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EARLY DETECTION OF GREY MOULD DEVELOPMENT IN 'RED GLOBE' GRAPES DURING STORAGE

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ABSTRACT

The monitoring of volatile compounds generated by diseased fruits and vegetables has been investigated as a means of detecting infections in storage compartments. To evaluate if infections of *Botrytis cinerea* could be detected prior to obvious disease symptoms on table grapes, the disease severity were assessed and the production of carbon dioxide, ethylene, ethanol, and acetaldehyde by 'Red Globe' grapes was measured by gas chromatography during storage at 20 °C following inoculation with *B. cinerea* as a spore suspension. The results indicated that visible decay became obvious between days 2 and 3 after inoculation, and a marked increase in ethylene production could be detected more than 24 h before the onset of the first signs of decay. However, a marked increase in carbon dioxide and ethanol was evident only on day 3 after inoculation and was correlated with decay development, whilst acetaldehyde could not be detected. It is estimated that ethylene could serve as an early indicator of infection of *B. cinerea* infection in table grapes, and may be used to predicate the presence of decayed table grapes and other fresh fruits during storage and transportation.

Key words: *Botrytis cinerea*; Postharvest; Red Globe grape; Decay; Prediction.

INTRODUCTION

Table grapes constitute a major world crop, which can be stored for as long as 4 months under optimal conditions (−1~0 °C and 90~95% RH). A primary difficulty associated with prolonged storage of table grapes is grey mold, caused by *Botrytis cinerea* (Franka *et al*., 2010; Jane, *et al*., 2010; Qin *et al*., 2015), invading grape flowers and berries by different infection pathways including directly penetration or through wounds (Elad 1997; Anna *et al*., 2012; Simona *et al*., 2012). Following the establishment of fungal hyphae, *B. cinerea* may become inactive for long periods with no symptoms in grapes until the fungus is reactivated during storage (Simona *et al*., 2012). Because of the latent nature of grey mould disease, its presence is not obvious to food handlers until visible decay is seen on table grapes, which results in significant losses (Li *et al*., 2011). Therefore, early detection technology that can identify pathogen infected grapes in storage would reduce postharvest losses.

More and more studies were performed to develop a rapid and non-destructive screening method to detect and predict postharvest pathogens infection. The detection of pathogenic activity during storage of many plant species through the analysis of volatile profiles has been proved to be a valuable way for non-destructive detection and prediction of postharvest disease (Pan *et al*., 2014; Luisa *et al*., 2015). Lyew *et al*. (2001) reported that a wider range of volatile compounds were produced by infected potatoes as compared to healthy ones, and differences between the volatile profiles of healthy and infected potatoes were observed as early as 24 h while the total quantity of volatiles produced was initially low and increased exponentially as infection became well established. Z-3-hexenyl 2-methylbutanoate could be a potential biomarker for 'Golden Smoothee' apples inoculated with *P. expansum* and *R. stolonifer* because it was quantified before these diseases were visible and was not detected in non-inoculated control fruit (Luisa *et al*., 2015). Six volatile compounds contributed to the distinguishing differences in volatiles emanating from the blueberry fruit due to infection (Li *et al*., 2010). It is also reported that ethylene might serve as an early indicator for infection in harvested fresh tomato fruit (Polevaya *et al*., 2002). Although these studies have attempted to identify volatile profiles that could be used as pathogen-specific markers for the presence of disease in fruit storage units, we need to identify some volatiles with simple structure that are easily and conveniently analyzed by modern rapid detection sensors. However, in *B. cinerea*, the causal agent of grey mould in table grapes, consistent results have not been found.

The objective of the present study was to detect early infection of *B. cinerea* in fresh 'Red Globe' grapes during storage by examining, using gas chromatography, the dynamics of carbon dioxide, ethylene, ethanol, and acetaldehyde production before the appearance of decay

symptoms. This approach could also serve as a possible early detection system for the development of gas sensor arrays for table grape disease detection and prediction.

MATERIALS AND METHODS

Grapes handling: Healthy 'Red Globe' grape berries of uniform size (11±1g) were harvested from a commercial non-heated greenhouse. Fruits were cleaned with 100 $mg.L^{-1}$ sodium hypochlorite and sterile distilled water before inoculation.

***B.cinerea* preparation:** *B. cinerea* utilized in the present study was from the culture collection of National Engineering and Technology Research Center for Preservation of Agricultural Products (Tianjin, P. R. China). The procedures of Fallik *et al*. (1993) and Zhu *et al*. (2012) were used to prepare *Botrytis* conidial suspension. The inoculum concentration (10^7 spore per mL) was similar to the natural levels of *B. cinerea* found on table grapes.

Inoculation and Incubation: Grape berries were divided into three groups. One group of fruits served as a control (unwounded). Two other groups of fruits were injected on both sides of the fruit equator with 25µl of distilled water or conidial suspension, respectively. Injections were made 2 mm deep into the pericarp tissue using a 25 gauge needle. Each group had 20 grape berries, and had 3 replicates.

Grape berries were weighed and placed inside 1,750 mL glass jars at 20 °C, RH>95%. The jars were sealed. Decay severity was assessed daily. Ethylene, ethanol and acetaldehyde (AA) were measured by gas chromatography and CO_2 was measured by O_2/CO_2 of PBI Dansensor Check Mate 9900, respectively. The first measurement was conducted 6 h following fruit inoculation, and each headspace was measured twice.

Disease assessment: The volume of diseased tissue (VD) was used to indicate the disease severity (Lui and Kushalappa, 2003). Each grape was cut vertically at the middle of each diseased berry, and then the height at the highest point, and diameters at three heights (top, middle and about bottom) were measured using a digital caliper. The volume of diseased grapes (mm^3) was calculated from the height and average diameter ($VD=\pi r^2 h$; r=radius, h=height). The wound volume of non-inoculated sites was measured and subtracted from the total diseased volume to calculate the net diseased volume.

