# Spatial distribution of flavor components and antioxidants in the flesh of 'Conference' pears and its relationship with postharvest pathogens susceptibility.

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## Highlights

- $C_2H_4$ and $CO_2$ production rates were similar along different sections of the flesh
- Sugars, malic and ascorbic acid contents in ‘Conference’ pear are spatial-dependent
- Higher amounts of fructose and malic acid may favor *R. stolonifer* growth
- Spatial susceptibility to fungal pathogens was related to the VOCs flesh content

**Abstract**

The spatial distribution of dry matter, ethylene production, respiration rate, organic acids, sugars, antioxidants, volatiles and fungal (*Penicillium expansum* and *Rhizopus stolonifer*) growth was evaluated analyzing four different slices of 'Conference' pear flesh taken along an equatorial radius. A common spatial distribution trend was found for ethylene emission, $CO_2$ production, antioxidant capacity and total phenolic compounds with a minimum in the slice under the skin and a maximum in the slice near the core. Fructose, which was the dominant sugar followed by sucrose and glucose, showed a quasi-linear decreasing profile from the outer slice towards the core. Malic and ascorbic acid had the highest content in the outer slice while citric remained practically constant over the different slices. Twenty-nine volatile organic compounds (VOCs) were identified using solid-phase microextraction (SPME), yet only six of them showed significant differences between flesh slices. The content in VOCs was further related to the tissue susceptibility to the above-mentioned postharvest pathogens using a multivariate approach. Fruit flesh from inner sections was more prone to *P. expansum* whereas flesh from the slice under the skin presented the highest incidence of *R. stolonifer*. A Partial Least Square (PLS) model showed that *P. expansum* growth was negatively correlated with malic acid, dry matter content, 2-ethyl-hexanal and butyl hexanoate concentrations and *R. stolonifer* was negatively correlated to sucrose and some volatiles such as hexanal and 1-butanol. Based on the results from the PLS, selected volatiles naturally present in the pear flesh were tested *in vitro,* at different concentrations, in order to investigate their effectiveness to control blue mold caused by *P. expansum* and soft rot caused by *R. stolonifer*. A completely control of *P. expansum* was found with 2-ethyl-hexanal application and hexanal while 1-butanol showed a total fungicide effect against *R. stolonifer*. This study is a step towards a better understanding of how biochemical compounds are spatially distributed among different slices of 'Conference' pears as well as in the development of natural compounds to fight major postharvest pathogens in pear fruit.

**Keywords:** 2-ethyl-hexanal, fungicide**,** *Penicillium expansum*, phenolic compounds, *Rhizopus stolonifer*, VOCs

## 1 Introduction

Pear is one of the most important fruit produced in Europe, with ‘Conference’ cultivar as the most commonly grown in north east of Spain. ’Conference’ is highly appreciated by consumers due to its flavor, juiciness and aroma (Saquet, 2018).

‘Conference’ pear as a climacteric fruit is a highly perishable product. The climacteric phase is characterized by a peak in ethylene production accompanied by a peak in fruit respiration. The burst displayed in the ethylene production is considered to set off biochemical and physicochemical processes (Moya-León et al., 2006; Rapparini and Predieri, 2003) leading to the biosynthesis of aroma compounds and stablishing the nutritional properties of the fruit.

The variability in aroma compounds of pear fruit is known to largely depend on the cultivar (Qin et al., 2012), maturity stage (Zerbini et al., 1993), agro-climatic conditions (Li et al., 2013) and storage conditions or postharvest handling (Zlatić et al., 2016). Volatile compounds, together with sugars and organic acids content (Defilippi et al., 2009), play an important role in fruit flavor. The major sugars in pears are fructose, glucose and sucrose (Colaric et al., 2006; Kolniak-Ostek, 2016; Lindo-García et al., 2019; Moriguchi et al., 2019) while malic and citric are the predominant organic acids in most pear cultivars. The ratio of sugar to organic acids is generally referred as a good indicator of flavor (Sha et al., 2011). However, scarce information is available on how volatile compounds, sugars and organic acids, are spatially distributed within the pear flesh. In other species such as peach, the volatiles concentration has been reported to notably differ from skin to flesh (Aubert and Milhet, 2007).

Despite present at relatively low concentration, pears are also a source of ascorbic acid (AsA) (Galvis Sánchez et al., 2003) and other bioactive compounds, including polyphenols, which positively contribute to human health. AsA content in ‘Conference’ pears changes during the fruit development and postharvest handling (Veltman et al., 2000) and higher concentration of this compound within the pear flesh has been linked to lower incidence of core browning in ‘Conference’ (Veltman et al., 1999) as well as superficial scald in ‘Blanquilla’ pears

97 (Larrigaudière et al., 2016). Phenolic compounds also contribute to the fruit aroma and flavor
98 (Imeh and Khokhar, 2002) and thanks to their anti-inflammatory and antimicrobial activity, can
99 help to prevent human diseases (Liaudanskas et al., 2017).

100 Pear major losses take place during the postharvest phase being mainly caused by physical,
101 physiological and pathological induced-changes. The main postharvest diseases of pears are
102 caused by *Botrytis cinerea*, *Penicillium expansum* and *Rhizopus stolonifer* (Sardella et al., 2016).
103 Traditionally, pears have been treated with chemical fungicide in order to control postharvest
104 decay. In the last years, new alternatives to curtail fungal growth such as the application of natural
105 compounds, including those emitted by pears, have also been studied. Neri et al. (2006b), applied
106 2-hexanal vapors to satisfactorily control blue mold growth caused by *P. expansum* and, Alla et
107 al. (2008) applied cinnamaldehyde vapors to control soft rot caused by *R. stolonifer*. Indeed, the
108 antifungic or fungistatic activity of a range of volatiles is well documented (Mari et al., 2016,
109 2002; Neri et al., 2006a; Sivakumar and Bautista-Baños, 2014). However, whether the
110 concentration of these 'antifungic' compounds along the pear flesh can account to improve
111 resistance to certain fungal postharvest pathogens is still elusive.

112 Accordingly, the aims of the present study were: 1) To investigate the spatial distribution of the
113 main flavor components and antioxidants in the flesh of 'Conference' pears. 2) To determine the
114 behavior of flesh samples from different spatial positions artificially inoculated with *P. expansum*
115 and *R. stolonifer* 3) To evaluate the protective effect of some naturally occurring volatile
116 compounds against both pathogens.

117 **2 Materials and methods**

118 **2.1 Plant material and experimental design**

119 'Conference' pears (*Pyrus communis L.*) were harvested in August 2018 from a commercial
120 orchard near Lleida (NE of Spain). Fruit was picked up at optimum commercial maturity
121 according to local growers recommendations which are basically assessed in terms of firmness
122 and sugars content (firmness≈ 55-65 N and total soluble solids >13 %). No pre-harvest fungicide

treatments were applied later than 30 days prior the commercial harvest. Thereafter, fruit were transported to IRTA facilities where 108 fruit free from defects and uniform size were selected and divided in 3 groups of 20 fruit each plus 2 groups of 24 fruit each. One group of 20 fruit was used to evaluate the dry matter content, sugars, organic acids, antioxidant capacity and phenols. Another group was used to evaluate ethylene production and respiration, and the last group of fruit was used to evaluate the VOCs content. The 2 groups of 24 fruit were used to evaluate the growth ability of *P. expansum* and *R. stolonifer* along different spatial locations.

From each fruit a pulp cylinder in the radial direction, equatorial zone, from the outside of the fruit to the heart was extracted (Fig. s1). Each cylinder was 11 mm in diameter and 24 mm in length. Then, the peel was removed, and the cylinder was cut into 4 equal slices, 6 mm high each, named I, II, III and IV and corresponding to the 4 spatial positions considered in this study (Supplementary Figure 1; Outer slice (slice 'I') until the core (slice 'IV')).

### 2.2 Dry matter content

The dry matter content profile was determined in 20 fruit, 4 replicates of 5 fruit each. Five slices per each location were placed in a petri dish, weighted ($m_{0i}$) and immediately frozen with liquid nitrogen. Slices were lyophilized for 72 h. After this time, each petri dish was weighted ($m_{1i}$) and the dry matter content was evaluated according to the formula: $(m_{1i}/m_{0i})\cdot 100$.

