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Interactions among grapevine disease-causing fungi. The role of reactive oxygen species

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Summary. *Botryosphaeria parva*, *Eutypa lata and Phomopsis viticola* are ascomyceteous fungi responsible for severe canker and dieback in numerous woody plants. In grapevine, these pathogens colonise the wood mainly through pruning wounds, and the diseases gradually develop, leading to partial or total vine death. In the present study, the three fungal species were grown in Czapek Dox modified medium. Under these conditions, fungal colonies are able to distinguish self from non-self. The production of reactive oxygen species (ROS) was analysed by specifically staining for superoxide ($O_2^{\cdot-}$) or peroxide (O_2^{2-}) radicals. The presence of ROS in both isolated cultures and fungal interactions was confirmed. All fungi produced both radicals, in every interaction. However, the patterns of ROS production depend on the fungus itself and on the fungal species with which it is interacting, being also dependent on the presence of antioxidant compounds in the surrounding medium. It is as though a fungal species hierarchy could be established for every interaction under each set of conditions (i.e. habitat). The results obtained suggest that fungi display more complex behaviours than generally acknowledged. They are able to recognize potential contestants and built up defence reactions, as well as weaken plant defences and structures to induce infection.

Key words: *Botryosphaeria parva*; *Eutypa lata*; *Phomopsis viticola*; wood diseases.

Introduction

It is well known that phytopathogenic fungi exude several premeditated products during the infection process that contribute to the establishment of the infection. These products can be as different as suppressors or host-specific toxins for suppression of host defence responses (Park and Ikeda, 2008); non-host-specific toxic metabolites (Daub and Ehrenshaft, 2000; Deighton *et al.*, 2001) that account for the development of symptoms; cell wall degradation enzymes (Mendegen *et al.*, 1996) for favouring fungal penetration into host cells; and reactive oxygen species (ROS) (Tiedemann, 1997; Deighton *et al.*, 1999; Daub and Ehrenshaft, 2000), acting as toxic agents for the induction of host cell death. Although formation of ROS by plants has been observed in countless plant-microbe interactions, their production by fungi has seldom been reported. Plant-derived ROS have been reported to be associated with defence mechanisms in several interactions (Lamb and Dixon, 1997; Wojtaszek, 1997). ROS generation is not only induced in pathogen-challenged host plants, but also in plants treated with several pathogen-produced molecules, such as elicitors (Lamb and Dixon, 1997; Wojtaszek,

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1997) and host-specific toxins (Shinogi *et al.*, 2002; Yao *et al.*, 2002; Shinogi *et al.*, 2003).

ROS are ions or very small molecules that include oxygen ions, free radicals and peroxides, both inorganic and organic. They are formed and degraded by all aerobic organisms as a natural by-product of oxygen metabolism (Fig. 1). The presence of unpaired valence shell electrons makes them highly reactive. In fungi, ROS play a role in cell signalling, cell proliferation and defence responses (Malagnac *et al.*, 2004). However, abiotic stresses (*e.g.*, UV light, temperature extremes, dehydration, salt, ozone and heavy metals) lead to enhanced levels of ROS, causing a situation known as oxidative stress, which can result in significant damage to cell structures and cause deleterious effects by damaging DNA, RNA, lipids and proteins (Rodriguez and Redman, 2005).

In fungi, ROS are known to regulate germination, development, and intercellular communications (Belozerskaya and Gessler, 2007). Fungi produce ROS in the course of metabolic activity and their production increases due to various stresses such as starvation, light, mechanical damage, and interactions with some other living organisms. Regulation of ROS levels appears to be very important during development of the fungal organism (Gessler *et al.*, 2007). For example, H2O2 acting as a signal molecule is involved in various processes, such as the change in fungal growth rate, differentiation, and proliferation (Hansberg *et al.*, 1990; Rayner *et al.*, 1999; Ivanova *et al.*, 2005; Belozerskaya and Gessler 2006; Georgiou *et al.*, 2006). It has been demonstrated that H2O2 influences the activity of *Aspergillus nidulans* genes, including antioxidant defence genes (Pocsi *et al.*, 2005), induces the differentiation of sclerotia in *Sclerotium rolfsii* (Sideri and Georgiou, 2000) and the transition to filamentous growth and pathogenicity in *Ustilago maydis* (Leuthner *et al.*, 2005), and activates the keratinogenesis in *Neurospora crassa* (Ligusa *et al.*, 2005). Hydrogen peroxide is able to react with a variety of valence metals such as, for example, Fe^{2+} (Fenton reaction), or with $O_2^{\bullet-}$ (Haber-Weiss reaction), to form the extremely reactive radical, OH•. Its ability to react with virtually all biological molecules, oxidizing DNA, proteins and lipids (Sigler *et al.*, 1999; Belozerskaya and Gessler, 2007), suggests that OH• can provoke significant damage to cells when present in excess.

