct from the reaction). Its efficiency was thoroughly assessed by complete standard European tests (mould fungi, blue stain fungi, white and brown rot fungi, soft rot fungi, longhorn beetle larvae and termites).

3.2. Resistance against fungal colonisation and decay

3.2.1. Mould fungi

Mould staining is the result of the surface growth of fungi (ascomycetes and Fungi imperfecti) that consume carbohydrates contained in the lumen of the cells on the surface of wood. However, they only affect wood aesthetic properties. The study of M-ASA-treated wood resistance against mould fungi proceeds in two steps, a screening test followed by a standard test according to British standard BS 3900 (1989). The screening consisted in the independent inoculation of the wood specimens with the five fungi cited in Section 2.2.1. In all the cases, the blocks treated with M-ASA (loading: $531 \pm 81\,kg\,m^{-3}$) having undergone leaching or not, were absolutely free from fungal coverage of the surfaces. On the contrary, RME (loading: $872 \pm 61\,kg\,m^{-3}$) showed only a weak protective effect. Therefore, crude M-ASA efficiency is not only related to its lipophilic properties, but also on a real antifungal character against moulds. It was observed that the control wood specimens having touched the M-ASA treated wood samples did not developed *Alternaria alternata*, which circumvented the contact zone.

After testing five fungi separately, larger-sized wood specimens were inoculated with a fungi cocktail to simulate the real attack conditions met in nature. The British standard BS 3900 (1989) consists in the inoculation of Scots pine sapwood by a mixture of seven fungi (*Penicillium purpurogenum, Alternaria alternata, Aspergillus versicolor, Stachybotrys chartarum, U. atrum, Sporobolomyces roseus, Phoma violacea)*. The results of the test are presented in Table 2.

After 6 weeks, M-ASA-treated wood, leached or not, showed a strong resistance to mould coverage (average coverage index 1.2 for unleached and 1.5 for leached) whereas the controls reached a value of 4. This value approaches the one observed in the case of very sensitive species like poplar (observed covering rate equal to 5). The microscopic examination revealed that the most resistant fungi against M-ASA was *Phoma violacea*, which is found on the majority of treated wood specimens in the form of small white fluff. On the other hand, untreated wood specimens were covered by all the fungi present in the mixture and they have a black-brownish colour. Ageing did not cause M-ASA to loose activity against the mould fungi.

3.2.2. Blue stain fungi

The agents responsible for blue staining induce discoloration of wood, which is the basis for a strong

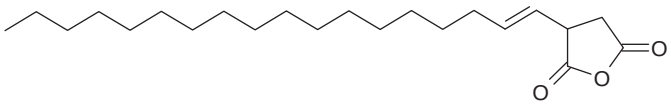

Fig. 2. Structure of a mineral ASA: example of *n*-octadecyl succinic anhydride.

Table 1
Efficacy of M-ASA components against decay by basidiomycetes

		Coniophora puteana (Scots pine sapwood)			*Coriolus versicolor* (Beech)		
		No ageing	Leaching	Control	No ageing	Leaching	Control
Pure M-ASA	Loading ($kg\,m^{-3}$)		519 ± 57	0		347 ± 29	0
	Corrected mass loss (%)	0.8 ± 0.4	1.5 ± 1.1	39.2 ± 17.0	1.6 ± 0.9	1.2 ± 1.0	37.8 ± 8.8
	Improvement factor	98	96	—	96	98	—
	α^a (<3%)	0.00	0.10	—	0.07	0.05	—
Crude M-ASA with 0% MAH (distilled)	Loading ($kg\,m^{-3}$)		538 ± 31	0		362 ± 38	0
	Corrected mass loss (%)	2.7 ± 0.5	1.5 ± 0.3	39.2 ± 17.0	2.4 ± 2.2	0.2 ± 0.1	37.8 ± 8.8
	Improvement factor	93	96	—	94	99	—
	α^a (<3%)	0.28	0.00	—	0.39	0.00	—
Crude M-ASA with 4% MAH (unmodified)	Loading ($kg\,m^{-3}$)		423 ± 25	0		350 ± 36	0
	Corrected mass loss (%)	-0.3 ± 2.6	1.9 ± 0.8	39.2 ± 17.0	7.8 ± 3.6	7.1 ± 3.8	37.8 ± 8.8
	Improvement factor	100	95	—	80	82	—
	α^a (<3%)	0.11	0.09	—	0.90	0.85	—
Crude M-ASA with 8% MAH (enriched)	Loading ($kg\,m^{-3}$)		520 ± 26	0		355 ± 43	0
	Corrected mass loss (%)	5.4 ± 3.6	8.1 ± 3.9	39.2 ± 17.0	6.9 ± 3.2	7.0 ± 3.2	37.8 ± 8.8
	Improvement factor	86	79	—	82	91	—
	α^a (<3%)	0.74	0.89	—	0.88	0.88	—
RME	Loading ($kg\,m^{-3}$)		449 ± 14	0		307 ± 18	0
	Corrected mass loss (%)	4.0 ± 1.9	6.6 ± 1.6	24.1 ± 10.0	4.1 ± 4.8	15.1 ± 6.8	39.2 ± 7.5
	Improvement factor	83	73	—	89	61	—
	α^a (<3%)	0.70	0.98	—	0.59	0.95	—