### 2.3 Ethylene production and respiration

Ethylene production and respiration were measured by enclosing 5 slices per each location in airtight tubes of a known volume (4 replicates) and placed in an acclimatized chamber at 20 ºC for two hours. After that time, ethylene concentration was measured by removing 1 mL of gas sample from the headspace of the tube and injecting it into a gas chromatograph fitted with a FID detector (Agilent Technologies 6890, Wilmington, Germany) and an alumina column 80/100 (2 m × 3 mm) (Teknokroma, Barcelona, Spain) as described by (Giné-Bordonaba et al., 2014). Oxygen and carbon dioxide concentrations within the tubes were measured with an $O_2/CO_2$ gas

analyzer (CheckPoint $O_2/CO_2$, PBI Dansensor, Ringsted, Denmark). Gas *i* (*i* = $O_2$, $CO_2$, ethylene) production rate, $r_i$ ($mol_i\ kg^{-1}\ h^{-1}$), was then calculated using Eq. (1),

$$r_i = \frac{\Delta P_i \cdot V_g}{R \cdot T \cdot M_f \cdot \Delta t}, \quad (1)$$

where $\Delta P_i = P_i^t - P_i^0$ (Pa) is the difference between the initial partial pressure, $P_i^0$ and the partial pressure $P_i^t$ after time $\Delta t$ (h), $V_g = V_0 - V_f$ ($m^3$) is the gas volume inside the closed tube obtained as the difference of the tube capacity $V_0$ and the volume occupied by the slices $V_f$, $R$=8.314 J $K^{-1}$ $mol^{-1}$ is the universal gas constant, $T$ (K) is the absolute ambient temperature and $M_f$ (kg) is the mass of slices inside the tube. Initial partial pressure of ethylene and $CO_2$ were assumed to be zero, while initial $O_2$ partial pressure was assumed to be $0.21 \cdot 10^5$ Pa. The respiratory quotient, RQ, was calculated as the molar ratio of $CO_2$ produced to $O_2$ consumed by the fruit, $RQ = -r_{CO2} / r_{O2}$.

### 2.4 Determination of fruit sugar content

Lyophilized slices used in dry matter content determination were ground with a stainless-steel blender and 100 mg of the powder were used for sugar content determination. Glucose, fructose and sucrose were extracted from lyophilized material as described by Giné-Bordonaba and Terry (2010). Briefly, 100 mg of lyophilized sample were dissolved in 2 mL of 62.5 % (v/v) aqueous methanol solvent and placed in a thermostatic bath at 55 °C for 15 min, mixing the solution with a vortex every 5 min to prevent layering. Then, samples were centrifuged at 20 000 *g* for 7 min at 20 °C. The supernatant from each extraction was recovered and used for enzyme-coupled spectrophotometric determination of glucose and fructose (hexokinase/phosphoglucose isomerase) and sucrose (β-fructosidase) as described by Famiani et al. (2012) using commercial kits (BioSystems S.A., Barcelona, Spain) and following the manufacturer instructions. All results are expressed on a fresh weight basis.

### 2.5 Determination of fruit organic acid content

Extracts for malic and citric acids determination, were prepared as described in Giné-Bordonaba and Terry (2010) with some modifications. One hundred mg of lyophilized frozen fruit tissue

174 from each location were added to 2 mL of HPLC-grade water. Samples were kept at room
175 temperature (20 °C) for 10 min and then centrifuged at 20 000 *g* for 7 min at 20 °C. The
176 supernatant from each extraction was recovered and used for enzyme-coupled spectrophotometric
177 determination of malic (L-malate dehydrogenase) and citric (citrate lyase / malate dehydrogenase)
178 acids as described by Famiani et al., (2012) using commercial kits (BioSystems S.A., Barcelona,
179 Spain) and following the manufacturer instructions.
180 Ascorbic acid (AsA) was determined using the freeze-dried material described above. One
181 hundred mg of freeze-dried fruit slices were diluted in 2 mL of 3% (v/v) meta-phosphoric acid
182 (MPA) and 8% (v/v) acetic acid aqueous solvent, mixing the solution for 1 min with a vortex.
183 Then, the samples were centrifuged at 24 000 *g* for 22 min at 4 °C. The supernatants of each
184 sample were filtered through a 0.45 µm filter for High Performance Liquid Chromatography
185 (HPLC) (Millipore, Bedford, MA, USA) and used for HPLC-UV determination as described by
186 Collazo et al. (2018). All results are expressed on a fresh weight basis.

187 **2.6 Determination of fruit antioxidant capacity and total phenolic content**

188 Fruit antioxidant capacity and total phenolic compounds (TPC) were quantified from the freeze-
189 dried material used in the dry matter content determination, as described earlier (Giné-Bordonaba
190 and Terry, 2008). One hundred mg of freeze-dried fruit sample were mixed with 2 mL of 79.5%
191 (v/v) methanol and 0.5% (v/v) HCl aqueous solvent. Sample extraction was held at 20 °C, mixing
192 the solution every 15 min with a vortex (Giné-Bordonaba and Terry, 2016). From the same
193 extract, TPC was measured by means of the Folin-Ciocalteu method calculated from a Gallic Acid
194 Equivalent (GAE) curve and total antioxidant capacity was measured by the Ferric Reducing
195 Antioxidant Power (FRAP) assay as described by Benzie and Szeto (1999). All results are
196 expressed on a fresh weight basis.

197 **2.7 Spatial distribution of volatiles in pears**

198 Headspace solid-phase microextraction (HS-SPME) was used to extract and to determine the
199 concentrations of volatile compounds along the cylinder of pear flesh. SPME fibers coated with
200 a 65-µm layer of polydimethylsiloxane–divinylbenzene (65 µm PDMS/DVB; Supelco Co.,

Bellefonte, PA, USA) were used. Fibers were activated before sampling according to the manufacturer's instructions.

Five slices per each spatial location and per replicate (4 replicates) were frozen in liquid nitrogen, crushed together and immediately transferred to −80 °C storage until the volatile compounds were analyzed. For each extraction, 5 g of homogenized sample per location were placed in a 20 mL screw-cap vial containing 2 g of NaCl to facilitate the release of volatile compounds. Prior to sealing the vial, 2 µL of 0.03 mL $L^{-1}$ 3-nonanone was added as an internal standard, and the solution was mixed with a glass rod. The mixture was incubated and agitated at 40 ºC during 20 min. Afterwards, the SPME fiber was injected into the headspace and exposed for 30 min at 40 ºC to absorb the volatiles as described by Qin et al. (20129. Volatile compounds were subsequently desorbed as described by Iglesias et al. (2018) and results expressed on a fresh weight basis.

**2.8 Fungal growth evaluation in pear tissue**

Both strains used in this study, *P. expansum* (CMP-1) and *R. stolonifer* (RSF) belong to the collection from the Postharvest Pathology group of IRTA (Lleida). They were the most aggressive isolates capable of infecting pome fruit, respectively. Conidial suspensions were prepared by rubbing the surface of 7 to 10-day-old cultures grown on potato dextrose agar (PDA) with sterile water containing 0.01 % (w/v) Tween-80 using a sterile glass rod. Concentration of each fungus was determined using a haemocytometer and prepared to obtain $3 \cdot 10^4$ conidia $mL^{-1}$ of *P. expansum* and $1 \cdot 10^3$ conidia $mL^{-1}$ of *R. stolonifer*.

Two groups of 24 fruit (8 replicates, 3 fruit each) were used to evaluate the growth of fungi. The first group was used to evaluate the severity and incidence of *P. expansum* and the second the incidence of *R. stolonifer*. Fruit were disinfected with 0.525% (v/v) sodium hypochlorite (NaClO) for 5 minutes and cleaned five times with tap water. Once dried, a pulp cylinder of the fruit was extracted and cut into 4 slices as explained in the plant material and experimental design section.

Each slice of the first group was inoculated with 5 µL of *P. expansum* and the ones of the second group were inoculated with 5 µL of *R. stolonifer*.

*P. expansum* incidence was evaluated by measuring the diameter of fungus growth and severity infection was evaluated as the % of mycelial presence on slices regarding the total of infected samples. *P. expansum* incidence was evaluated after 72 h post the inoculation while *R. stolonifer* incidence was measured after 44 h post the inoculation.

### 2.9 Evaluation of fungistatic or fungicide activity of synthetic pear volatiles *in vitro*

Fungistatic and fungicide activity of the four most representative VOCs found in the Principal Component Analysis (PCA) of detected pear volatiles was evaluated as reported by Gotor-Vila et al. (2017) with some modifications. Briefly, pure standards of these four volatiles were purchased from Sigma-Aldrich (Madrid, Spain) and individually tested for suppressing mycelial growth of target pathogens. For this purpose, 10 µL of conidial suspension containing each pathogen were placed in the center of petri dishes containing PDA. Then, a paper filter (85 mm diameter) containing an aliquot of pure compound was positioned inside the cover of the dishes and the petri dishes were immediately sealed with parafilm and incubated at 25 °C. The aliquots of pure compounds introduced in the petri dishes were: 5, 10, 20, 40, 80, 160 and 320 µL corresponding to 0.027, 0.055, 0.11, 0.22, 0.44, 0.88, 1.76 mL $L^{-1}$ headspace, respectively. Measures for *P. expansum* were made after three, four, five and seven days post the inoculation and *R. stolonifer* after one, two and three days. The sample unit was represented by four replicates for each dose and pathogen and dishes with paper filter with water at 1.76 mL $L^{-1}$ were used as control. The percentage of mycelial inhibition (PMI) of fungal growth was calculated after 5 and 3 d from inoculation for *P. expansum* and *R. stolonifer*, respectively. Percentage mycelial inhibition (PMI) was determined according to the formula $(\%)=[(d_c-d_t)/d_c)]\cdot 100$, where $d_c$ is the diameter growth average of control and $d_t$ is the treatment diameter average (Li et al., 2016). The effect of VOC's on fungus were tested by determining the effective concentration values that reduced the mycelial growth by 50% ($EC_{50}$) as reported by Alexander et al. (1999).

252 **2.10 Statistical analyses**

253 Means were compared by analysis of variance (ANOVA). When the analysis was statistically
254 significant, the Tukey's Honestly Significant Difference (HSD) test at $P \leq 0.05$ was performed
255 for separation of means.

