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Chitosans and grapevine trunk diseases: surprising results from laboratory and vineyard studies

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The potential of chitosans to control grapevine trunk diseases (GTDs) has been studied in a public-private partnership. *In vitro* tests, vineyard efficacy trials and laboratory analyses were carried out to understand their mode of action. *In vitro,* low molecular weight (MW) chitosans, the most soluble, were often the most effective. In the vineyard, the most effective were those with a medium MW, in particular chitosan hydrochlorides which form a protective film. They could be used to dress wounds caused by pruning, re-trunking or curettage.

Chitosan is a natural product extracted from the chitin in the walls of fungi or the shells of crustaceans and insects. It is used in many fields (medicine, wastewater treatment, cosmetics, etc.) including enology[1]. In crop protection, chitosan is also known for its antiviral, antibacterial and antifungal properties and for contributing to natural plant defense mechanisms[2]. However, its mode of action in direct control of plant bio-aggressors remains poorly understood. For grapevines, chitosan of fungal origin is a basic substance approved as a stimulator of plant defense mechanisms. To better understand the variability in chitosan efficacy and identify the best candidate for use on grapevines, a partnership study was initiated in 2016. A total of 23 chitosans were assessed, but only 16 were subjected to multiple tests, namely: 7 chitosans extracted from crustaceans (OCe3, Ce20, Ca50, J80, S674 and two chitosan hydrochlorides, HA and HS), 5 from fungi (OF3, Fda30, LF43, F329 and chitosan hydrochloride H90) and 4 from insects (OY5, Y40, Y350 and Y903). In each case, the letters identify the chitosans while the numbers indicate their MW (kDa) when known.

In vitro the lightest are the most effective!

The ability of chitosans to inhibit the mycelial growth of two fungal pathogens responsible for trunk diseases (*Eutypa lata,* eutypa dieback, and *Phaeomoniella chlamydospora*, esca) was first assessed in the laboratory. Given that only oligochitosans and the hydrochlorides are soluble in water, a novel test method was devised (Figure 1).

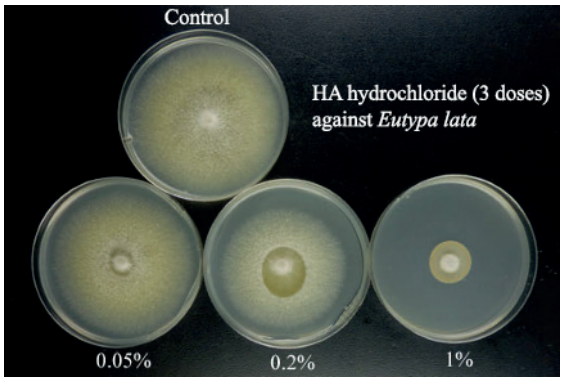

FIGURE 1. Novel method used to test chitosans. A 300µL aliquot is deposited at the center on the surface of an agar medium in a Petri dish. After drying, a disk of mycelium is deposited in the center of the circle containing the product. Efficacy is calculated by measuring the diameter of the mycelial colony and comparing it with that of an untreated control.

For the 3 concentrations tested (0.05, 0.2 and 1%), chitosans S674, F329, Y350 and Y903, with high PM (>300 kDa), always showed the lowest efficacy whatever the pathogen. Conversely, the best percentage efficacy was observed with oligochitosans (lowest MWs, ≤5 kDa). The origin of the chitosan (fungus, crustacean or insect) did not appear to influence efficacy. Hence, the best candidates against these two pathogenic fungi were oligochitosans and chitosans of medium MW (between 5 and 130 kDa) (Table 1).

TABLE 1. Effect of chitosans of different origins on mycelial growth of *E. lata* and *P. chlamydospora* when tested *in vitro* at 1%. In most cases, the products were tested two or three times.