Accelerated test.
MAH, maleic anhydride; RME, rapeseed methyl esters.
[a]Percentile of the significance (*t*-Student) test for corrected mass loss to be below 3%.

depreciation even though the mechanical properties are affected only to a very limited extent. Several species are at the origin of the blue staining. The most important are the following: *Aureobasidium pullulans* and *Sclerophoma pithyophila*.

This study was undertaken by initially carrying out an accelerated screening test in order to show the efficacy of the crude M-ASA on mini-blocks. Screening tests showed that Scots pine sapwood samples treated with crude M-ASA (531 kg m^{-3}) were not colonized by blue stain fungi.

The EN 152 (1988/1989) standard test confirmed that crude M-ASA protects Scots pine sapwood against blue stain (Table 3) and RMC provided only limited protection. Ageing has no negative impact on the efficacy of M-ASA treatment. The untreated blocks were totally blue stained (index 5).

3.2.3. Basidiomycete fungi

Brown and white rot fungi cause a strong structural degradation of wood involving an important loss of mass and a strong reduction of mechanical properties. The mini-blocks test (see Section 3.1) showed good improvement factors for crude M-ASA (Table 1). In order to give evidence of the effectiveness of the crude M-ASA the standard test (EN 113, 1996) was performed.

The European standard EN 113 (1996) uses four fungi and two wood species: Scots pine sapwood associated to *Coniophora puteana*, *Poria placenta* and *G. trabeum* and beech in combination with *Coriolus versicolor*. Some of the wood blocks were aged by one of the two European standard methods, namely leaching (EN 84, 1996) and evaporation (EN 73, 1988/1992).

Table 2
Average covering index of treated Scots pine sapwood tested with a mixture of 7 mould fungi (BS 3900)

Treatment agent		Loading (kg m^{-3})	Ageing	Coverage index
Crude M-ASA		300 ± 19	None	1.2
			Leaching	1.5
RME		872 ± 61	None	2.4
			Leaching	3.4
Controls	Pine sapwood	—	None	4
	Poplar		Leaching	5

RME, rapeseed methyl esters.

M-ASA fully protected Scots pine sapwood against brown rot fungi. For all fungi tested, the corrected mass loss was less than 3%, the threshold indicated in the European standard EN 599 (1996) (Table 4).

Beech decay resistance was improved with approximately 80% by a M-ASA treatment but, contrarily to Scots pine sapwood, the corrected mass loss was superior to 3% for the unleached blocks and EN 73 (1988/1992) aged blocks (Table 4).

At the end of the test, the colour of the beech blocks treated by M-ASA remained the same as at the beginning of the test. Generally, *Coriolus versicolor* attacks the lignin component clearly causing a discoloration of beech, as noted in control blocks after the test. In this case, it seems that *Coriolus* did not fully attack the lignin.

In spite of the hydrophobic character of M-ASA, the latter penetrates easily in wood showing retentions of 501 kg m^{-3} for Scots pine sapwood and 346 kg m^{-3} for beech. The carrier function of RME may account for these high values. Moreover, the polar moiety in M-ASA: the methyl ester function may play a favourable role. Finally, it also must be noticed that after grafting, the M-ASA molecule creates a carboxylic group that contributes to the hydrophilicity of the compound.

Thus the protection of wood is not only due to filling up the voids in wood by M-ASA. RME is a more hydrophobic product than M-ASA but it does not protect Scots pine sapwood and beech when applied at comparable loading values (389 kg m^{-3}). In fact, the blocks treated by RME, without ageing, presented a corrected mass loss superior to 3 of, respectively, 17 for *Coniophora* and 9% for *Coriolus* (Table 4).

3.2.4. Soft rot fungi

Ascomycetes and fungi imperfecti such as *Chaetomium globosum* and *Phialophora hoffmanii* are responsible for soft rot decay. Tests were carried out according to the European standard ENV 807 (1993). In general, crude M-ASA does not appear to be an effective wood preservative against soft rot fungi. The protection takes effect after the 24th week of testing (improvement of 11% for Scots pine sapwood and 29% for beech) and the mass loss seems to be stabilized starting from the 32nd week (Table 5).