256 A hierarchical cluster analysis dendrogram was done applying Ward method of minimum
257 variance. The objective function was the error of the sum of the squares or variance (Ward, 1963).
258 The dendrogram and the constellation graph were constructed in order to establish a preliminary
259 relationship between sugars, organic acids and antioxidants in order to find relationships between
260 different pear 'Conference' slices spatially distributed. The analyzed data included the 4 slices
261 along the spatial distribution (I, II, III and IV) and 40 variables representing the components being
262 analyzed.

263 Two partial least square (PLS) regression models were used to correlate organic acids, sugars,
264 antioxidants and volatile compounds (as X variables or explanatory variables) with fungal
265 infections as response variables, *P. expansum* as ($Y_1$) and *R. stolonifer* as ($Y_2$). The non-linear
266 iterative partial least squares (NIPALS) algorithm was used for computing the first few factors.
267 KFold validation was used to select the number of factors that minimize the Root Mean PRESS
268 statistic. As a pre-treatment, data were centered and weighed by the inverse of the standard
269 deviation of each variable in order to avoid dependence on measured units. All analyses were
270 carried out with the PLS platform of JMP® 13.1.0 SAS Institute Inc. (SAS, 2013).

271 **3 Results and discussion**

272 **3.1 Dry matter content, ethylene emission and respiration**

273 Dry matter (DM) of pip fruit is basically formed by carbohydrates (90 %) (Travers et al., 2014),
274 in soluble and insoluble forms, and the remaining part are mainly organic acids (Suni et al., 2000).
275 Our results showed that the DM content was minimum in slice II and III but with no significant
276 differences between them (p=0.1891) (Fig. 1A). The average of DM content reported herein
277 (17.8 %) was in accordance with the ones reported by Costa et al. (2015) in pear fruit from four

278 different varieties (average 17.9 %). The moisture content profile, which is its complementary
279 ($m_c$=100-DM), had thus a maximum in slice II, which can be explained by the fact that moisture
280 diffuses outwards to the fruit surface at a higher flux rate than it does inwards, to the core of the
281 fruit, hence resulting in a lower gradient towards the center.

282 Several studies have already analyzed the ethylene emission of whole pears at different maturities,
283 temperatures and storage periods (Knee, 1987; Lindo-García et al., 2019; Villalobos-Acuña and
284 Mitcham, 2008) as well as its respiration rate (Ho et al., 2018; Lammertyn et al., 2001; Saquet
285 and Streif, 2017). To our knowledge no studies are available investigating the spatial distribution
286 of ethylene production and respiration rates in pears. The ethylene production profile (Fig. 1B)
287 presented a minimum at intermediate slices, II and III, with a significant increase towards the
288 core. A similar profile, but with a better defined minimum at slice III, was found in the respiration
289 rate (Fig. 1C). Our results showed a relatively poor correlation between respiration rate and
290 ethylene production ($r^2$=0.546) likely due to the different diffusivity of both compounds (ethylene
291 and $CO_2$) within the pear flesh. Rudell et al. (2000) found that ethylene production had a
292 maximum in the carpellary tissue in 'Fuji' apple at all harvest dates, which is in accordance to
293 our results found for the inner slice (referred as IV). Moreover, Rudell et al. (2000) reported a
294 minimum in $CO_2$ production in the hypanthial tissue, hence also in accordance with our results
295 (Fig. 1C).

296 **3.2 Sugar and organic acid composition**

297 Fructose, glucose and sucrose are known to be the main sugars in 'Conference' pear fruit and
298 according to Colaric et al. (2007), in general, fructose represents more than 50 % of the pear sugar
299 content. Our results are in accordance with this statement, fructose accounted for 60 % of the total
300 sugar content, but clearly showed that these sugars were not uniformly distributed within the flesh
301 of the fruit. Fructose content showed a quasi-linear decreasing profile with content values in the
302 inner slice (slice IV Fig. 2A) about 40 % lower than in the outer slice, while sucrose showed an
303 opposite trend with its lowest values under the fruit skin. Glucose content was minimum at slice

II (Fig. 2A) and significantly higher ($p > 0.022$) in the slice near the core (slice IV). Measured fructose values, 46.3 g $kg^{-1}$ as weighted average, were similar to the ones reported by Colaric et al. (2007) for 'Conference' pears harvested in 2004, however, these values were 1.5-fold lower than the ones obtained in the same study for fruit harvested in 2005. The measured glucose content (11.6 g $kg^{-1}$, weighted average) was nearly 2-fold higher than the values reported by Colaric et al. (2007) in fruit harvested in 2004 and Hudina and Štampar (2004) in Williams pears. Hudina and Štampar (2004) reported that the fruit sugar content was affected by climatic and soil conditions leading to differences as high as 50 %.

Malic acid is the predominant organic acid in 'Conference' pears followed by citric acid (Hudina and Stampar, 2000). The ratio between malic acid content and citric correlates with sensory perception of fruit taste (Colaric et al., 2007). In our measurements (Fig. 2B) malic was the predominant acid (3.6 g $kg^{-1}$ as weighted averages) and its distribution profile presented a minimum in slice II. Hudina and Štampar (2004) reported similar results (3.7 g $kg^{-1}$) for 'Conference' pears harvested at south-east of Slovenia. Kou et al., (2014) reported that malic acid content in the peel (3.6 g $kg^{-1}$) of 'Huang guan' pear was higher than in the pulp (2.2 g $kg^{-1}$) which is in line with our results. Citric spatial distribution followed a similar trend than the one observed in malic acid content although no significant differences were found between slices (Fig. 2B). Citric acid (1.2 g $kg^{-1}$ as weighted average) was 2.5-fold lower than malic acid in all slices.

In our study, only slice 'IV' had the lowest AsA content and showed significant differences if compared to the other slices ($p=0.0393$) (Fig. 2B). Johnson et al. (2013) found that AsA content in pulp (0.093 g $kg^{-1}$) of '*Citrullus Lanatus*' watermelon was higher than in rind and seed (0.076 and 0.053 g $kg^{-1}$, respectively). AsA content and fructose showed a quite good correlation with $r^2=0.764$. This result was in agreement with that found by Franck et al. (2003) who reported that AsA and fructose content had a similar pattern in 'Conference' pear, suggesting a close relationship between both components.

### 3.3 Antioxidant capacity and total phenolic compounds

According to different studies, pear fruit has beneficial health effects, protecting against different diseases, thanks to its antioxidant properties (Imeh and Khokhar, 2002; Kolniak-Ostek, 2016; Liaudanskas et al., 2017). Even though antioxidant capacity and total phenolic compounds in pears are low when compared to other fruit such as berries (Määttä-Riihinen et al., 2004), orange, kiwifruit and apples (Wang et al., 1996), the contribution of pear to the daily consumption of antioxidants and phenolics is relatively high (Chun et al., 2005). If compared to apples, total phenolic content in pear flesh is 3-fold lower (Leontowicz et al., 2002) and great variability exist among different pear cultivars (Brahem et al., 2017).

To our knowledge, little information is available about how antioxidant capacity and TPC are distributed along the flesh of fruit, and especially in pear. The fruit antioxidant capacity (1210.5 mg $Fe^{3+}$ $kg^{-1}$ as weighted average) had a minimum in slice III with a sharp increase in the slice near the core (Fig. 2C).

A similar profile was also found for TPC content (Fig. 2D). Imeh and Khokhar, (2002) analyzed TPC in different apple, pear and stone fruit cultivars and reported that 'Conference' pear had the lowest values (3023 mg $kg^{-1}$ GAE). However, their values were two-fold higher than that obtained in this study. This could be because in their analysis they included the peel, which is reported to have higher amounts of TPC.

### 3.4 Volatiles spatial distribution

While several studies have been focusing on 'Conference' pear volatiles emission under different circumstances (Goliáš et al., 2015; Hendges et al., 2018; Saquet, 2017) no information is available describing the VOC's concentration in different locations inside the pear flesh. Aubert and Milhet (2007) investigated the distribution of VOCs in different parts of a white-fleshed peach (cv. Maura) and found that volatiles content in skin were significantly higher than in flesh.

In our study twenty-nine volatile compounds were identified and quantified in the different locations of the slices in 'Conference' pear (Table s1). These volatile compounds included 16 esters, 6 alcohols, 3 aldehydes, 2 terpenoids, 1 acid and 1 ketone. Esters play an important role

356 providing a characteristic fruity aroma (Zlatić et al., 2016) when volatiles are released from intact
357 fruit. However, when fruit is cut or crushed different enzymatic processes can be activated, some
358 of which are extremely rapid once cellular disruption begins (Rapparini and Predieri, 2003). In
359 this context, aldehydes are major components in pulp extracts, but not in the headspace of intact
360 pears.

