

Effects of electromagnetically signalized media on host-pathogen interaction

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SUMMARY

Up to date, limited data are available about electromagnetic phase signaling effects on host-pathogen interactions during the postharvest of horticultural commodities. Inspired by the last striking works on water physics, quantum signaling through phase transfer and its impact on biological and histological structures, we studied the effect of different electromagnetic signals on pome blue mold (*Penicillium expansum*) pathogenesis. Tags with different electromagnetic-signals (EmS) were used to generate 3 Coherent ElectroDynamic (CED) environments. Artificially wounded ‘Coscia’ pears, placed onto 3 EmS tags (QF, QA and QR), were employed for the *in vivo* experiment. Whereas, a set of wounded-fruit placed onto an un-electromagnetic-signalized tag (QN) or kept without tag were used as blank or control, respectively. Inoculation was performed 2 or 24 hrs post-wounding with *P. expansum* conidia. The same tags placed under Petri dishes containing dot-inoculated PDA served for the *in vitro* experiment. Both experiments performed at 25°C endured 7 days. The percentage of infected wounds was calculated and the radial growth measured *in vitro*. Concerning the *in vivo* experiment, 100% of control and blank fruit inoculated 2 hrs post-wounding was infected after 5 days, while, 97% after 7 days, when inoculation occurred 24 hrs post-wounding. Compared to control and blank, the pathogenesis in fruit placed on the EmS tags resulted inhibited, and when fruit was inoculated 2 hrs post-wounding, the infection degree on QF, QA and QR tags resulted 19, 52 and 64%, respectively. The degree for the same EmS tags was significantly lower when fruit was inoculated 24 hrs post-wounding (9, 32 and 42%, respectively). The *in vitro* experiment evidenced a notable inhibition of the radial growth by all EmS tags in comparison to control and blank (51 mm), while the QF tag provided the greatest inhibition (12 mm).

Key words: *Penicillium expansum*, pome fruit, EM potentials, coherent water, phase transfer.

INTRODUCTION

The just passed century has witnessed a complete understanding of the structures, interactions and dynamical properties of atoms and molecules. It was then concluded that the properties of complex systems follows their molecular structure. This idea led to the development of *molecular biology*. From the point of view of physics, biological materials are extremely complex systems that, on occasion, show sensitivities equal to the highest available in modern technology. An example can be the sensitivity at low intensities of some visual systems in animals (including humans) which are close to the theoretical limit [Attneave, 1954] or the sensitivity of certain fishes or bacteria to electrical signals as well [Bullock, 1977; Blank & Goodman, 2009]. The lowest electric field observed to evoke a response is of the order of 10^{-8} V/cm [Blank & Goodman, 2009]. Theoretical investigations, based on Quantum Electro-Dynamics (QED) suggested the spontaneous emergence of coherence in liquid water [Arani et al., 1995; Del Giudice & Tedeschi, 2009]. So, an appealing possibility is that the electromagnetic (EM) field responsible for the coherent molecule structures in water (which are in principle very long lasting) could explain the peculiar coherence in biological structures (like chloroplasts), and their sensitivity to very weak EM stimuli as evidenced also for bacteria [Del Giudice et al., 2011]. As deeply studied in these recent years [Henry, 2005], our talking about “coherence” and “phase stimuli” is possible and consistent thanks to the extraordinary role of water in living systems. Liquid water is the fundamental ingredient of living organisms [Jørgensen, 2005], and its decrease below a concentration threshold irreversibly affects cell life. In the advanced conceptual frame of the Quantum field Theory (QFT), water is regarded as a large ensemble of molecules (matter field) interacting through a long-range field (the only possible candidate is the electromagnetic field). This led to understand and to discover the existence of many mathematical