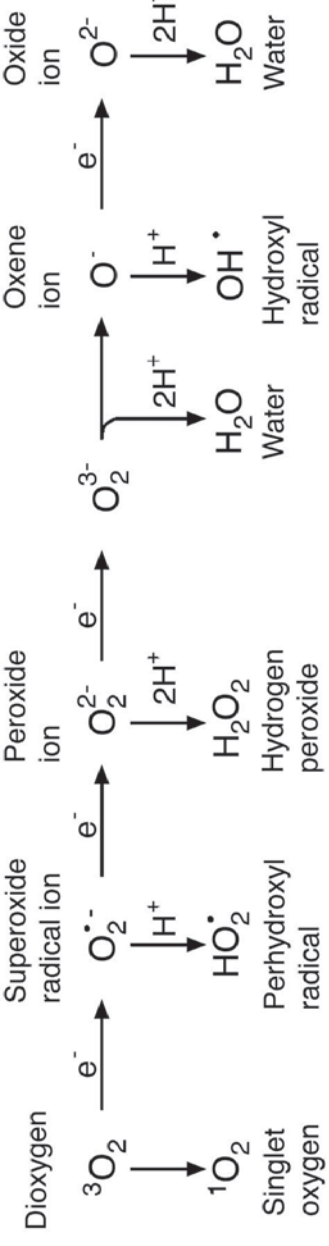

Fig. 1. Generation of different ROS by energy transfer or sequential univalent reduction of ground-state triplet oxygen (Rodriguez and Redman, 2005).

In some cases, ROS destructive potential can play a positive role in the organisms that produce them. For example, ROS, and, particularly, hydroxyl radical – the most reactive one produced – can be used to disrupt cellulose and lignin in wood-degrading fungi (Hammel *et al.*, 2002). Hydroxyl radical formation was detected chemically, and by other methods, in eight out of ten fungi tested (Hammel *et al.*, 2002; Tornberg and Olsson, 2002). It may be assumed that phytopathogenic fungi also produce OH• to penetrate the cell wall of the host plant (Gessler *et al.*, 2007). Indeed, even though many wood decay basidiomycetes dispense oxidative and hydrolytic enzymes that participate in lignocellulose biodegradation, it is generally recognized now that these enzymes cannot penetrate solid wood, and that fungi must utilize smaller agents to commence decay. ROS are probable candidates and evidence is accumulating that some wood decay fungi produce these oxidants (Hammel *et al.*, 2002).

Recognition of self and alien species upon hyphal contact appears to be a crucial point in development of mycelial fungi. Silar (2005) searched for regulated ROS production at the contact zone between eumycetes and other micro-organisms and then evaluated the possible role of NADPH oxidases in ROS production. This author analyzed H_2O_2 generation by contacting mycelia in a large group of fungi, presenting evidence that some filamentous fungi are indeed able to accumulate large amounts of peroxide when challenged, an occurrence markedly similar to an oxidative burst in plants. As observed in plants and animals, the specificity of the interaction between the partners suggests that a recognition mechanism is involved to detect a potential threat and that this oxidative burst may be associated, in some combinations, with the death of one of the partners. It has been concluded that H_2O_2 serves as one of the signals for identification of alien organisms by hyphae (Silar, 2005; Gessler *et al.*, 2007).

The present study dealt with the production of extracellular ROS, namely superoxide and peroxide, by three pathogenic wood inhabiting fungi, *Botryosphaeria parva* Pennycook & Samuels, *Eutypa lata* (Pers.: Fr) Tul. & C. Tul. and *Phomopsis viticola* (Sacc.) Sacc., three fungi that are the causal agents of severe trunk diseases in grapevine. The fungi were tested in axenic cultures or during intra- and interspecific fungal interactions, and also in the presence of antioxidants. These fungi are capable of growth in the same grapevine plant, apparently, without interfering with each other. An attempt was made to determine whether ROS production is used to recognise the presence of other micro-organisms or to promote the wood structure breakdown for the infection process of these wood inhabiting fungi.

Materials and methods

Fungal strains and growth conditions

Botryosphaeria parva (CBS 110301), *E. lata* (INRA, P. Lecomte isolate 8D) and *P. viticola* (Portuguese isolate from Azeitão, Portugal) isolates obtained from grapevine were grown on solid Czapek Dox modified medium (Oxoid, Hants, UK) dishes, at 24°C, in the dark.

Mycelium plugs (3 mm diameter) were aseptically removed from the edge of actively growing colonies and transferred, singly or as fungal pairs, to Petri dishes containing the medium referred above. For single cultures, plugs were placed at the centre of each dish, or in opposite sides of the dish, 1 cm from the dish border, when they were paired. Tests with fungal growth in 9 cm diameter Petri dishes were incubated for 5 days. Tests with fungal growth in 7 cm diameter dishes were incubated between 3 and 7 days, for *B. parva* or *E. lata* / *P. viticola* interactions, respectively. Each test had between 7 and 10 replicates and the experiments were repeated twice.

Antioxidant trials were made adding 2.5 mg ml^{-1} antioxidant (L-ascorbic acid, catechin and beta-carotene - solubilised in oil, due to its lipophilic nature) to the Czapek Dox modified medium before pouring into the Petri dishes. For control, dishes without antioxidant were used. A vehicle-treated control was used because beta-carotene, a lipotropic antioxidant, is insoluble in the growth medium utilized and so had to be solubilised in a vehicle (an oil), previously to its addition to the medium. It was also used as control, a medium consisting of pulverized grapevine cane with 1.5% (w/v) agar.

Superoxide and peroxide detection

The localization of extracellular superoxide and peroxide relative to colony growth were qualitatively detected by flooding dishes with appropriate test solutions (Munkres, 1990; Malagnac *et al.*, 2004). Briefly, after each incubation period, the dishes were flooded with 5 ml of 2.5 mM Nitro Blue

Tetrazolium (NBT) in 5 mM (N-morpholino) propanesulfonate-NaOH at pH 7.6 to detect superoxide (reacts with NBT to form a blue precipitate), or with 5 ml of 2.5 mM diaminobenzidine (DAB) and 5 purpurogallin units ml^{-1} of horseradish peroxidase (grade II; Sigma, St. Louis, MO, USA) in 5 mM potassium phosphate buffer at pH 6.9 to detect peroxide (triggers the accumulation of a red precipitate). After 30 min, the supernatant was removed and results were assessed after incubation for a few hours (Silar, 2005).