361 Our research showed that hexanal was the main volatile detected with its highest concentration in
362 the 'II' slice (140 µg $kg^{-1}$) but with no significant differences between locations of the slices
363 ($p=0.1278$). Aldehydes are known to be the main responsible of grassy aroma (Zlatić et al., 2016)
364 and green flavor (Rapparini and Predieri, 2003). Besides being a typical fruit volatile, hexanal is
365 also formed when cellular structures are disrupted (Clark et al., 2014) and hence this compound
366 is detected at its highest concentrations in fresh-cut fruit or when using similar methodologies to
367 the one described herein (SPME);. For instance, Rizzolo et al. (2005), found that hexanal was one
368 of the main volatile in 'Conference' pears under controlled atmosphere and it was the most
369 prominent in odor units. Lindo-García et al. (2019) also found that hexanal was the principal
370 aldehyde in 'Blanquilla' pears during on and off-tree ripening. Similarly, Makkumrai et al. (2014)
371 reported that hexanal was the main aldehyde in 'Barlett' pears stored at 20 ºC for 11 d and Horvat
372 et al. (1992) found that hexanal was one of the main volatiles in five Asian pear cultivars. All
373 these studies used similar methodologies as the one described in this study.

374 The main ester detected was butyl butanoate which has been already reported as an impact volatile
375 in 'Conference' pears (Rizzolo et al., 2005). Even though, no significant differences between
376 locations of the slices were found, its maximum concentration was found in slice B. Butyl
377 butanoate is largely known to contribute to sweet or fruity odors.

378 From the 29 identified volatiles only six presented significant differences between locations of
379 the slices; butyl acetate, 2-ethy-hexanal, 3-methylbutyl 3-methyl-butanoate, (E)-2-hexenyl
380 acetate, hexyl butanoate and hexyl 2-methylbutanoate. Some of these compounds have been
381 previously identified as important character-impact volatiles in whole 'Conference' pears (El

382 Hadi et al., 2013; Saquet, 2017; Torregrosa et al., 2019) contributing, among others, to sweet and
383 fruity odors. The spatial distribution of flavor components and antioxidants along the flesh of pear
384 fruit may be of use to the fresh-cut industry to supply fruit with improved flavor and nutritional
385 value by selecting not only the appropriate fruit but also specific parts of it.

386 **3.5 Susceptibility to *P. expansum* and *R. stolonifer* along the pear flesh**

387 *P. expansum* and *R. stolonifer* fungus are important destructive fungal pathogens of pome fruit.
388 Many studies analyzed blue mould and soft rot in entire pears (López et al., 2015; Neri et al.,
389 2010). However, no information is available about the fungal growth on flesh from different
390 locations in 'Conference' pear.

391 *P. expansum* showed an incidence of 100 % in all locations of the evaluated slices, in contrast
392 severity was significantly different between slices ($p < 0.001$), slice (I) close to the peel had the
393 lower fungal severity (Fig. 3A). Rot incidence was evaluated in inoculated slices with *R. stolonif*er
394 since measuring severity for this type of pathogen is not an easy task mainly due to the black and
395 loose mycelium with white aerial fruiting structures (Sardella et al., 2016). Slice 'I' had the
396 highest incidence of *R. stolonifer* (Fig. 3B).

397 **3.6 Relationship between tissue composition and susceptibility to major postharvest**
398 **pathogens**

399 In order to know which variables were characteristics of each slice and determine those that were
400 key to differentiate slices, a first multivariate analysis considering all the analyzed variables,
401 except those of fungal susceptibility to *P. expansum* and *R. stolonifer*, was done. A dendogram
402 graph was used to further obtain a global overview of the relationship between ethylene emission,
403 respiration, sugars, organic acids, antioxidants, phenols and the profile of volatile compounds in
404 a reduced dimension plot. In this data set, 42 variables were used (Fig. 4A). The hierarchical
405 heatmap showed that slices 'I' and 'IV' had similar amounts of the components in cluster 1 (C1),
406 except for sucrose and hexanal (Fig. 4B). This cluster encompasses some major pear character-

impact compounds such as butyl butanoate. Components encompassed in cluster 3 (C3) had a similar behavior in slices 'II' and 'III', except for 1-hexanol.

On the other hand, and given the different susceptibility of the different slices locations to blue mold and soft rot, two partial least square regression (PLS) models were performed in order to identify which variables had higher correlation with the susceptibility of *P. expansum* and *R. stolonifer* growth. The PLS models were done to correlate respectively *P. expansum* growth ($Y_1$ variable) and *R. stolonifer* growth ($Y_2$ variable) with a set of potentially explanatory variables: sugars and organic acids content, ethylene production, respiration, dry matter, volatiles compounds, antioxidant capacity and total phenolic content (X variables). Based on PLS method, the X data set was reduced to two principal factors. The first factor explained more than 99% for both fungi, *P. expansum* (Fig. 5A) and *R. stolonifer* (Fig. 6A). The correlation between measured and predicted blue mold severity and soft rot incidence were higher than 0.99, demonstrating the goodness of the model (Fig. 5B, 6B). *P. expansum* growth showed a positively correlation with the sucrose content and some VOC's such as (E)-2-hexenyl acetate, ethyl octanoate, pentyl hexanoate, hexanal, 1-butanol, 2-methyl-1-butanol and 6-methyl-5-hepten-2-one (Fig. 5C). With such a background, 'II' and 'III' slices followed by 'IV' and 'A' were more prone to the growth of this fungus. However, *R. stolonifer* was positively correlated with fructose, malic acid and dry matter content and with ethyl acetate, butyl hexanoate, 2-ethyl-hexanal, butyl hexanoate, (Z)-b-farnesene and α-farnesene (Fig. 6C). 'I' is the most suitable slice for its fungus to growth.

### 3.7 Antifungal efficacy *in vitro* of VOCs against *P. expansum* and *R. stolonifer*

Based on our PLS results (Fig. 5 and 6), 2-ethyl-hexanal and butyl hexanoate were the most effective compounds against *P. expansum* and hexanal and 1-butanol against *R. stolonifer* and their effects were further studied *in vitro* with different concentrations (Fig. s2). The *in vitro* results of exogenous applied compounds, commonly emitted by 'Conference' pears, and their capacity to suppress the mycelial growth of both pathogens is shown in Table 1. All tested oncentrations of 2-ethyl-hexanal, completely controlled *P. expansum* growth while control fruit had a diameter growth of 3 cm after 3 d (Fig. s2A). Moreover, any used concentration of butyl

hexanoate was capable to completely control mycelial growth (Fig. s2B). A concentration of 0.22 µL $mL^{-1}$ of hexanal completely controlled the infection (Fig. s2C) and hexanal had an $EC_{50}$ of 0.055 µL $mL^{-1}$ on *R. stolonifer* growth (Table 1). Soft rot was completely controlled by 1-butanol application at 1.76 µL $mL^{-1}$ (Fig. s2D). These results agreed with those found by Neri et al. (2006), who investigated the effect of nine plant volatiles *in vitro* and *in vivo* trials against blue mold on pears and found that *trans*-2-hexanal and carvacol had prominent effects, while hexanal had a less marked effect. Sáenz-Garza et al. (2013) also reported that the hexanal released from microcapsules on the surface of PDA inhibit blue mold growth and it was viable to preserve apple slices. As reviewed by Mari et al. (2016), other aldehydes and alcohols such as benzaldehyde and ethanol have shown promising results controlling different fungal growth in a wide range of fruit and vegetables and hence future studies are warrant.

## 4 Conclusions

The results from this study demonstrate that flavor components including sugars and organic acids are non-uniformly distributed along the flesh of Conference pears. Not only components but also the capacity of the tissue to produce ethylene and $CO_2$ was different along the equatorial location. Some VOCs also presented significant differences among slices. *In vitro* experiments showed that components naturally present along the pear flesh had antifungal activity. Thus, 2-ethyl-hexanal revealed an antifungal effect against *P. expansum* while hexanal and 1-butanol acted against *R. stolonifer*. Overall, the results presented herein give added value to the fresh-cut industry (fruit with improved nutritional quality and flavor) and could improve food security using natural compounds capable of inhibiting major postharvest pathogens.

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**Supplementary table 1:** Mean ± standard deviations (n=4) values of VOC's concentration (µg $kg^{-1}$) of equatorial slices of 'Conference' pear from different radial locations. Means within the slices preceded by the same small letters are not significantly different at $p \leq 0.05$ (HSD test). No letter indicates the absence of significant differences.