solutions, corresponding to the plurality of phases (in the thermodynamic sense) required by a living self-organized organism, and therefore containing an information reservoir [Del Giudice et al., 2005]. In practice, considering phase dynamics of liquid water makes the understanding of its peculiar role in self-organization of living organisms and ecosystems possible and allows us to conceive how phase-changing stimuli can affect biological processes, namely their bio-electromagnetic features. In a Quantum Field (QF) frame we can appreciate the rather unique role of water in living organisms (actually water cannot be replaced by any other H-bonded liquid), and the differences between the normal bulk water and “special waters” such as those close to the hydrophilic surfaces, typical in biological systems. In order to discuss the kind of dynamics and stimuli involved in such issues and in the experimental study presented here, we need to portray the conceptual framework within which it's possible to describe the actual feature of water (intended as “collective system of molecules”) and consider living structures not only as molecular aggregates, but rather as *space-time-evolving phase* systems. In the case of water, the excited state involved in the coherent oscillation (12.06 eV) lies just below the ionization threshold of the molecule (12.60 eV) [Arani et al., 1995]. An oscillation of 12.06 eV corresponds to a water Coherent Domain (CD) size of 0.1 microns. The onset of the coherent oscillation gives rise to the appearance of one quasi-free electron per molecule in the coherent state; therefore, the CD becomes a reservoir of quasi-free electrons that are easily excitable. In this way, the water CDs would become a structure able to collect the energy coming from the environment and transform it into energy able to induce electronic excitations in biomolecules. This property is the key to explain biological systems especially about the possibility of long lasting coherent excitation at room temperature and the electron transfer in biological reactions. The possibility of manifold coherent excited states of water coherent domains (CDs) opens a fascinating perspective: the possibility of coherence among water CDs. In this way, many coherent regions, each having a size of 0.1 μ , could give rise to much more extended coherent regions, as in living organisms. A hierarchy of nested organized regions would emerge. In fact, this possibility holds only for liquids where the excited component of the coherent state lies just below the ionization threshold and this is just the peculiar case of water. In order to produce coherence among CDs, it is necessary to induce oscillation, which means that CDs should be able to discharge energy outwards. A possible way out could be a chemical discharge of energy. If an external non-aqueous molecule present in the liquid contains in its own spectrum a frequency close to the oscillation frequency of the water CD, this molecule could become a *guest* participant in the water coherent dynamics and would settle on the surface of the CD. The EM self-trapping inside CD produces strong potentials peaked at the interface, this because fields are space derivative of potentials. So, from electro-dynamics we know that these potential peaks provoke dispersion forces able to act on charges present in the surrounding environment [Arani et al., 1995; Henry, 2009]. Two outcomes may occur: one electrostatic and one electro-dynamic (dependent on frequency). The first induce a polarization in molecules and atoms attracted on the water CD surface, and molecules result strongly polarized and chemically reactive (static force is always repulsive). The second with a CD surface sharply selective for particular molecules and forces can be both attractive and repulsive depending on the algebraic sign of the difference in frequency between CD and the molecule. Very little tunings in CD frequency may switch the behavior from attractive to repulsive. This is the very key-point of our researches involving the stimuli in the interaction between fruits and signalized substrates. These stimuli produce a phase shift in water CDs, so that resonance frequencies change and biochemical paths in the system are affected, for example slowing/fastening ripening, or activating secondary metabolisms associated to natural resistance. This last point plays a crucial role in biological functioning: it's the first physical consistent explanation of how in cytosol or in the extra-cellular matrix reactants can be “convoked” at the right moment and at the right place in a reaction cycle, without creating spurious products. That's why biological systems have such a high energetic yield (65-70%) with respect to thermodynamic machines. Coherence can support an ordered functioning where system is able to evolve by changing its own work-frequencies not randomly: that's the basis for *homeostasis*. The aim of this research was to shed light on the effect of

different phase signals on the pathogenesis of *Penicillium expansum* the cause of pome postharvest blue mold.

MATERIALS & METHODS

Signalized Substrates. The substrates placed below fruit and PDA dishes were tags made of ordinary PP (polypropylene). [Arani et al., 1995; Blank & Goodman, 2009]. Different phase signals (*information*) were employed to signalize 3 cards (QA, QF, QR). The information was transmitted by exposure of the cards to physical signals carrying phase patterns for an average of 48 h. The coherent systems used as a source of phase signal for the QA cards were different solutions of alkaline carbonates; for the QF cards, carbonated aqueous dispersion of finely chopped leaves, fruits and seeds of several plant and algae species were employed and high frequency sound waves produced by piezoelectric excitation of crystals were used for the QR cards. Following signalizing, the cards were tested by monitoring the variations in conductivity and enthalpy of a hydro-alcoholic solutions (kept in small vials put in touch with the signalized cards), un-signalized cards (blank - QN) did not produce any variation [Renati, 2014].