Results and discussion

Fungal interactions

All organisms in their natural habitat are very often forced to come in contact or proximity with a diversity of others, so that frequent interactions must be a salient feature of their pattern of life. The interactive behaviours of wood-decay fungi on simple, mineral media, often correlate well with their patterns of occurrence in the natural environment (Bruno and Sparapano, 2006). The pairing of micro-organisms on artificial substrates can lead either to a deadlock, in which neither mycelia can enter the other's domain, to replacement of one by the other or to co-habitation of the same site. Free chemical diffusion of compounds through the substrate in an interaction might induce fungal growth inhibition, implying specific recognition and the involvement of antimicrobial molecules (Tsujiyama and Minami, 2005) and generation of an unfavourable pH (Feofilova, 2003).

Botryosphaeria parva, *E. lata* and *P. viticola* are three fungal species responsible for wood diseases in grapevine. The uncertainty remains in what concerns their capacity to co-infect the same patches of wood, with or without overlapping between adjacent, non-self colonies.

When grown singly, *B. parva* colonies exhibited a much higher growth rate than either *E. lata* or *P. viticola* colonies (Fig. 2A, 2B, 2C). When two colonies of the fast growing fungus *B. parva* meet, they seem to recognize each other as self, and their mycelia intertwine (Fig. 2D). The interaction does not evidence any sort of competition, with both colonies appearing to integrate with one another. This might be due to the fact that they belong to the same strain.

However, when a *B. parva* colony is challenged by an *E. lata* colony, they somehow sense each other as non-self and their mycelia stop growing, forming a twilight zone at the border between the two separate colonies (Fig. 2E). It is as though a mutual exclusion boundary is established, probably due to the presence of inhibitory chemicals secreted by both fungal species. Although not readily visible in Fig. 2, the boundaries between each pair of colonies turned dark, thickened and aerial hyphae formed a ridge-like barrier between them. Interestingly, in the case of *B. parva*, aerial hyphae stopped growing at an invisible boundary and did not cross over at the confrontation region with both *E. lata* and *P. viticola*, as if an invisible wall existed there, indicating that *B. parva* is able to differentiate between self vs. non-self.

Plant pathogen interactions are perceived through pathogen-derived compounds which elicit defence reactions. In the case of the wood fungi, some of these molecules may be ROS, which might be produced by both the plants, as defence mechanism, and the fungi, as attack strategy against the plant, to instigate wood degradation allowing its invasion, or against other competing fungi, as defence. Thus, the fungi with the greatest attacking and defence capacity may acquire dominance over rival fungi.

The observations referred above and the well known production of reactive oxygen species (ROS) by fungi prompted the investigation of the production of superoxide and peroxide by isolates of *B. parva*, *E. lata* and *P. viticola* as well as during their interaction, in the presence or absence of antioxidant compounds.

Superoxide and peroxide detection.

The capacity of isolated cultures of *B. parva*, *E. lata* and *P. viticola* to produce peroxide and superoxide radicals was investigated both in the presence and absence of a fungal challenger. It was shown before (Silar, 2005) that some fungi produce a high amount of peroxide when challenged by another fungus, while superoxide suffers no obvious modification at the confrontation zones.

In Fig. 3 and 4, fungal colony patterns identical to those depicted in Figure 2 were stained with NBT (nitro blue tetrazolium) (Fig. 3) or DAB (diaminobenzidine) (Fig. 4) to yield a deep blue or yellow colour at the sites of superoxide or peroxide

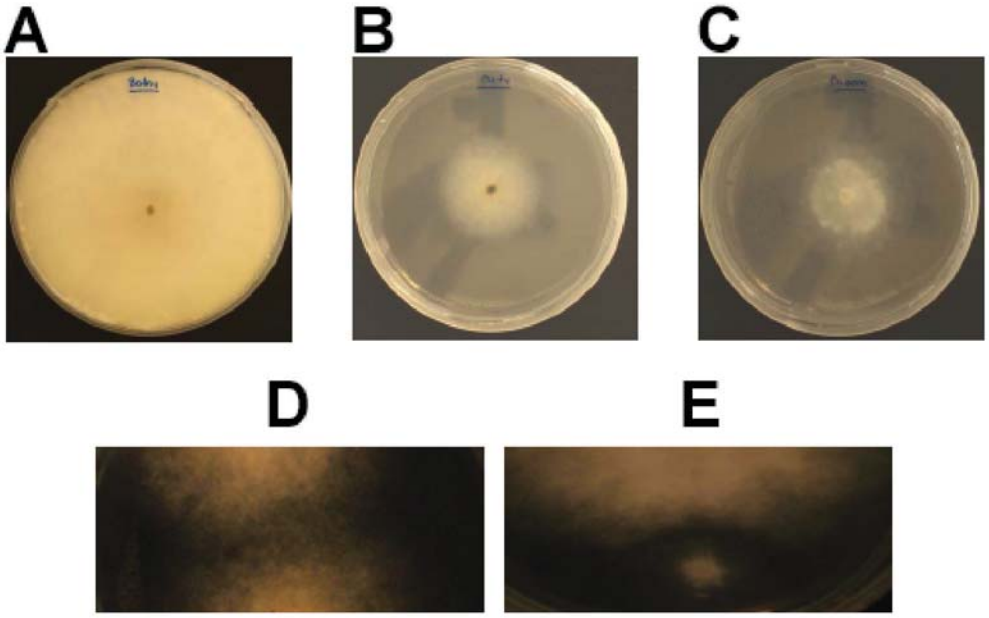

Fig. 2. Fungal growth for 5 days. (A) *Botryosphaeria parva*; (B) *Eutypa lata*; (C) *Phomopsis viticola*; and detailed view of the interface between *B. parva-B. parva* (D) or *B. parva-E. lata* colonies (E). Due to the lower growth rate of *E. lata*, the centre of its colony is visible in 2E.