| | Slice | | | |
|---|---|---|---|---|
| **Volatile compounds** | **I** | **II** | **III** | **IV** |
| ***Esters*** | | | | |
| Ethyl Acetate | 7.1±0.8 | 6.6±0.1 | 6.4±0.1 | 5.0±3.3 |
| Tert-Butyl propionate | 3.4±3.0 | 2.4±3.5 | 3.7±2.5 | 2.4±2.9 |
| Methyl butanoate | 1.6±2.9 | 2.4±3.5 | 2.4±2.9 | 1.2±2.5 |
| Butyl acetate | [a]3.6±3.2 | [a]5.5±0.2 | [a]5.5±0.3 | [b]0.0±0.0 |
| Pentyl acetate | 1.2±2.2 | 3.7±0.0 | 1.0±2.1 | 1.3±2.7 |
| Butyl butanoate | 27.2±8.8 | 33.4±3.8 | 29.1±20.9 | 24.6±17.0 |
| Hexyl acetate | 1.3±2.3 | 3.8±0.0 | 2.7±1.9 | 0.0±0.0 |
| 3-Methylbutyl 3-methyl-butanoate | [b]0.0±0.0 | [b]0.0±0.0 | [a]2.7±1.8 | [b]0.0±0.0 |
| (E)-2-Hexenyl acetate | [c]0.0±0.0 | [c]0.0±0.0 | [a]4.1±0.2 | [ab]2.7±1.8 |
| Butyl hexanoate | 4.2±0.1 | 2.0±2.9 | 2.0±2.4 | 3.4±2.4 |
| Hexyl butanoate | [b]0.0±0.0 | [b]0.0±0.0 | [b]0.0±0.0 | [a]2.3±1.6 |

| | | | | |
|---|---|---|---|---|
| Hexyl 2-methylbutanoate | [b]0.0±0.0 | [a]3.5±0.0 | [b]0.0±0.0 | [b]0.0±0.0 |
| Ethyl octanoate | 0.0±0.0 | 0.0±0.0 | 2.9±2.0 | 2.7±1.9 |
| Octyl acetate | 2.0±0.0 | 1.0±1.4 | 2.0±0.1 | 1.5±1.0 |
| Pentyl hexanoate | 0.0±0.0 | 0.0±0.0 | 1.2±2.4 | 1.1±2.3 |
| Hexyl hexanoate | 3.0±2.7 | 4.1±0.4 | 4.4±0.5 | 2.2±2.6 |
| ***Alcohols*** | | | | |
| 1-Butanol | 2.7±2.3 | 4.1±0.0 | 4.0±0.1 | 3.0±2.0 |
| 2-Methyl-1-butanol | 3.0±2.7 | 4.6±0.5 | 3.6±2.4 | 3.2±2.2 |
| 1-Hexanol | 3.3±0.1 | 3.6±0.2 | 2.7±1.8 | 3.4±0.1 |
| 2-Ethyl-1-hexanol | 5.0±1.0 | 5.1±0.3 | 5.0±0.8 | 4.2±0.5 |
| 1-Octanol | 1.1±1.9 | 0.0±0.0 | 1.6±1.9 | 0.8±1.6 |
| Benzyl alcohol | 1.4±2.5 | 0.0±0.0 | 3.2±2.2 | 1.0±2.0 |
| ***Aldehydes*** | | | | |
| Hexanal | 104.4±91.9 | 140.2±15.5 | 128.8±29.7 | 128.7±86.3 |
| 2-Ethyl-hexanal | [a]5.1±1.5 | [b]0.0±0.0 | [b]0.0±0.0 | [ab]2.2±2.7 |
| Benzaldehyde | 2.8±2.5 | 4.2±0.2 | 4.2±0.1 | 2.1±2.4 |
| *Terpenoids* | | | | |
| (Z)-β-farnesene | 6.5±6.8 | 5.4±0.0 | 5.9±4.4 | 6.0±4.3 |
| α-farnesene | 4.7±0.3 | 4.4±0.1 | 3.6±2.4 | 3.3±2.2 |
| ***Acid*** | | | | |
| Acetic acid | | | | |
| ***Ketone*** | 8.9±6.0 | 2.8±4.0 | 16.1±27.2 | 5.4±6.4 |
| 6-Methyl-5-hepten-2-one | 2.5±2.3 | 3.8±0.2 | 3.8±0.2 | 2.7±1.8 |

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**Table 1:** Antifungal activity of pure volatile organic compounds at different concentrations on the in vitro mycelial growth inhibition (%) tests against *P. expansum* after 5 d and *R. stolonifer* after 3 d. When possible, $EC_{50}$ values were calculated according to Alexander et al. (1999) (mL $L^{-1}$ headspace).

| **Pathogen** | **Compound** | **Concentration (mL $L^{-1}$ headspace)** | **Mycelial growth inhibition (%)** | **$EC_{50}$ (mL $L^{-1}$)** |
|---|---|---|---|---|
| *P. expansum* | 2-Ethyl hexanal | 0.027 | 100.0 | - |
| | | 0.055 | 100.0 | |
| | | 0.11 | 100.0 | |
| | | 0.22 | 100.0 | |
| | | 0.44 | 100.0 | |
| | | 0.88 | 100.0 | |
| | | 1.76 | 100.0 | |
| | Butyl hexanoate | 0.027 | 9.5 | 0.61 |
| | | 0.055 | 29.7 | |
| | | 0.11 | 10.9 | |
| | | 0.22 | 26.6 | |
| | | 0.44 | 37.6 | |
| | | 0.88 | 56.1 | |
| | | 1.76 | 54.9 | |
| *R. stolonifer* | Hexanal | 0.027 | 4.4 | 0.055 |

| | | | |
|---|---|---|---|
| | 0.055 | 50.0 | |
| | 0.11 | 95.9 | |
| | 0.22 | 100.0 | |
| | 0.44 | 100.0 | |
| | 0.88 | 100.0 | |
| | 1.76 | 100.0 | |
| 1-Butanol | 0.027 | ni | 0.48 |
| | 0.055 | ni | |
| | 0.11 | ni | |
| | 0.22 | 9.1 | |
| | 0.44 | 43.4 | |
| | 0.88 | 97.6 | |
| | 1.76 | 100.0 | |

ni: no mycelial growth inhibition observed
-: insufficient data to calculate $EC_{50}$ values.

**List of figures**

**Figure 1:** A) Spatial distribution among slices of dry matter content, B) ethylene production rate, C) $O_2$ consumption rate (black bars, left axis), $CO_2$ production rate (grey bars, left axis) and RQ (○, right axis). Error bars indicate standard deviation for n=4. For each graph, mean values with the same letter are not significantly different according to analysis of variance (ANOVA) and Tukey's HSD test ($P < 0.05$). Horizontal lines represent weighted averages, and were calculated weighting the value at each location by the difference of spherical volumes corresponding to the radius of both extremes of the sample.

**Figure 2:** Contents, referred to unit of pulp fresh mass, of: A) sugars: fructose, glucose and sucrose, and B) acids: malic, citric (black and grey with diagonal lines bars, left axis) and ascorbic (grey dotted bars, right axis), C) antioxidant capacity measured by the FRAP assay and D) total

711 phenolic compounds in different slices of 'Conference' pears spatially distributed. Error bars
712 indicate standard deviation for n=4. For each graph, mean values with the same letter are not
713 significantly different according to analysis of variance (ANOVA) and Tukey's HSD test
714 ($P < 0.05$). Horizontal lines represent weighted averages.

715 **Figure 3:** Fungal susceptivility, A) blue mold (*Penicillium expansum*) severity and B) soft rot
716 (*Rhizopus stolonifer*) incidence in the different locations of 'Conference' pear flesh. For each
717 graph, mean values with the same letter are not significantly different according to analysis of
718 variance (ANOVA) and Tukey's HSD test ($P < 0.05$).

719 **Figure 4:** A) Hierarchical heatmap based on the normalized quantities of the analyzed elements
720 and identified volatiles in each 'Conference' section. The lowest content is in the lightest green
721 and the highest in the darkest red. * indicate significant differences ($P < 0.05$) and ** indicate
722 significant differences ($p < 0.01$) between sections. B) Constellation plot of the different clusters.

723 **Figure 5:** A) Partial Least Squares (PLS) correlation loading plots of the 2 factors of *P. expansum*
724 severity. B) The measured vs the predicted *P. expansum* severity through the model and its
725 correlation coefficient. C) Variable importance plot (VIP), the number of VIP>1.

726 **Figure 6:** A) Partial Least Squares (PLS) correlation loading plots of the 2 factors of *R. stolonifer*
727 incidence. B) The measured vs the predicted *R. stolonifer* incidence through the model and its
728 correlation coefficient. C) Variable importance plot (VIP), the number of VIP>1.

729 **Figure supplementary 1:** Methodology used for the equatorial cylinder extraction and slices
730 division in 'Conference' pear. Fruit skin was adhered to the left side of sliceI.

731 **Figure supplementary 2:** Effects of different concentrations of VOCs, A) 2-ethyl-hexanal and
732 B) butyl hexanoate on mycelia diameter (cm) of *P. expansum* growth during 5 d and C) hexanal
733 and D) 1-Butanol on mycelia diameter (cm) of *R. stolonifer* growth during 3 d. Error bars indicate
734 standard deviation for n=4. For each graph, mean values with the same letter are not significantly
735 different according to analysis of variance (ANOVA) and Tukey's HSD test ($p < 0.05$).