However, when the wood blocks do not undergo leaching, the M-ASA treatment diminishes the mass loss. When they undergo an initial leaching, the efficiency of the treatment decreases. It is even interesting to note that the treated beech

Table 3
Average blue staining index for Scots pine sapwood treated with crude M-ASA or RME (EN 152)

	RME			Crude M-ASA			Controls		
Loading (kg m^{-3})		387 ± 22			442 ± 53			—	
Duration of ageing (weeks)	0	1	2	0	1	2	0	1	2
Surface blue staining	3	4	2	0	0	0	5	5	5

RME, rapeseed methyl esters.

specimens are more degraded by the micro-organisms than controls after leaching. These results are surprising since they are opposite to those found for the standard EN 113 (1996) basidiomycete test. The elimination of non-grafted M-ASA or MAH could explain this difference. The loss of these components during leaching would cause an improvement of the protection properties of wood in the case of basidiomycetes whereas their elimination is unhelpful in the case of exposure to ascomycetes. In addition, the decay resistance of wood against soft rot is improved when RME was used alone: improvement after 32 weeks 47% for Scots pine sapwood and 36% for beech. Thus, it appears that the RME is more important to protect against soft rot than M-ASA regardless the leaching procedure.

In the case of soft rot, only RME seem to limit the development of the organisms. Although the crude M-ASA contains RME, it seems that its efficacy is not enough or it is inhibited by the presence of the other components in the solution.

3.3. Resistance against insects

The insects are predators that can cause considerable damage to wood. It is essential that a wood preservative afford resistance to the attack of xylophagous insect larvae in order to be approved. Two types of insects were retained for testing: longhorn beetle (*H. bajulus*) larvae and termites (*R. santonensis*).

3.3.1. Longhorn beetle larvae

The tests were carried out according to the European standards EN 46 (1992) and EN 47 (1992). The EN 46 (1992) (larvae of *H. bajulus* placed on the wood specimens) allows to appreciate the preventive effectiveness of a preservative applied on the surface. The EN 47 (1992) defines the content from which the product prevents the survival of the larvae in a completely impregnated wood (larvae placed in wood). It is possible to determine if M-ASA confers to wood insecticidal or repulsive properties.

The results were similar for treated wood specimens and for treated samples artificially aged by leaching or evaporation. A 100% death rate and no attempt of attack were observed for both M-ASA and RME. Both products are effective against the attacks by longhorn beetle larvae at a load of 235–298 $kg\,m^{-3}$. However, in spite of the death of the larvae at the end of the first month, the insecticidal activity of the M-ASA could not be

Table 4
Efficacy of crude M-ASA and rapeseed methyl esters (RME) against decay by basidiomycetes (EN 113)

	Treatment	Loading ($kg\,m^{-3}$)	Ageing	Corrected mass loss (%)	IF (%)	α^a (< 3%)
Coniophora puteana (Scots pine sapwood)			None	2.8±1.6	92	0.45
	M-ASA	501±50	Leaching (EN 84)	1.6±0.2	95	0.00
			Evaporation (EN 73)	2.7±1.0	92	0.39
			None	17.3±2.4	50	1
	RME	384±25	Leaching (EN 84)	12.5±0.4	64	1
			Evaporation (EN 73)	6.7±3.7	81	0.83
		Controls		34.9±10	—	—
Poria placenta (Scots pine sapwood)			None	1.0±1.9	97	0.16
	M-ASA	501±50	Leaching (EN 84)	0.9±0.2	97	0.00
			Evaporation (EN 73)	0.1±0.5	100	0.00
	RME	384±25	None	6.8±1.5	79	0.98
			Leaching (EN 84)	6.7±2.2	79	0.93
			Evaporation (EN 73)	7.9±6.9	76	0.75
		Controls		32.7±11.7	—	—
Gloeophyllum trabeum (Scots pine sapwood)			None	2.9±1.7	91	0.48
	M-ASA	501±50	Leaching (EN 84)	1.8±0.2	95	0.00
			Evaporation (EN 73)	1.2±1.1	96	0.07
	RME	384±25	None	14.8±4.2	56	0.99
			Leaching (EN 84)	14.7±9.4	56	0.88
			Evaporation (EN 73)	20.8±4.2	38	1
		Controls		33.4±11.2	—	—
Coriolus versicolor (beech)			None	7.9±2.9	74	0.94
	M-ASA	346±26	Leaching (EN 84)	2.4±0.9	92	0.26
			Evaporation (EN 73)	7.3±2.7	76	0.93
	RME	300±19	None	8.6±1.8	72	0.99
			Leaching (EN 84)	18.9±1.2	38	1
			Evaporation (EN 73)	3.1±4.3	90	0.51
		Controls		30.6±5.8	—	—

IF, improvement factor.
[a]Percentile of the significance (*t*-Student) test for corrected mass loss to be below 3%.

shown. It is possible that the larvae did not die by poisoning but rather by lack of food. During the tests the larvae tried to escape by drilling holes in the paraffin. This phenomenon was not observed with the RME, which indicates that the crude M-ASA would play a repulsive role (Table 6).