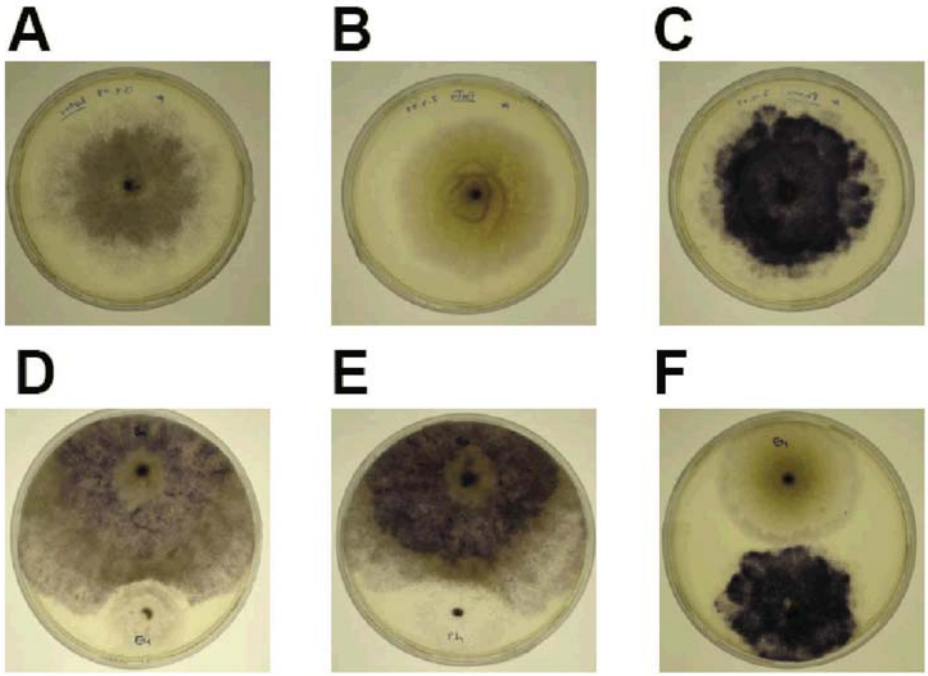

Fig. 3. Superoxide detected with NBT coloration (fungal growth for 4–7 days): (A) *Botryosphaeria parva*; (B) *Eutypa lata*; (C) *Phomopsis viticola*; and interactions between the fungi: (D) *B. parva-E. lata*; (E) *B. parva-P. viticola*; (F) *E. lata-P. viticola*.

radical accumulation, respectively. *B. parva* shows a superoxide production centred at the colony thallus, branching out to the border hyphae. The outer stretches of the colony produced a lighter and faint blue colour due to a lower mycelia density. This production appears to be more intense when challenged by other fungal species. *E. lata* seems to be a weaker superoxide producer, with the radical concentrated at the centre of the colony and a ring at the border hyphae. This pattern remains unchanged in the vicinity of other fungal species. *P. viticola*, on the other hand, shows an extremely high production of superoxide, which indicates a high NADPH oxidase activity (Bloomfield and Pears, 2003), when grown either alone and challenged with *E. lata* (Fig. 3F). Strangely enough, *P. viticola* seems to lose all its superoxide production capacity (including in the centre of the colony – Fig. 3E) when *B. parva* is nearby, suggesting a possible decrease in NADPH oxidase activity. Peroxide production was detected mainly at the border hyphae of all isolated fungal colonies (Fig. 4A, 4B, 4C). In addition, *E. lata* also presents a strong signal at the centre of the thallus. When challenged, *B. parva*, in the pairings *B. parva* / *E. lata* (Fig. 4D) and *B. parva* / *P. viticola* (Fig. 4E), specifically accumulated higher levels of peroxides at the confrontation zones, but in the pairing *E. lata* / *P. viticola* (Fig. 4F), fungal challenging did not affect the pattern of peroxide accumulation.

In summary, in a contact situation, *B. parva* increases its superoxide production when challenged with either *E. lata* or *P. viticola*, while *E. lata* maintains the pattern and *P. viticola* reduces the formation of superoxide, but maintain it when faced with *E. lata*. Similarly, in peroxide production, both *E. lata* and *P. viticola* maintain their production patterns while *B. parva* increased peroxide production in both interactions.

However, it may not always be advantageous to stockpile peroxide. For example, fungal peroxide accumulation may copy the early oxidative burst involved in plant defence to biotic stress and ought to be counteracted in fungi establishing parasitic or symbiotic association with plants (Silar, 2005). On the contrary, in fungi that come across many fungal challengers in their environment, this process may be positively selected (Silar, 2005).