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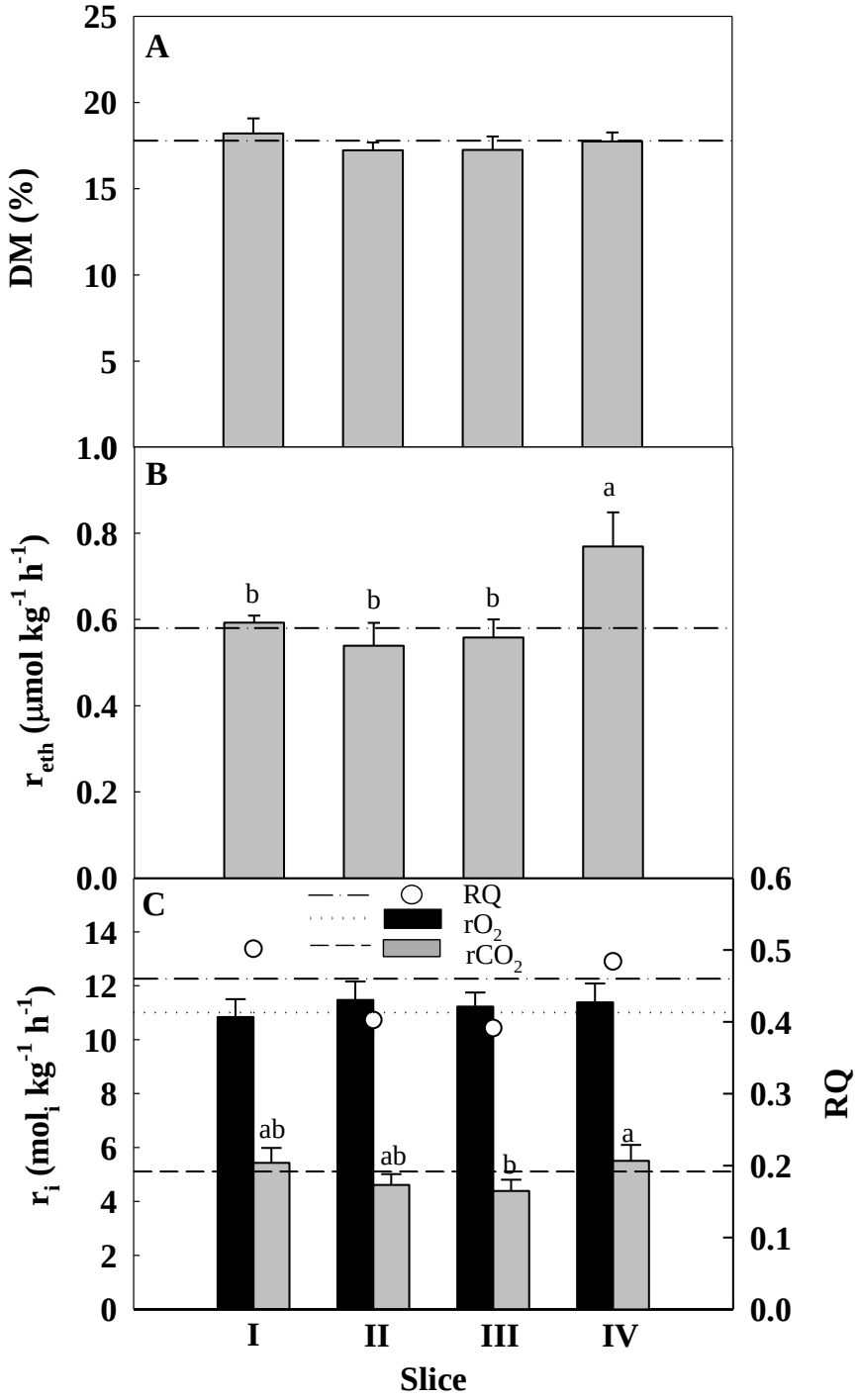


748

749 **Figure 1**

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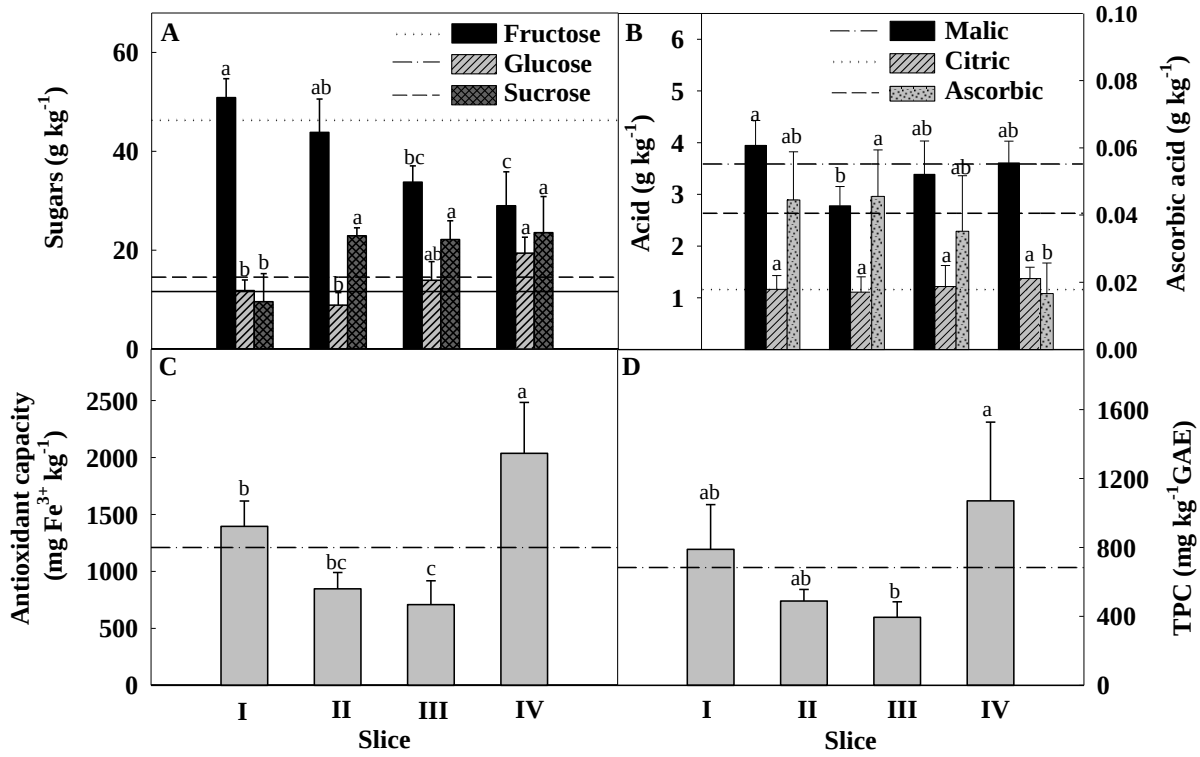

A
Fructose
Glucose
Sucrose
Sugars (g kg-1)
B
Malic
Citric
Ascorbic
Acid (g kg-1)
Ascorbic acid (g kg-1)
C
Antioxidant capacity (mg Fe3+ kg-1)
D
TPC (mg kg-1GAE)
I
II
III
IV
Slice

**Figure 2**

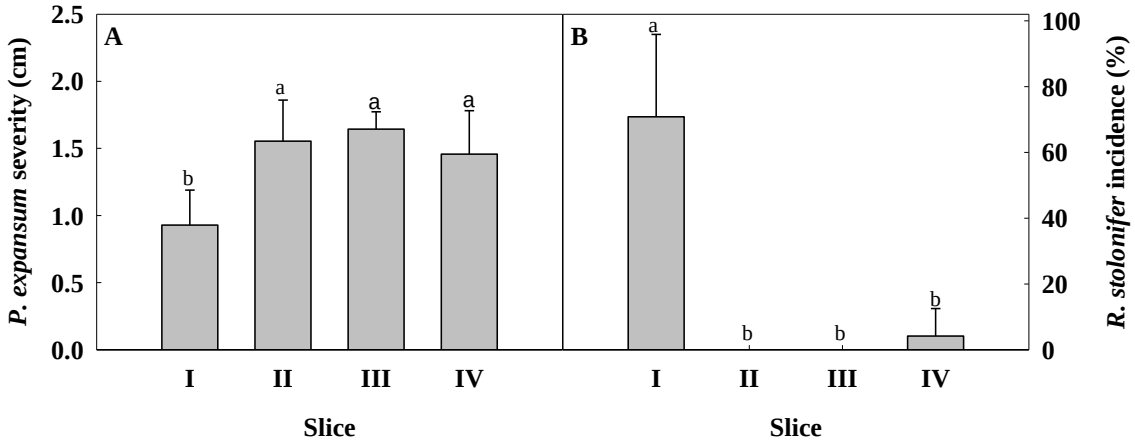
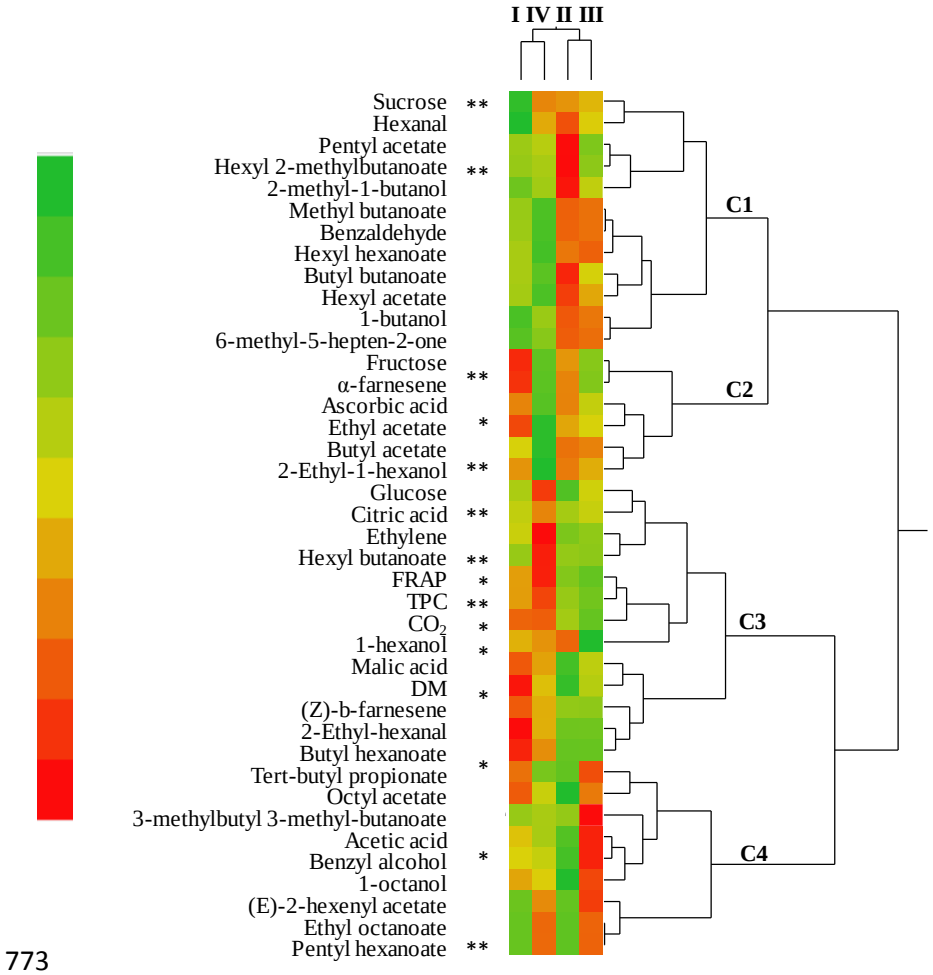

