

# PROPAGATION OF PIONEER FUNGI ASSOCIATED WITH ESCA DISEASE BY VEGETATIVE MATERIAL IN FRENCH GRAPEVINE NURSERIES

Esca disease of grapevines is caused by white rot in the wood, which is also correlated with various foliar symptoms (mild form, apoplexy). The white rot results from the action of several micro-organisms : pioneer fungi leading to the formation of a brown necrosis in central position (*Phaeoacremonium aleophilum* (*Pal*)) or in sectorial position (*Eutypa lata*), which is then colonized by *Fomitiporia mediterranea* responsible for the white rot. Because no control measures are known for vineyards, measures must be taken in nurseries to manage this syndrome. In this aim, we studied the life cycle of fungi associated with esca disease in nurseries and tested control measures for the production of healthy plants.

## OCCURRENCE OF PIONEER FUNGI AT THE SURFACE OR INSIDE THE PROPAGATION MATERIAL

A study in nurseries on fungi associated with wood diseases showed the presence of pioneer fungi of esca disease in the woody tissues of canes intended for nurseries (grafts, rootstocks). But *Eutypa lata* and *Fomitiporia mediterranea* were not found. The analysis of isolations of segments located between 15<sup>th</sup> and 16<sup>th</sup> mm from the base of each cane of 3 840 grafts collected from a strongly esca-infected vineyard (35 % in 1997), showed that *Pch* was present in 0.44 % and *Pal* in 0.89 % of the isolations. Molecular tools such as PCR also revealed the presence of fungi on the surface of the propagation material (grafts, rootstock), which could constitute a considerable source of inoculum for the contaminations observed in nurseries.

Table 1 : Isolation from canes of rootstock at different distances from the top of the plants, collected from 12 vineyards and on 8 varieties

Distance from the top of the plants (cm)	Number of canes examined	<i>Pch</i> (%)	<i>Pal</i> (%)
5	1 150	1.04	0.17
40	300	0	0
80	300	0	0
400	350	0	0
600	350	0	0

Table 2 : Detection by PCR of fungi on the surface of the vegetal material : tests of the rinsing water of 4 cm segments taken at the base of canes

Primers		PCL <sup>1</sup>	PCH <sup>2</sup>	PAL 1/2 <sup>3</sup>	PAL 3/4 <sup>3</sup>	
Canes	Number examined	% with <i>Pch</i>	% with <i>Pal</i>			
Rootstock	41 B 153	100	9	20	11	12
	SO4 102	50	30	10	4	24
Graft	Cabernet Sauvignon N	100	10	5	9	3
	Sauvignon B	400	14	1,8	1,5	3,3

<sup>1</sup> Groenewald et al. (2000), <sup>2</sup> Tegli et al. (20000), <sup>3</sup> Péros (personal communication)

## IDENTIFICATION AND LOCALIZATION OF PIONEER FUNGI IN THE PLANTING MATERIAL

A survey in nurseries of the south-east of the France showed that the pioneer fungi of esca disease were isolated from plants after nursery field. Their presence in the plants is more or less important according to the sample. This variability is probably due to the origin of the plant material and/or the nursery process. *Eutypa lata* and *Fomitiporia mediterranea* were not found in the plants. Fungi had different locations in the plants. *Pal* was found preferentially at the top of the grafted cutting (graft, graft union, top of the rootstock) whereas *Pch* was mainly isolated from the zones located at their base (1 cm from the basal end, basal end).

Samples	<i>Pch</i> (%)	<i>Pal</i> (%)	Samples	<i>Pch</i> (%)	<i>Pal</i> (%)
Grenache N 435 / 110 R 151	4	12	Mourvèdre N 369 / 110 R 151	16.7	11.1
Syrah N 300 / 3309 C 144	34	4	Mourvèdre N 369 / 110 R 151	1.9	0
Alicante Bouschet N 804 / 140 Ru 265	2	2	Mourvèdre N 369 / 110 R 237	10	2
Syrah N 525 / 161-49 C 176	2	0	Mourvèdre N 369 / 110 R 151	0	0
Chardonnay N 95 / Fercal 242	34	4	Mourvèdre N 369 / 110 R 151	6	2
Grenache N 135 / 110 R 151	2	0	Mourvèdre N 369 / 110 R 151	10.4	8.3

Table 3 : Isolation from 12 samples of 50 plants collected from commercial nurseries  
Total percentage of infected plants : *Pch* 10.3 % and *Pal* 3.8 %