Chitosan		Efficacy (%)	
		E. lata	*P. chlamydospora*
Crustacean	OCe3	**100 ± 0**	**57 ± 18**
	Ce20	**91**	**75**
	Ca50	**85 ± 3**	**78 ± 3**
	J80	**35**	**46**
	HA*	**92 ± 1**	**46 ± 22**
	HS*	**90 ± 3**	**43 ± 7**
	S674	**0 ± 0**	**3 ± 4**
Fungus	OF3	**100 ± 0**	**59 ± 11**
	Fda30	**87**	**41**
	LF43	**87 ± 5**	**69 ± 24**
	H90	**86 ± 6**	**45 ± 24**
	F329	**4 ± 6**	**5 ± 7**
Insect	OY5	**92 ± 1**	**74 ± 17**
	Y40	**90 ± 1**	**62 ± 28**
	Y350	**2 ± 3**	**0 ± 0**
	Y903	**2 ± 3**	**18 ± 6**

* Unknown MW

In the vineyard, the lightest are the least effective!

Since 2016, vineyard trials have assessed the ability of chitosan to protect pruning wounds against trunk diseases, using the official method CEB 155[3] [4], based on artificial infection. Efficacy is judged by wound infection rates (30 canes per product) and the number of infected wood chips per wound (25 wood chips analyzed per cane), compared with an untreated control.

LF43 (fungal) was the first to be tested in the vineyard, against *E. lata* (2016, 2017) and *P. chlamydospora* (2017). Applied as a preventive paste on pruning wounds at a dose of 30%, LF43 proved ineffective against *P. chlamydospora* but very effective against *Eutypa lata* (71% and 77% efficacy in 2016 and 2017 respectively). Unfortunately, the propensity of the product to crack on drying and its high cost discouraged further study.

The choice thus fell on the oligochitosan OCe3, which performs well *in vitro*, and the hydrochloride H90, for exploratory purposes (Table 2).

TABLE 2. Results of preventive trials performed in the vineyard in 2018, 2019 and 2021 to assess the preventive efficacy of different chitosans.

	Eutypa lata		*Phaeomoniella chlamydospora*	
2018 and 2019 trials	215 ascospores inoculated (2018)		1,786 conidia inoculated (2018)	
	355 ascospores inoculated (2019)		1,960 conidia inoculated (2019)	
	Infection rate **Efficacy (%)**		Infection rate **Efficacy (%)**	
	Pruning wounds* n = 30	Wood chips** n = 750 (30 x 25)	Pruning wounds* n = 30	Wood chips** n = 750 (30 x 25)
Inoculated control (2018–2019)	80 [a] – 83.3 [a]	27.2 – 32.9	96.6 [ab] – 100 [a]	47.3 – 50.8
OCe3 sprayed at 5% (2018)	60 [a] **25**	16.1 **NS** **40.7**	86.7 [ab] **10.2**	38 **NS** **19.7**
OCe3 sprayed at 10% (2019)	83.3 [a] **0**	36.4 **NS** **0**	96.7 [a] **3.3**	47.7 **NS** **6**
OCe3 sprayed at 15% (2019)	86.7 [a] **0**	34 **NS** **0**	93.3 [a] **6.7**	44.7 **S** **12.1**
H90 pasted at 10% (2019)	33.3 [b] **60**	13.8 **S** **57.9**	33.3 [b] 66.7	8.9 S 82.4
	Eutypa lata		***Phaeomoniella chlamydospora***	
2021 trial	431 ascospores inoculated (2021)		1,965 conidia inoculated (2021)	
	Pruning wounds* n = 30	Wood chips** n = 750 (30 x 25)	Pruning wounds* n = 30	Wood chips** n = 750 (30 x 25)
Inoculated control	63.3 [a]	22	88 [a]	24.3
HA sprayed at 2%	24.1 [b] **61.8**	6.9 **HS** **68.6**	75 [ab] **14.8**	13.6 **NS** **44.2**
HA sprayed at 5%	23.3 [b] **63.2**	1.1 **THS** **95.2**	76.9 [b] **12.6**	15.8 **NS** **34.8**

* For pruning wounds, the letters ([a, ab, b]) indicate the homogeneous groups resulting from the analysis of variance or a Kruskal-Wallis test (the 30 pruning wound results were broken down into 3 repetitions of 10 wounds).