A
B
P. expansum severity (cm)
R. stolonifer incidence (%)
2.5
2.0
1.5
1.0
0.5
0.0
100
80
60
40
20
0
a
a
a
b
a
b
b
b
I
II
III
IV
I
II
III
IV
Slice
Slice

**Figure 3**

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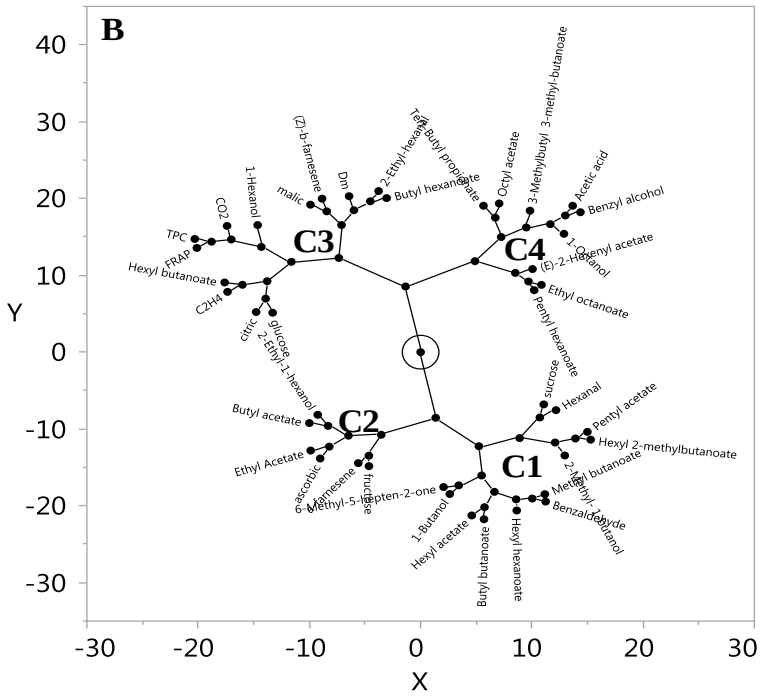


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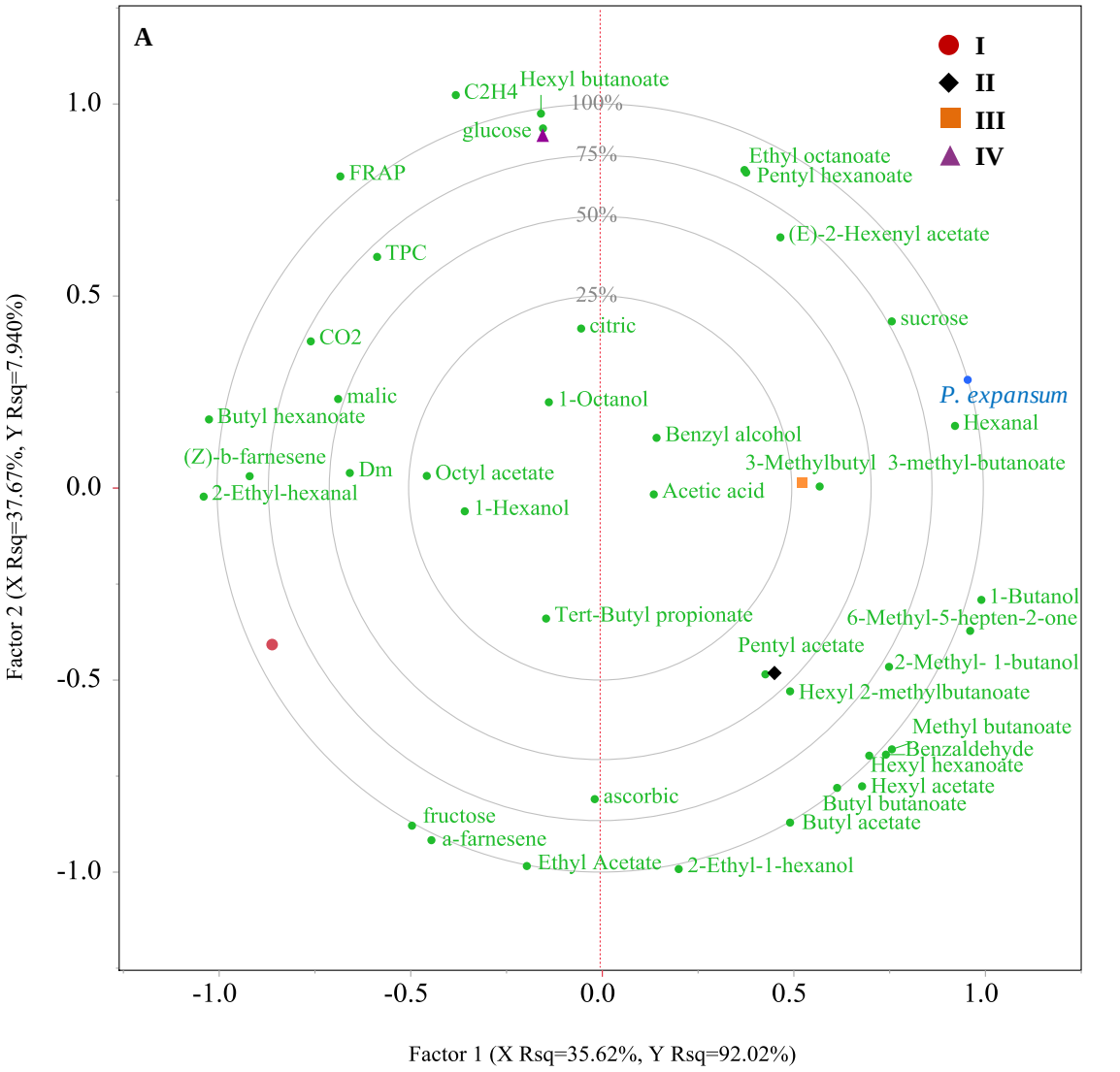
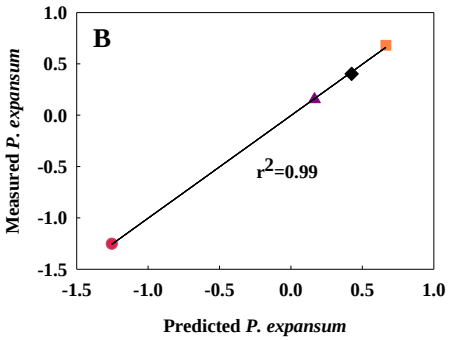
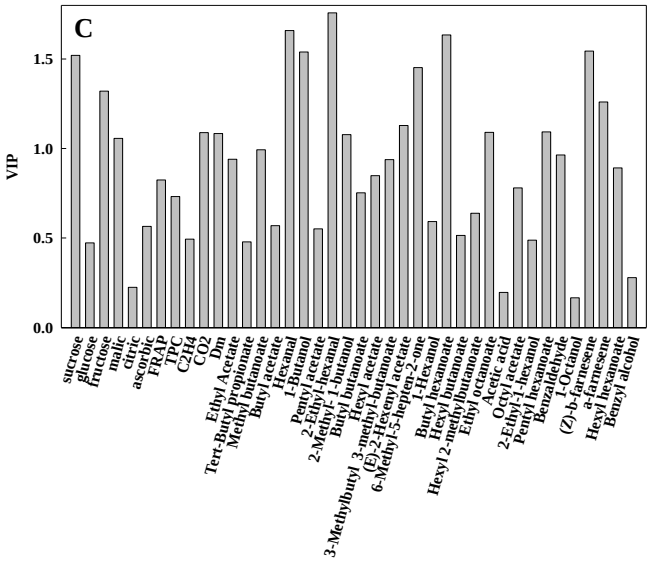
775 **Figure 4**