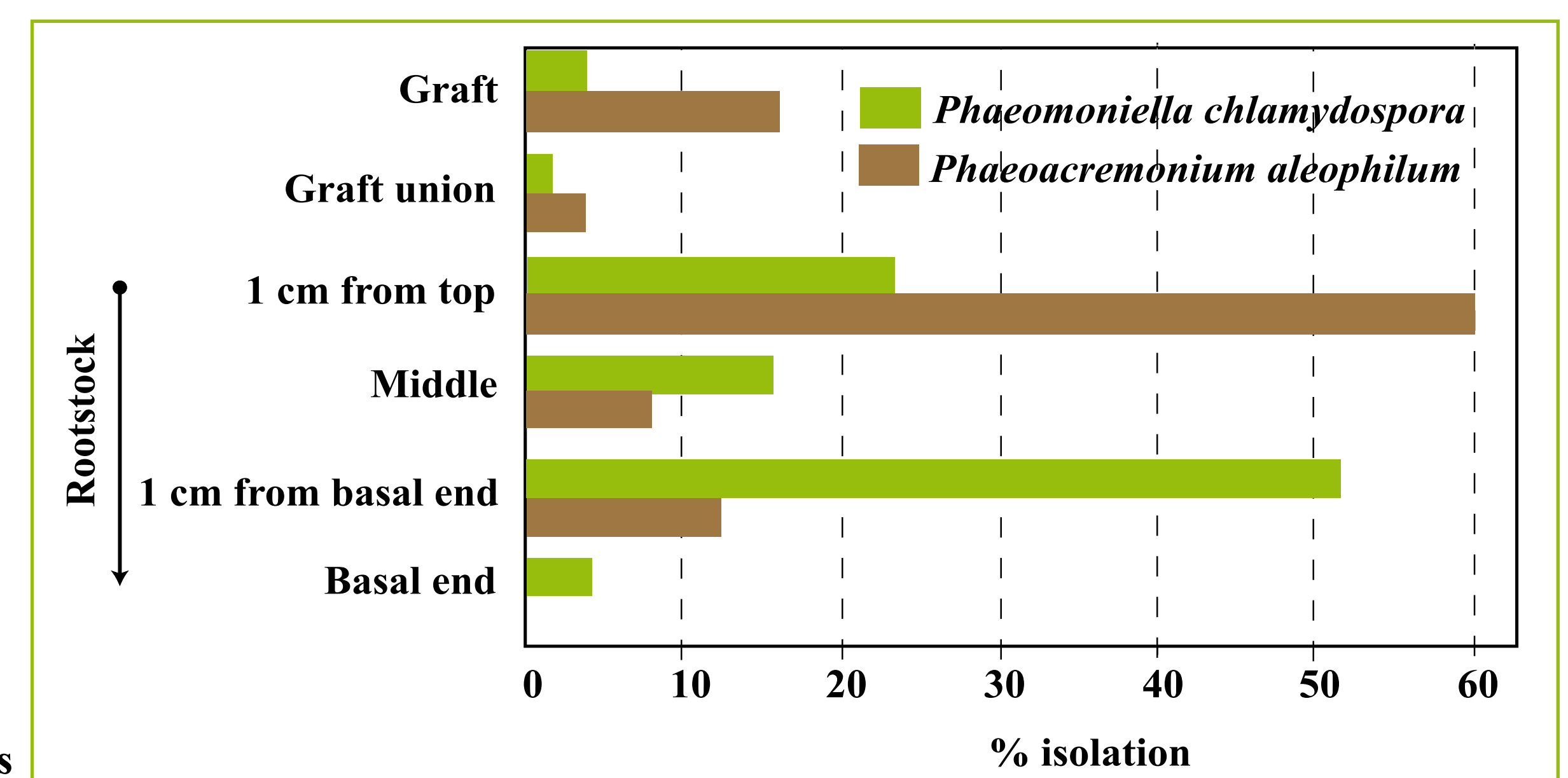


Figure 1 : Localization of fungi in plants

## STEPS DURING WHICH THE CONTAMINATIONS TAKE PLACE IN NURSERIES <sup>(1)</sup> AND CONTROL

The assumption of a contamination in nurseries was examined during two steps in the grapevine propagation process : callusing and planting. The isolations were taken after callusing or planting from non-inoculated cuttings (Syrah N 470 / Ru 140) which were put in the presence of cuttings inoculated at the graft union (extremities of the graft and rootstock being put in contact with a colony of *Pch* isolate SO28 or *Pal* isolate SO34). The experiment was replicated two times.

For *Pch* : the contaminations of cuttings took place only during callusing, caused by wounds located at their base. For *Pal* : the contaminations took place only during planting by aerial parts of the plant. It is difficult to know which sources of inoculum were responsible : endo-inoculum or exo-inoculum.

Among the different treatments tested, only hot water treatment (45 min, 50° C) showed good efficiency against *Pch*. No treatment was efficient against *Pal*.

Table 4 : Effect of different treatments against *Pch* and *Pal*

Material treated	Treatments	Number of plants examined	% effectiveness	
			<i>Pch</i>	<i>Pal</i>
Propagation material (before grafting)	Bleach 0.5 % active chlorine (dipping, 2 h)	50	0	20
	Cryptonol 3.5 % (dipping, 4 h)	50	0	0
	Extract vegetal (dipping, 4 h)	50	0	0
	<i>Trichoderma atroviride</i> strain T10 (spraying, 10 <sup>6</sup> spores/ml)	50	0	6.7
Plants after nursery field	Hot water treatment (50° C, 45 min.)	190	92.1	11.3

## CONCLUSION

These studies allowed to improve the knowledge on the life cycle of pioneer fungi in nurseries and on the control for the production of healthy planting material. Further studies should concern the identification of the state of fungi at the surface of propagation material and their localization, the identification of sources of inoculum of *Pal* responsible for contaminations in nursery fields (endo-inoculum or exo-inoculum). Further studies will test the efficiency of a combination of hot water treatment with other treatments in nurseries. Also, a change in nursery practices such as herbaceous grafted cuttings could be envisaged.