** For wood chips (25 examined per pruning wound), the results were divided into 3 classes according to the number of infected wood chips per wound: [0–5], [6–10], [11–25]. The distributions were then compared using a χ^2 test. Each treatment was compared with the inoculated untreated control. NS = not significant; S = significant (α = 5%); HS = Highly Significant (α = 1%); VHS = Very Highly Significant (α = 0.1%).

FIGURE 2. Pruning wound on Cabernet Sauvignon treated with a chitosan hydrochloride paste. Photograph taken immediately after application.

Tested in 2018 and 2019 at doses of 5, 10 and 15%, OCe3 did not sufficiently reduce pruning wound infections by *E. lata* and *P. chlamydospora* (efficacy always below 25%). This result was very surprising in the light of the *in vitro* tests, where it shows good efficacy. In contrast, H90, applied in 2019 as a 10% paste, significantly controlled infections by both pathogens (60% and 58% efficacy on pruning wounds and wood chips respectively against *E. lata*, and 67% and 82% efficacy against *P. chlamydospora*). While the high viscosity of the hydrochloride made it difficult to apply by spraying, it formed a protective barrier when applied as a paste (Figure 2).

A new trial was set up in 2021 with hydrochloride HA applied preventively as a 2% spray or 5% paste (Table 2). With *E. lata*, HA significantly reduced infections (62% efficacy for the spray and 63% for the paste). This effect was confirmed by the rate of infected wood chips per wound: 68% efficacy for the spray and 95% for the paste. For *P. chlamydospora*, HA did not significantly reduce wound infections (14% and 12% efficacy). In contrast, the efficacy of the product appeared to be better in terms of the rate of wood chips infected by the pathogen: 44% for the spray and 34% for the paste, but not statistically confirmed. This difference in efficacy, depending on the application technique and the fungus studied, suggested a barrier effect, with its efficacy linked to the MW and solubility of the product and the size of the spores deposited on the protective film generated by the product after application.

Research into the mode of action of chitosan hydrochloride

To better understand the contradictory results from the *in vitro* and *in vivo* trials, a complementary study was carried out, also based on method CEB 155, by cutting the tissue underlying the pruning wounds into ten disks approximately 1 mm thick. This method makes it possible to see the depth to which the fungi penetrate. Six formulations, all at 5%, were applied as a preventive paste to pruning wounds: oligochitosans OF3 and OCe3, chitosans LF43 and Ca50, and hydrochlorides H90 and HS. The results showed total efficacy (100%) of H90 and HS against *E. lata* and *P. chlamydospora*, as the products prevented the spores from penetrating below the wound surface. Chitosans LF43 and Ca50 showed moderate effects (43–60% efficacy) against *E. lata* and no effect against *P. chlamydospora*. Oligochitosans OCe3 and OF3 proved ineffective (0 to 34% efficacy). This additional study thus confirmed the barrier effect of medium MW chitosans, and in particular of water-soluble hydrochlorides.

Conclusion

While initial laboratory trials showed that oligochitosans had good potential to control two fungi responsible for trunk diseases, medium MW chitosans, particularly the hydrochloride because of its solubility in water, proved the most effective in protecting pruning wounds in the vineyard, with an average efficacy of 57% against *E. lata* for HA applied as a paste (2 trials in 2021). As it dries and probably agglomerates, the hydrochloride seems capable of trapping any spores that may be deposited on the wound surface. It acts as a dressing, so its mode of action is mainly physical, more fungistatic than fungicidal. It could help prevent several pathogenic fungi – ascomycetes, characterized by their large spores, and basidiomycetes – from penetrating the wood, thus protecting wounds caused by pruning or re-trunking, or even curettage, from over-rapid re-infection. It should be noted that no toxicity was observed with this product in the study. This work has been the subject of French patent application number FR2004922. However, no formulation is yet available. ■

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