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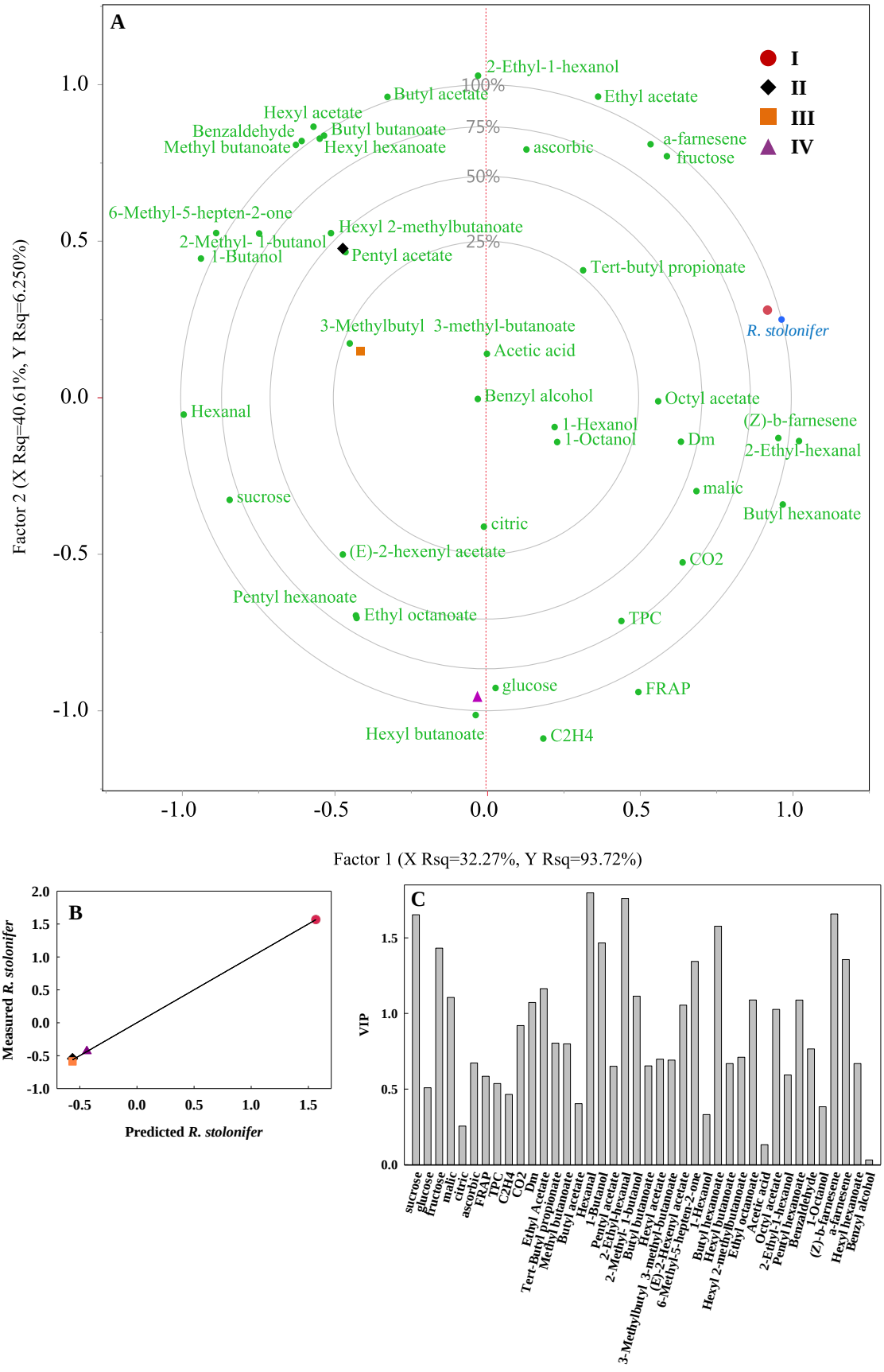
777 **Figure 5**

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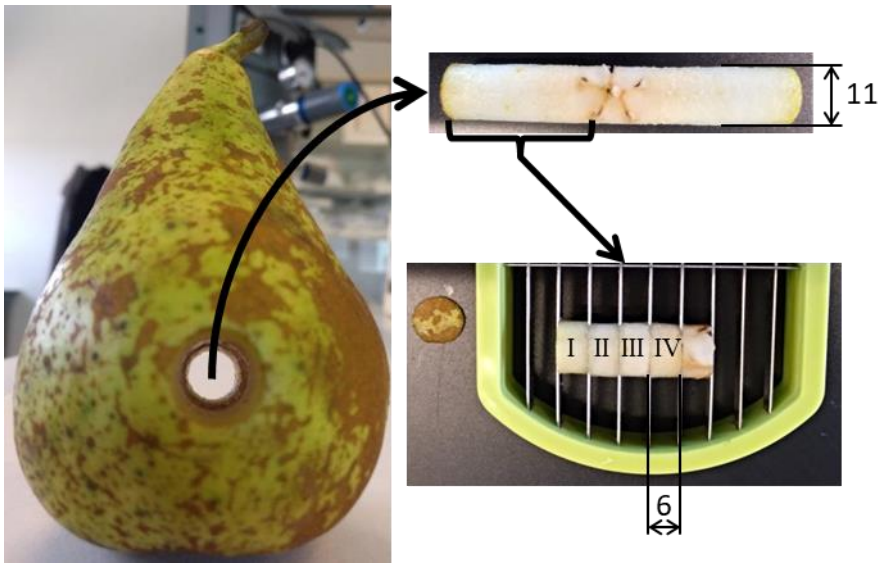
779 **Figure 6**

780 


781 **Figure s1**

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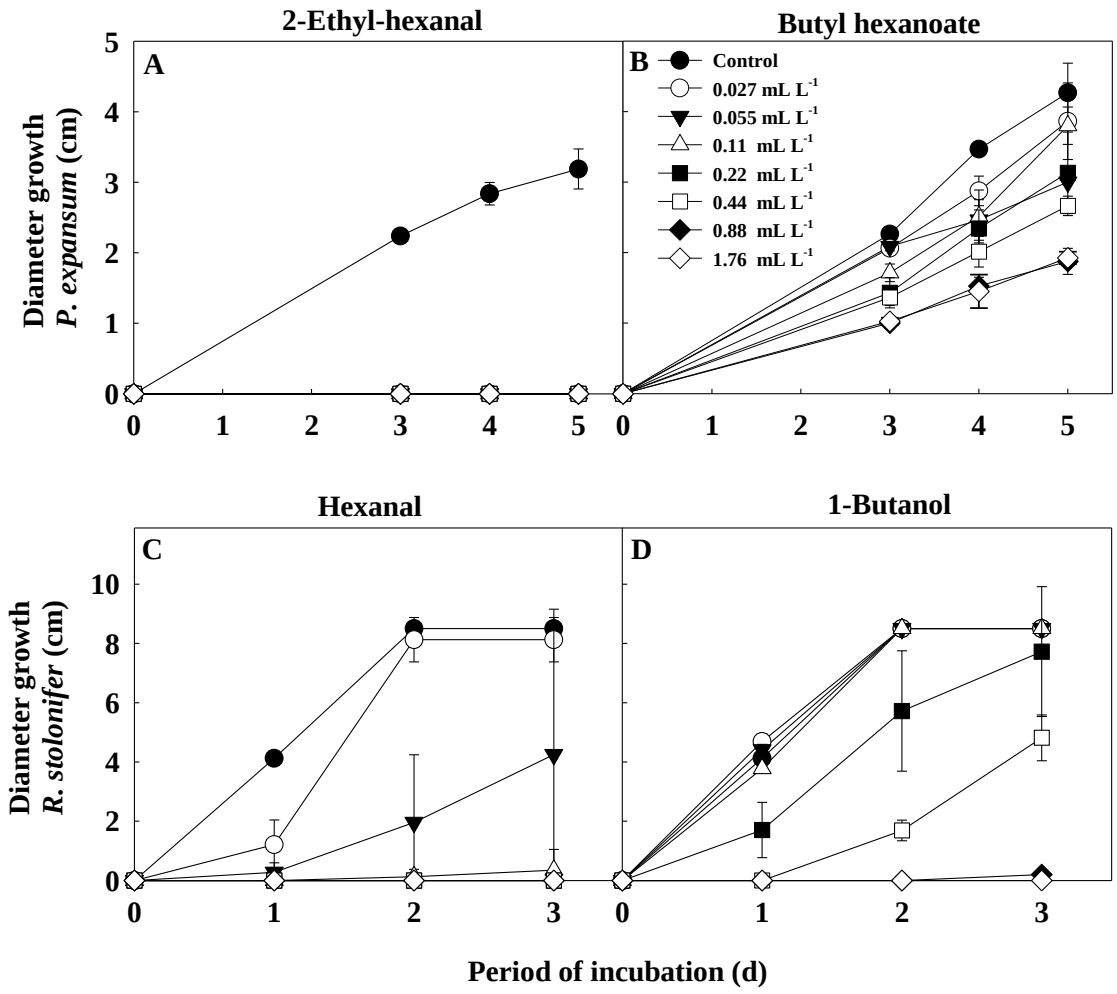


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795 **Figure s2**