Fruit. Pear fruit (*Pirus communis* L. cv 'Coscia') was harvested in 2013 when commercially mature (July) in an *ex situ* germplasm conservation orchard belonging to the ISPA-CNR located in Oristano (Sardinia - Italy). After harvest, fruit (350) were selected and randomized in order to obtain 4 homogeneous sets (80 fruit each), three to be placed on the signalized cards and one on the un-signalized one (blank). A set of 30 fruit was selected and used as control. Fruit surfaces were disinfected by a 2 min immersion in a 2% (v/v) sodium hypochlorite solution, followed by a rinse with deionized water. Once dry, fruit were wounded once at the equatorial area with a sterile stainless rod (2 mm wide by 2 mm deep) and placed on the cards (80 fruit each) or into a cardboard (30 fruit). All fruit was stored at 25°C and 95% RH in the dark for 7 d.

Pathogen, fruit and media inoculation. Artificial inoculation of fruit and PDA media took place employing a wild isolate of *Penicillium expansum* Link, obtained from a decayed endemic pear stored at 2°C for 2 months. The inoculum was prepared from a 10-day-old sporulating culture by adding 5 mL of sterile water with 0.05% (v/v) Tween 80 and by gently scrubbing the agar. After filtering, spore concentration was brought to 1×10^4 conidia/mL, and 10 μ L of this suspension was used in all experiments. Concerning the *in vivo* trail, half of the fruit in each set was inoculated 2 hrs post-wounding, while the remaining after 24 hrs. The *in vitro* experiment was carried out employing Potato Dextrose Agar (PDA 39g/L) poured into 9 cm Petri dishes. After pouring, 30 plates were placed onto each of the 4 cards and dot inoculated after 2 or 24hrs, control plates were placed without a card. Then, plates were moved into an incubation chamber at 25°C for 7 d. Daily, the colony radial growth was monitored.

Statistical analysis. ANOVA was applied to all registered data using open stat. Data from disease incidence were arcsine transformed to the square root of the proportion of decayed fruit. When appropriate, means were separated by Fisher's protected least significant difference test with a significance level of $P = 0.05$.

RESULTS & DISCUSSION

Five days post-inoculation the degree of infected wounds in blank and control fruit was significantly influenced by the inoculation time, reaching 100% and 63%, when inoculated 2 or 24 hrs post-wounding, respectively (Table 1). Two days later, the degree had grown from 63 to 97%. These results indicate that un-signalized cards had no effect on pathogenesis nor on fruit resistance, while natural resistance was affected by a 24 hr delay of inoculation. This statement is supported by the *in vitro* results where the inoculation delay did not affect pathogenesis (Table 2). Seven days post-inoculation, the pathogenesis in fruit placed on the EmS cards resulted significantly inhibited compared to blank and control, and when inoculated 2 hrs post-wounding 19, 52 and 64% of the wounds was infected in fruit placed on QF, QA and QR cards, respectively. The infection degree for