3.3.2. *Termites*

The tests were carried out according to European standard EN 117 (1981) protocol, this allows to determine the efficiency of a preservative against *R. santonensis*. A load of 235 kg m^{-3} of M-ASA brings about a death rate of 100% of termites in all the cases (ageing and no ageing). The wood specimens degree of attack is always included between 0 (for the non-aged wood specimens) and 1 (aged wood specimens), which shows a real activity of the product. The efficacy of M-ASA is mainly observed when compared with the results obtained with control blocks. In the latter case the degree of attack rises to a level of 4 (the maximum index of the quotation) whereas it approaches the minimum level in the former case. Results show that the solution of M-ASA is a product that allows wood preservation against termites even after ageing. However, it cannot be considered as a "termiticide" because insects were still alive at the end of the tests. Contrarily to the case of *H. bajulus*, RME showed only a limited efficiency since RME-treated samples were partially attacked by termites and no mortality was observed (Table 6).

4. Conclusion

Among the components of the crude raw material only "pure M-ASA" (methyl oleate and linoleate succinic anhydrides) has an effective fungicidal activity except against soft rot. RME also slows down the wood degradation process even if its impact is limited. Because of its fluidity, it plays the role of carrier facilitating the diffusion and the penetration of M-ASA in the core of the wood pieces. On the contrary, MAH (tested on basidiomycetes) facilitates fungal decay. However, only a small residual quantity of MAH remains in the crude M-ASA solution.

Crude M-ASA seems to be a promising wood preservation product acting against the majority of wood degradation agents: mould fungi, blue staining organisms, white and brown rot fungi, and xylophagous insects as the longhorn beetle larvae and termites. This wide range of protection sharing complementarity between the efficiency against moulds, blue stain and basidiomycetes fungi is original for a wood preservation treatment by chemical grafting.

Scots pine sapwood becomes highly durable after M-ASA treatment. In the same way, beech durability is improved substantially by the M-ASA treatment. However, protection against soft rot is insufficient. The potential utilisation of M-ASA-treated wood in use class 4 situations may require increasing levels of M-ASA grafted in cell wall components (but not necessarily an increase in the M-ASA loading). The investigation on the dose-response effect is in progress and will be reported in the next future.

The ageing of treated samples by water leaching does not affect wood resistance against basidiomycetes. Contrary to this, for soft rot fungi, the ageing decreases the efficiency of the M-ASA treatment. It would be interesting to specify the nature of the eliminated product that is responsible of this lack of performance. Therefore, M-ASA solution can be indexed as an efficient wood preservative for use in classes 2 and 3, not in ground contact, the obligatory and optional criteria being acquired.

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Table 5
Efficacy of crude M-ASA against soft rot fungi (ENV 807)

Species	Loading (kg m^{-3})	Soil exposure (weeks)	Mass loss (%)			IF (%)	Durability class
			Controls	No ageing	Leaching (EN 84)		
Scots pine sapwood	376±61	8	6.4±1.3	1.7±0.8	9.1±1.2	−42.2	4–5
		16	11.0±1.6	6.8±1.2	10.5±2.5	4.5	
		24	15.1±4.6	8.4±1.1	13.5±3.2	10.6	
		32	14.8±8.0	9.6±3.1	13.8±2.1	6.7	
Beech	333±33	8	18.1±7.5	7.6±1.5	21.3±1.7	−17.7	4–5
		16	24.7±4.7	14.0±3.8	24.7±5.3	0	
		24	24.5±5.3	16.3±2.9	31.6±1.5	19.6	
		32	24.1±3.0	18.8±4.2	33.4±3.5	38.6	

IF, improvement factor.

Table 6
Efficacy of crude M-ASA and rapeseed methyl esters (RME) against attack from termites and longhorn beetle larvae (EN 117 and EN 46/47)

		Termites (*Reticulitermes santonensis*)		Longhorn beetle (*Hylotrupes bajulus*)	
		No ageing	Ageing (EN 73/84)	No ageing	Ageing (EN 73/84)
Crude M-ASA loaded at $298 \pm 82\,kg\,m^{-3}$	Death rate (%)	50	0	100	100
	Degree of destruction	0	1	0	0
RME loaded at $235 \pm 82\,kg\,m^{-3}$	Death rate (%)	0	not done	100	100
	Degree of destruction	2.5	not done	0	0
Controls	Death rate (%)	0	—	23 (EN 46)	—
	Degree of destruction	4	—	22 (EN 47)	—

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