Influence of antioxidants in *B. parva*

The fungus-to-fungus interaction is interesting from the point of view of fungal ecology and pathogenicity. It is well known that these fungi are capable of growing together in a small piece of grapevine wood, so with all probability, when confronted, they do not contact directly with one another, but interfere with each other using metabolites. Since ROS may play a role in this interference, another set of experiments was set up. The substrate media was supplied with selected antioxidants and their effect on the growth of *B. parva* was assessed (Fig. 5).

When grown unchallenged on antioxidant supplied media, *B. parva* was able to grow, when compared to the control, at a higher rate in the presence of ascorbic acid and beta-carotene, while in catechin supplied medium the rate was equivalent to that of the control. The vehicle-supplied control did not affect *B. parva* growth. A grapevine control was also tested, and the results clearly demonstrate that *B. parva* was able to grow much faster in the presence of grapevine cane powder than in any other media analysed.

Production of superoxide and peroxide by *B. parva* grown in the presence of antioxidants

The results obtained for *B. parva* grown in the presence of ascorbic acid or catechin are shown in Fig. 6. When compared to the control, superoxide production appears to be less intense but more widespread towards the outer edges of the colony in the case of ascorbate, but produced only in the centre of the fungal colony in the case of catechin. Conversely, peroxide production, which accumulates at the hyphae of *B. parva* colony border, is reduced in the case of ascorbate, but enhanced in the case of catechin.

Accumulation of ROS during fungal interactions

Considering the results presented in Fig. 2 to 6, the effect of selected antioxidants (i.e. ascorbic acid and catechin) in ROS accumulation during fungal interactions was studied. Table 1 summarises the results obtained, where the three types of media, control and control supplemented with 2.5 mg ml-1 of ascorbic acid or catechin, were used as the growth support for all three possible dual interactions among the three fungi under study: *B. parva*, *E. lata* and *P. viticola* For comparative purposes, the interaction between two *B. parva* colonies was also assessed.

Superoxide and peroxide detection during fungal interactions in the presence of antioxidants

Superoxide production for interactions *B. parva* / *B. parva* followed a pattern similar to that presented earlier (see Fig. 3), suggesting that the nearby presence of another self-colony does not interfere with superoxide accumulation. A mild fungal accumulation of superoxide was detected all over the mycelia in *B. parva* / *B. parva* interactions, except in the catechin medium, where the production was more concentrated in the thallus, is illustrated in Table 1. Production of superoxide by *B. parva* is greatly enhanced in the centre of the colony and reduced in its edges in all cases of interactions with the other fungi, either in the presence or absence of antioxidants. As for *E. lata* and *P. viticola*, when interacting with *B. parva*, accumulation of superoxide is not affected by the presence of antioxidants and apparently concentrates in the centre of the colony. With respect to the *E. lata* / *P. viticola* interaction, *P. viticola* increased peroxide accumulation in the presence of ascorbic acid and both mycelia stained a vivid red colour instead of the expected blue (from NBT staining) in the presence of catechin.

The corresponding results obtained for peroxide production are also displayed in Table 1. In *B. parva* / *B. parva* interaction (in ascorbic acid medium), the mycelia coloration, increased at the colony borders but also gave a strong reaction at the colony thallus. In catechin medium, the interaction showed a similar pattern to that of the control. In the pairing *B. parva* / *E. lata* the peroxide production increased at the border *B. parva* hyphae for both antioxidant supplemented media. *B. parva* / *P. viticola* exhibited a control-like production of peroxide, with only a slight decrease for *B. parva* peroxide production in catechin medium.

The pairing *E. lata* / *P. viticola* exhibited peroxide production similar to the control situation (see Fig. 4), except on catechin medium, where both fungi presented a red stained colony instead of the expected orange halo of peroxide production.

It was noted that superoxide and peroxide production followed standard production for these pairings, on antioxidant media with some exceptions. Superoxide production was, in general, similar to control, except in catechin medium, where *E. lata* and *P. viticola*, responded to NBT by producing a red colouration, instead of the expected blue. This effect is not due to any pH variation in the growth media (results not shown). As for peroxide production, there is a strong analogy to the control situation in the majority of the pairings analysed, with exception to the pairing *B. parva* / *E. lata*, which increased in both antioxidant media. *B. parva* in *B. parva* / *B. parva* pairing showed a more intense reaction to the peroxide staining at the centre of the colony in ascorbic acid media, when compared with control, and a reduced one in the *B. parva* / *P. viticola* interaction in catechin medium. *E. lata* and *P. viticola* reacted to each others presence in catechin medium by colouring red at the colony centre with the peroxide staining. Once again, this effect is not due to a change in pH in the fungal growth media.

Considering that catechins are polyphenolic antioxidant plant metabolites, many of which exhibit antifungal properties and are naturally present in wood (Balaban, 2004), the natural habitat for these fungi, it is reasonable to assume that they have developed specific mechanisms to deal with them. This observation, taken together with the data presented in Table 1 and the fact that this red colouring effect appear to occur exclusively to *E. lata* and *P. viticola*, seem to suggest that it might be due to specific requirements or manner of action of each fungus and its interaction with catechins throughout the infection process of the wood material.

Fungal competition for organic resources can be further sub-divided into primary resource capture and combat; the former consists of gaining initial access to and influence over an available uncolonized resources, whereas the latter involves capturing territory from fungi which are already colonizing a resource or defending a territory from potential invaders (Bruno and Sparapano, 2006).

