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SHORT NOTES

Survival of fungi associated with grapevine decline in pruned wood after composting

Pascal Lecomte[1], Gwénaëlle Louvet[1], Bertrand Vacher[1] and Pascal Guilbaud[2]

[1] Institut National de la Recherche Agronomique, UMR Santé Végétale (INRA-ENITAB), CR Bordeaux-Aquitaine, Avenue Edouard Bourleaux, BP 81, 33883 - Villenave d'Ornon cedex, France
[2]Chambre d'Agriculture de la Gironde, 39 rue Michel Montaigne, BP 115, 33294 – Blanquefort, France

Summary. Recycling vine wood pruned in winter in the vineyard, after grinding and composting, might pose a risk of recontamination with fungi associated with grapevine decline. The survival of four ascomycete fungi (*Botryosphaeria obtusa*, *Phaeomoniella chlamydospora*, *Phaeoacremonium aleophilum* and *Eutypa lata*) in composted material was investigated in a 3-year study conducted in the Bordeaux area. Naturally and artificially infested material was examined before and after composting using classical isolation procedures. Results clearly showed that a composting process can eradicate the four target fungi efficiently.

Key words: wood diseases, Eutypa dieback, esca, cultural practices, survival.

Introduction

A range of management practices are used to deal with canes or grapevine wood removed from vines during the pruning season. One of the most useful methods for recycling this waste involves grinding, composting and then reapplying it to the vineyard (Fig. 1). This study was designed to determine if fungi associated with vine decline survive the composting procedure and pose a risk of vineyard recontamination.

Corresponding author: P. Lecomte
Fax: +33 557122621
E-mail: lecomte@bordeaux.inra.fr

A 3-year study was initiated during 2002 in a commercial vineyard located at Pauillac (Château Latour - Médoc) to examine the ability of four ascomycete fungi (*Eutypa lata*, *Phaeomoniella chlamydospora*, *Phaeoacremonium aleophilum* and *Botryosphaeria obtusa*), which are associated with vine decline (Larignon and Dubos, 1997), to survive composting of pruned wood.

Materials and methods

In each of the three years (2002–2004), about 140 m^3 of pruned vine material was ground and mixed (Fig. 2) with 125 m^3 of sheep manure and 60 m^3 of stalks and garden residues (leaves, grass). Temperatures inside the compost were measured

Fig. 1. View of the compost produced in 2004 at Château Latour (Médoc).

Fig. 2. Turning the ground pruning compost.

with a thermometer introduced 1 meter into the compost. Six random measures per date of observation were done at least every 10 days. Data were collected from late April to August in 2003 and from early March to the end of July in 2004. The compost was wetted in summer and turned.

In 2002, the ground pruning material used was sampled for fungal development only after composting. In 2003 and 2004, the ground pruning material was sampled for fungi before and after composting. To accurately assess the effect of composting on the survival of fungi, naturally or artificially infected wood material (at least three labelled pieces of wood per treatment) was incorporated into the compost mixture prior to composting. Samples with characteristic symptoms of *E. lata, Pa. chlamydospora, P. aleophilum* and *Botryosphaeria* sp. were removed from diseased vines in local vineyards. These natural samples were put in small nets and introduced in the compost at the beginning of spring. Artificial material was also prepared by inoculating autoclaved grapevine wood pieces with *Pa. chlamydospora*.

To check the presence of wood decline fungi, samples (ground canes, pieces of wood, debris) were analysed with classical isolation methods (10–30 wood chips per sample) on malt-agar medium (20 g l^{-1} of agar-agar and 15 g l^{-1} of malt). Before plating, in order to limit the development of common and rapidly growing fungi, part of the wood chips was disinfected with calcium hypochlorite (1.8□% of active chlorine) according to a previously described procedure (Lecomte *et al.*, 2000).

Fungal identification was based on morphological characters and by comparison with local reference strains. *E.□lata* germination was assessed by classical tests of viability on malt-agar medium. Small pieces of infected grapevine wood bearing perithecia were cut with a scalpel and immersed in sterile water. Ascospore release and identification on the basis of the characteristic allantoid form were checked using a haemacytometer. About 200 μl of each ascospore suspension was plated and repeated on malt-agar medium supplemented with chloramphenicol (50 m g l^{-1}) and one replicate was also done on malt-agar medium without chloramphenicol. After 24–48 h of incubation, germination of ascospores was observed using a microscope (magnification 10×15) on the surface of the medium.

Fig. 3. Wood samples bearing *E. lata* stromata used in this study (left: not composted; right: after composting).

Table 1. Survival of fungal pathogens in composted pruned wood in 2003: wood colonization or spore germination (*Eutypa lata*) before and after composting. Colonization was expressed as number of wood chips allowing the isolation of one of the fungi studied, in samples disinfected (A) or not disinfected (B) with calcium hypochlorite.