the same EmS cards was additionally contained, when fruit was inoculated 24 hrs post-wounding (9, 32 and 42%, respectively). The *in vitro*, results indicate a clear influence of the EmS cards on pathogenesis and again the time of inoculation played an important role. These *in vitro* results suggest that the phase signals affect not only the pathogen but also the growth media (Table 2). In fact, when a phase stimuli is received by a coherent system (media/fruit), if thermodynamically favorable, a re-arranging of electro-dynamics occurs. Being coherent, all the components have to change all together in order not to break out of coherence, which would imply a significant energy expense, but shift the phase towards other equally stable hierarchically ordered configurations. Dissipative dynamics are managed and this process needs an amount of time, being involved very low frequencies (radio wave and lower ones too), depending also on the chemical oscillations due to coherent re-arranging of system occurring in the biological matter (fruit, media, pathogen). Indeed, the radial growth difference observed between the plates inoculated after a 2 or 24 hrs can be attributed only to media properties. The differences observed among the EmS cards are related to the different kinds of signals able to couple with various coherent systems especially the weak ones. In order to increase our knowledge on media coherence properties it may be interesting to test pathogen growth on different artificial media subjected to various phase signals. The results attained *in vitro* make it difficult to understand if the EmS cards affected fruit natural resistance. Still, stimuli involved in the interaction between fruit and signalized substrates are implied to produce a phase shift in water CDs constituting the fruit systems. In this way, the resonance frequencies change and biochemical paths can be affected, for example slowing senescence processes as well as the biochemical reactions associated to natural resistance. Affecting the phase of CDs (extremely sensitive to weak stimuli), implies to dialogue with their EM potentials directly. Acting on the phase, drives the system towards other biochemical paths (secondary metabolisms), or slows down the ordinary ones. This is exactly what we intended to do by our signalized substrates in their interaction with inoculated pome fruit. To ascertain any effect on natural resistance other experiments are required in order to shed light on host cell behavior as triggered by wounding, pathogen and phase signals. The *in vitro* and *in vivo* results indicate a clear difference among the tested phase signals and *P. expansum* pathogenesis was greatly affected by the QF phase signal.

CONCLUSION

The *in vitro* and *in vivo* experiments have evidenced that phase signals affect pathogenesis and that the signals provided by the QF cards are the most effective, followed by QA and QR. From the trails it results that exposure time to the EmS cards before inoculation influences pathogenesis. From the *in vitro* experiment we may conclude that the growth media is influenced by the phase signals. While, it is not clear if host resistance factors are directly involved in the reduction of decay. The promising results attained by the QF phase signal will implement new researches on different postharvest pathogens and easier to apply systems (*e.g.* phase signalized packages). We're going forward to study various "improving information" sources and methods by transferring information signals able to produce exploitable effects on biological goods.

ACKNOWLEDGEMENT

The project was funded by the "Conoscenze Integrate per la Sostenibilità e L'innovazione del Made in Italy Agroalimentare" (CISIA) Legge 191/2009 and by www.freebioenergy.it.

Table 1. Percentage \pm SD of infected wound by *Penicillium expansum* in 'Coscia' pear fruit subjected to different phase signals for 2 or 24 hrs before inoculation and up to 7 days post-inoculation^{xy}

Time		Phase Signals (<i>informations</i>) ^z				
Observation (day post- inoculation)	Inoculation (hours)	None	QN	QF	QA	QR
5	2	95 \pm 4	100	10 \pm 4	38 \pm 5	49 \pm 2
	24	63 \pm 5	60 \pm 2	4 \pm 2	21 \pm 4	28 \pm 4
7	2	100	100	19 \pm 5	52 \pm 3	64 \pm 2
	24	97 \pm 2	97 \pm 3	9 \pm 3	32 \pm 4	42 \pm 4

^x N= 80 for QN, QF, QA, QR and N= 30 for None. ^y inoculation by 10 μ L of a 1×10^4 conidia/mL, fruit kept at 25°C and 90% RH. ^z un-signaled card (QN); cards differently signaled (QF, QA, QR).

Table 2. Radial growth and spore production of *Penicillium expansum* on PDA media subjected to different phase signals for 2 or 24 hours before dot-inoculation and up to 7 days post-inoculation^{xy}

Time		Radial growth (mm)				
Observation (day post- inoculation)	Inoculation (hours)	None	QN ^z	QF	QA	QR
5	2	35 \pm 2	32 \pm 3	13 \pm 4	25 \pm 5	29 \pm 5
	24	32 \pm 4	33 \pm 2	05 \pm 2	18 \pm 4	31 \pm 4
7	2	51 \pm 4	49 \pm 3	25 \pm 5	34 \pm 4	46 \pm 5
	24	50 \pm 2	51 \pm 4	12 \pm 3	25 \pm 3	45 \pm 6

^x N= 30. ^y inoculation by 10 μ L of a 1×10^4 conidia/mL. ^z non phase signaled card (QN); cards differently signaled (QF, QA, QR).

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