This work reveals interesting aspects of wood fungal pathogen metabolism. More studies are needed to determine the role of ROS in wood fungal infection and data concerning these interactions might increase our understanding of the insidious character of the diseases by analysing the relationship between fungal biosynthesis and the changing environment. Correlation between these studies and field observations is desired in order to better comprehend the biology of these diseases and implement more efficient agronomical practices.

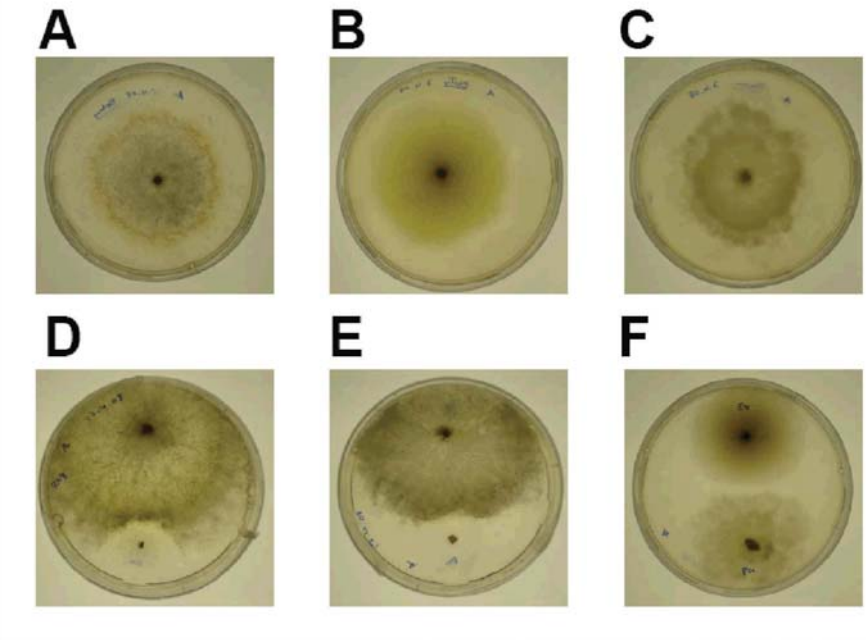

Fig. 4. Peroxide detected with DAB coloration (fungal growth for 4–7 days): (A)- *Botryosphaeria parva*; (B) *Eutypa lata*; (C) *Phomopsis viticola*; and interactions between the fungi: (D) *B. parva-E. lata*; (E) *B. parva-P. viticola*; (F) *E. lata-P. viticola*.

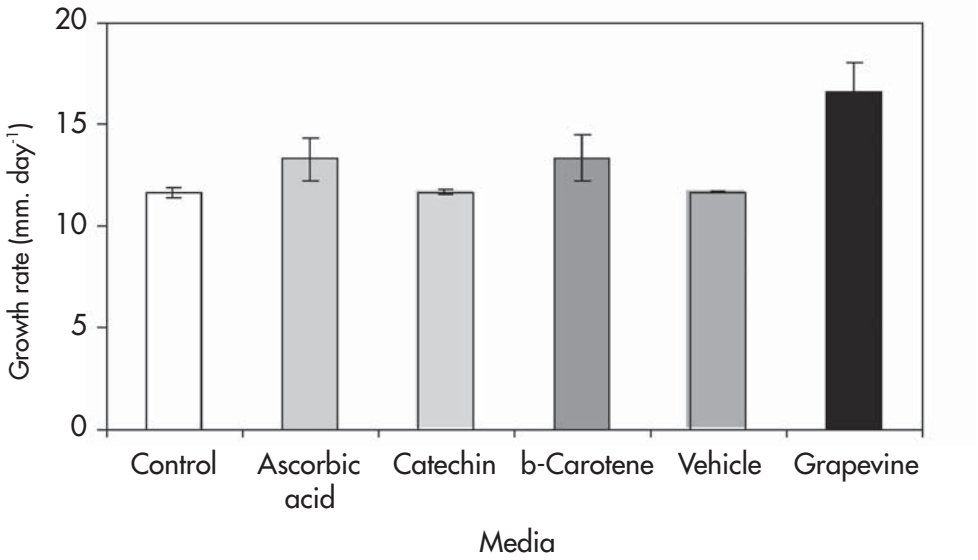

Fig. 5. *Botryosphaeria parva* growth in the presence of antioxidants. The fungus was grown in Czapek Dox modified solid medium with 2.5 mg ml-1 of antioxidant supplement: ascorbic acid, catechin and beta-carotene. As control, simple Czapek Dox medium, Czapek Dox medium supplemented with the vehicle used to solubilize beta-carotene, and grapevine medium were used. The cultures were incubated for 3 days, in 7 cm diameter Petri dishes.