Fungus	Composted ground material						Composted artificially or naturally infected material						Control sampled after 6 months		
	Before			After			Before			After					
	A	B	%[a]	A	B	%[a]	A	B	%[a]	A	B	%[a]	A	B	%[a]
Pa. chlamydospora mycelium	0/200	0/200	0	0/200	0/200	0	17/40	-	42.5	0/90	-	0	-[b]	-[b]	-[b]
Botryosphaeria sp. mycelium	45/200	69/200	28.5	0/200	0/200	0	20/30	9/10	72.5	2/35	0/125	1.2	10/30	4/10	35
E. lata mycelium	0/200	0/200	0	0/200	0/200	0	-	-	-	0/180	-	0	3/45	-	6.6
E. lata ascospores	-	-	-	-	-	-	-	-	64 to 96	-	-	0	-	-	30 to 70

A, Number of positive (allowing the isolation of one of the fungi studied) wood chips/Total number of wood chips examined that were not disinfected with calcium hypochlorite.
B, Number of positive (allowing the isolation of one of the fungi studied) wood chips/Total number of wood chips examined that had been disinfected with calcium hypochlorite.
[a] Percentage over total (A+B) wood chips examined
[b] Samples were highly contaminated by *Penicillium* sp. and not usable.

Table 2. Survival of fungal pathogens in composted pruned wood in 2004: wood colonization or spore germination (*Eutypa lata*) before and after composting. Colonization was expressed as number of wood chip (or sample*) allowing the isolation of one of the fungi studied, in samples disinfected (A) or not disinfected (B) with calcium hypochlorite.

Fungus	Composted ground material						Composted artificially or naturally infected material										Control sampled after 6 months				
	Before			After			Necrotic wood						Inoculated wood				Necrotic wood			Inoculated wood	
							Before			After			Before		After						
	A	B	%[a]	A	B	%[a]	A	B	%[a]	A	B	%[a]	A	%[a]	A	%[a]	A	B	%[a]	A	%[a]
Pa. chlamydospora mycelium	0/750	-	0	0/100	-	0	14/15	-	93	0/180	0/90	0	6/6*	100	0/180	0	-	0/120[b]	0	0/6*[b]	0
Botryosphaeria sp. mycelium	11/375	15/375	3.5	0/50	0/50	0	-	3/5	60	0/180	0/60	0	-	-	-	-	10/20	3/5	52	-	-
Pm. aleophilum mycelium	0/750	-	0	0/100	-	0	-	5/10	50	0/60	0/80	0	-	-	-	-	3/10	-	30	-	-
E. lata mycelium	0/750	-	0	0/100	-	0	95/105	-	90.5	0/180	-	0	-	-	-	-	49/85	-	57.6	-	-
E. lata ascospores	-	-	-	-	-	-	-	-	88 to 99	-	-	0	-	-	-	-	-	-	44 to 73	-	-

A, Number of positive (allowing the isolation of one of the fungi studied) wood chips/Total number of wood chips examined that were not disinfected with calcium hypochlorite.
B, Number of positive (allowing the isolation of one of the fungi studied) wood chips/Total number of wood chips examined that had been disinfected with calcium hypochlorite.
[a] Percentage over total (A+B) wood chip or sample (*) examined.
[b] These samples were highly contaminated by *Penicillium* sp.

Results

In 2003, the mean internal temperatures of this compost was about 65°C a few days after composting and varied between 50 and 65°C after May. In 2004, internal temperatures reached 75°C a few hours after mixing and then fluctuated between 40 to 50°C.Whatever the year, the temperatures never went below 40°C during the six-month period of composting.

Disinfection with calcium hypochlorite generally limited the development of contaminating fungi such as *Penicillium* sp., *Mucor* sp., *Rhizopus* sp.

In 2002, no fungi associated with grapevine decline were isolated from composted material.

Results obtained in 2003 and 2004 are summarized in the Table 1 and 2, respectively. Before composting, only *B. obtusa* was isolated from ground canes. The absence of *Pa. chlamydospora* or *P. aleophilum* from our samples may be partly due to the grinding protocol, possibly favouring the development of saprophytes that grow more rapidly than the vine decline fungi.

All the fungi in the naturally infected samples were found to be viable before their introduction in the compost. Moreover mycelium development was observed from infected wood stored in the laboratory during 6 months for control.

No mycelial development was observed from composted ground cane material or introduced samples for any of the fungi with the exception of some colonies of *B. obtusa* in 2003, that were derived from a single piece of wood. Samples of wood containing stromata of *E. lata* were black-coloured and their surface appeared highly damaged (Fig. 3). Perithecia were often open and ascospores were rare and not viable.

Discussion

The composting process efficiently eradicated the four target fungi associated with vine decline. Therefore, the composted ground material can be reintroduced in the vineyard with no risk of contamination.

Eutypa lata causes Eutypa dieback, a serious disease in temperate and humid areas. The development of this wood disease can be influenced by inoculum pressure (Carter, 1991). For the other fungi associated with grapevine decline and those fungi that are more common in grapevine, such as *Botryosphaeria* sp., *Pa. chlamydospora* and *P. aleophilum,* there is agronomic benefit in composting as a control method. We conclude that this treatment will reduce inoculum and thereby the incidence and spread of grapevine decline. Composting of pruning debris may ease growers concern of pruning wood becoming a potential source of vineyard inoculum.

Further studies would be useful to investigate other composting procedures, in particular composts that are produced at lower temperature. To our knowledge, this study is the first to present results on this compost recycling process.

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Literature cited

Carter M.V., 1991. The status of *Eutypa lata* as a pathogen. Monograph - *Phytopathological Paper* 32.

Larignon P. and B. Dubos, 1997. Fungi associated with esca disease in grapevine. *European Journal of Plant Pathology* 103, 147–157.

Lecomte P., J-P. Péros, D. Blancard, N. Bastien and C. Délye, 2000. PCR assays that identify the grapevine dieback fungus *Eutypa lata*. *Applied Environmental Microbiology* 66, 4475–4480.

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