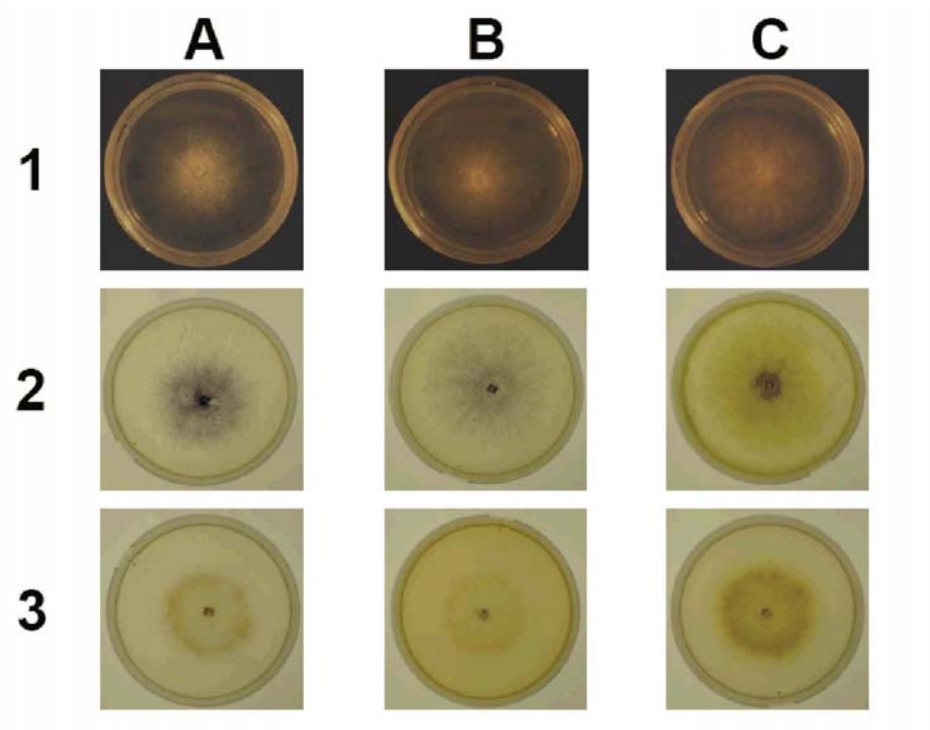

Fig. 6. *Botryosphaeria parva* growth on a Czapek Dox modified solid medium with 2.5 mg ml-1 antioxidant supplement: (A) control; (B) ascorbate; (C) catechin; 1- control; 2- NBT coloration; 3- DAB coloration. Fungal growth, for 3 days in 7 cm diameter Petri dishes.

Table 1. Fungal interactions in Czapek Dox solid medium with 2.5 mg ml-1 of antioxidant supplement (ascorbic acid and catechin) related to the control situation (without antioxidant supplement). Superoxide species were detected with NBT and peroxide species were detected with DAB. =, similar to the control; +, increased production; –, decreased production; ∗, red stained mycelia. Fungal growth for 3–5 days, in 7 cm diameter Petri dishes. The pattern of ROS spreading in each colony is represented between brackets for each fungal interaction: (●) entire mycelium and (⦿) focused at the centre of the colony.

Fungal interaction	Superoxide				Peroxide			
	Ascorbic acid		Catechin		Ascorbic acid		Catechin	
B. parva / *B. parva*	=	(●/●)	+	(⦿/⦿)	+	(⦿/⦿)	=	(⦿/⦿)
B.parva / *E. lata*	=/=	(⦿/⦿)	=/=	(⦿/⦿)	+/+	(●/⦿)	++/=	(⦿/⦿)
B. parva / *P. viticola*	=/=	(●/⦿)	=/∗	(⦿/⦿)	=/=	(⦿/⦿)	-/=	(⦿/⦿)
E. lata / *P. viticola*	=/+	(●/●)	∗/∗	(●/●)	=/=	(●/●)	∗/∗	(●/●)

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Literature cited

Balaban M., 2004. Identification of the main phenolic compounds in wood of *Ceratonia siliqua* by GC-MS. *Phytochemical Analysis* 15, 385–388.

Belozerskaya T.A. and N.N. Gessler, 2006. Oxidative stress and cell differentiation in *Neurospora crassa*. *Microbiology* 75, 427–431.

Belozerskaya T.A., and N.N. Gessler, 2007. Reactive oxygen species and the strategy of antioxidant defense in fungi: a review. *Applied Biochemistry and Microbiology* 43, 506–515.

Bloomfield G. and C. Pears, 2003. Superoxide signalling required for multicellular development of *Dictyostelium*. *Journal of Cell Science* 116, 3387–3397.

Bruno G. and L. Sparapano, 2006. Effects of three esca-associated fungi on *Vitis vinifera* L.: III. Enzymes produced by the pathogens and their role in fungus-to-plant or in fungus-to-fungus interactions. *Physiological and Molecular Plant Pathology* 69, 182–194.

Daub M.E. and, M. Ehrenshaft, 2000. The photoactivated *Cercospora* toxin cercosporin: contributions to plant disease and fundamental biology. *Annual Review of Phytopathology* 38, 461–490.

Deighton N., I. Muckenschnabel, A.J. Colmenares, I.G. Collado and B. Williamson 2001. Botrydial is produced in plant tissues infected by *Botrytis cinerea*. *Phytochemistry* 57, 689–692.

Deighton N., I. Muckenschnabel, B.A. Goodman and B. Williamson 1999. Lipid peroxidation and the oxidative burst associated with infection of *Capsicum annuum by Botrytis cinerea*. *Plant Journal* 20, 485–492.

Feofilova E.P. 2003. Deceleration of vital activity as a universal biochemical mechanism ensuring adaptation of microorganisms to stress factors: a review. *Applied Biochemistry and Microbiology* 39, 1–18.

Georgiou C., N. Patsoukis, I. Papapostolou and G. Zervoudakis 2006. Sclerotial metamorphosis in filamentous fungi is induced by oxidative stress. *Integrative and Comparative Biology* 46, 691–712.

Gessler N.N., A.A. Aver'yanov, and T.A. Belozerskaya 2007. Reactive oxygen species in regulation of fungal development. *Biochemistry* (*Moscow*). 72, 1091–1109.

Hammel K.E., A.N. Kapich, K.A. Jensen Jr., and Z.C. Ryan 2002. Reactive oxygen species as agents of wood decay by fungi. *Enzyme and Microbial Technology* 30, 445–453.

Hansberg W. and J. Aguirre 1990. Hyperoxidant states cause microbial cell differentiation by cell isolation from dioxygen. *Journal of Theoretical Biology* 142, 201–211.

Ligusa H., Y. Yoshida, and K. Hasunuma 2005. Oxygen and hydrogen peroxide enhance light-induced carotenoid synthesis in *Neurospora crassa*. *FEBS Letters* 579, 4012–4016.

Ivanova A.E., K.B. Aslanidi, Y.Y. Karpenko and T.A. Belozerskaya 2005. The effect of hydrogen peroxide on the growth of microscopic mycelial fungi isolated from habitats with different levels of radioactive contamination. *Microbiology* 74, 655–663.

Kicka S., and P. Silar 2003. PaASK1, a MAPKKK that controls cell degeneration and cell differentiation in *Podospora anserina*. *Genetics* 166, 1241–1252.

Lamb C. and R. Dixon 1997. The oxidative burst in plant disease resistance. *Annual Review of Plant Physiology and Plant Molecular Biology* 48, 251–275.

Leuthner B., C. Aichinger, E. Oechmen, E. Koopmann, O. Muller, P. Muller, R. Kahmann, M. Bolker, and P.H. Schreier 2005. A H_2O_2-producing glyoxal oxidase is required for filamentous growth and pathogenicity in *Ustilago maydis*. *Molecular Genetics and Genomics* 272, 639–650.

Malagnac F., H. Lalucque, G. Lepère and P. Silar, 2004. Two NADPH oxidase isoforms are required for sexual reproduction and ascospores germination in the filamentous fungus *Podospora anserina*. *Fungal Genetics and Biology* 41, 982–997.

Mendegen K., M. Hahn and H. Deising, 1996. Morphogenesis and mechanisms of penetration by plant pathogenic fungi. *Annual Review of Phytopathology* 34, 367–386.

Munkres K.D., 1990. Histochemical detection of superoxide radicals and hydrogen peroxide by Age-1 mutants of Neurospora. *Fungal Genetics Newsletter* 37, 24–25.

Park P., K. Ikeda 2008. Ultrastructural analysis of responses of host and fungal cells during plant infection. *Journal of General Plant Pathology* 74, 2–14.

Pocsi I., M. Miskei, Z. Karanyi, T. Emri, P. Ayoubi, T. Pusztahelyi, G. Balla and R.A. Prade, 2005. Comparison of gene expression signatures of diamide, H_2O_2 and menadione exposed *Aspergillus nidulans* cultures - linking genome-wide transcriptional changes to cellular physiology. *BMC Genomics* 6, 182.

Rayner A.D.M., Z.R. Watkins and J.R. Beeching, 1999. Self-integration – an emerging concept from the fungal mycelium. In: *The Fungal Colony* (N.A.R. Gow, G.D. Robson, G.M. Gadd, eds), Cambridge University Press, Cambridge, UK, 1–24.

Rodriguez R. and R. Redman, 2005. Balancing the generation and elimination of reactive oxygen species. *Proceedings of the National Academy of Sciences* 102, 3175–3176.

Shinogi T., T. Suzuki, T. Kurihara, Y. Narusaka and P. Park, 2003. Microscopic detection of reactive oxygen species generation in the compatible and incompatible interactions of *Alternaria alternata* Japanese pear pathotype and host plants. *Journal of General Plant Pathology* 69, 7–16.

Shinogi T., T. Suzuki, Y. Narusaka and P. Park, 2002. Ultrastructural localization of hydrogen peroxide in host leaves treated with AKtoxin I produced by *Alternaria alternata* Japanese pear pathotype. *Journal of General Plant Pathology* 68, 38–45.

Sideri M. and C.D. Georgiou, 2000. Differentiation and hydrogen peroxide production in *Sclerotium rolfsii* are induced by the oxidizing growth factors, light and iron. *Mycologia* 92, 1033–1042.

Sigler K., J. Chaloupka,, J. Brozmanova, N. Stadler and M. Hofer, 1999. Oxidative stress in microorganisms - I - Microbial vs. higher cells - Damage and defenses in relation to cell aging and death. *Folia Microbiologica* 44, 587–624.

Silar P., 2005. Peroxide accumulation and cell death in filamentous fungi induced by contact with a contestant. *Mycological Research* 109, 137–49.

Tiedemann A.V., 1997. Evidence for a primary role of active oxygen species in induction of host cell death during infection of bean leaves with *Botrytis cinerea*. *Physiological Plant Pathology* 50, 151–166.

Tornberg K., and S. Olsson, 2002. Detection of hydroxyl radicals produced by wood-decomposing fungi. *FEMS Microbiology Ecology* 40, 13–20.

Tsujiyama S. and M. Minami, 2005. Production of phenol-oxidizing enzymes in the interaction between white-rot fungi. *Mycoscience* 46, 268–271.

Wojtaszek P., 1997. Oxidative burst: an early plant response to pathogen infection. *Biochemical Journal*. 322, 681–692.

Yao N., Y. Tada, M. Sakamoto, H. Nakayashiki, P. Park, Y. Tosa and S. Mayama, 2002. Mitochondrial oxidative burst involved in apoptotic response in oat. *Plant Journal* 30, 567–580.

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