Gas chromatogram analysis: The Agilent gas chromatograph (DB-5 column: 30m×0.25mm, 0.25µm coating thickness) with the carrier gas, ultra-purified nitrogen (99.99%) at a constant flow rate of 30mL/min was used. The temperature program started at 80°C, maintained for 2 minutes, and was then raised at a rate of 6℃/min to 230℃ and held for one minute. The injector temperature was 120°C and the FID detector was set to 230°C.

Statistical analysis: Statistical analysis was performed using SPSS 17.0 software. The data were analyzed with analysis of variance (ANOVA) followed by a Duncan's multiple range test for means comparison with a significance level of 0.05.

RESULTS

Between days 2 and 3 after *Botrytis*-inoculation, visible decay could be observed on the fruit (Fig. 1). From day 2 forward, a significant increase in the severity of decay was found. Water-inoculated and non-inoculated control grapes did not show visible decay during first 6 days.

Notably, a marked increase in ethylene generation of *Botrytis*-inoculated fruits was noticed between days 1 and 2 following inoculation, more than 24 h before the appearance of visible decay (Fig.1). Afterward, ethylene production increased continuously, reaching its maximum on day 6 after inoculation, and then declined. The amount of ethylene in water-inoculated and non-inoculated control fruits were significantly lower than *Botrytis*-inoculated fruit between days 2 and 6 ($p<0.05$), while significant difference ($p<0.05$) was not found between water-inoculated and non-inoculated control fruits during this period.

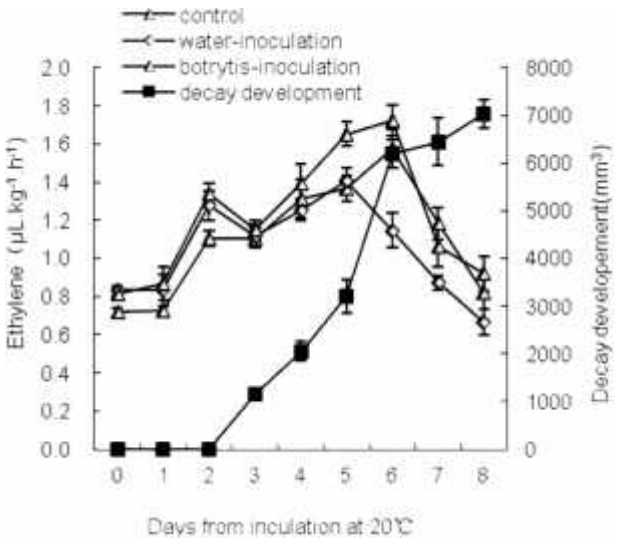

Fig. 1. Ethylene production in *Botrytis*-inoculated 'Red Globe' grape in comparison with control (wounded, not inoculated) and water-inoculated fruit, and in correlation with decay development of *Botrytis* inoculated fruit during 8 days of post-inoculation incubation at 20 °C.

The amounts of ethanol production measured in all treated fruits were low after 2 days inoculation (Fig. 2). From day 2, a sharp increase in ethanol production was observed in the control, water-inoculated and *Botrytis*-inoculated fruits, correlated with decay development, with a peak on day 8. In water-inoculated fruits, a relatively high amount of ethanol was observed 3 days after inoculation, followed by a decrease on day 4. The ethanol content then rose up to a maximum amount on day 7 followed by a decline on day 8. Ethanol production in non-inoculated control fruit was steady and low during storage. The amount of ethylene in water-inoculated and non-inoculated control fruits were significantly lower than *Botrytis*-inoculated fruit between days 3 and 6 ($p<0.05$).

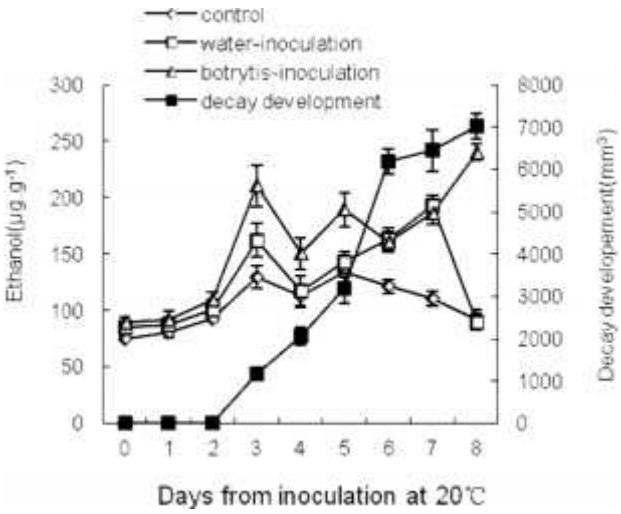

Fig.2. Ethanol production in *Botrytis*-inoculated 'Red Globe' grape in comparison with control (wounded, not inoculated) and water-inoculated fruit, and in correlation with decay development of *Botrytis* inoculated fruit during 8 days of post-inoculation incubation at 20 °C.

During the first 2 days of incubation following *B. cinerea* inoculation, a steady and relatively higher amount of CO_2 was observed, which sharply increased from day 3 to day 5, coinciding with the decay development. After 5 days, CO_2 production decreased (Fig. 3). In water-inoculated and non-inoculated control fruits, levels of CO_2 generally declined except for a slight increase between the 2 and 3 day after inoculation. The amount of CO_2 in *Botrytis*-inoculated fruit was significantly higher than water-inoculated and non-inoculated control fruits after day 3 ($p<0.05$).

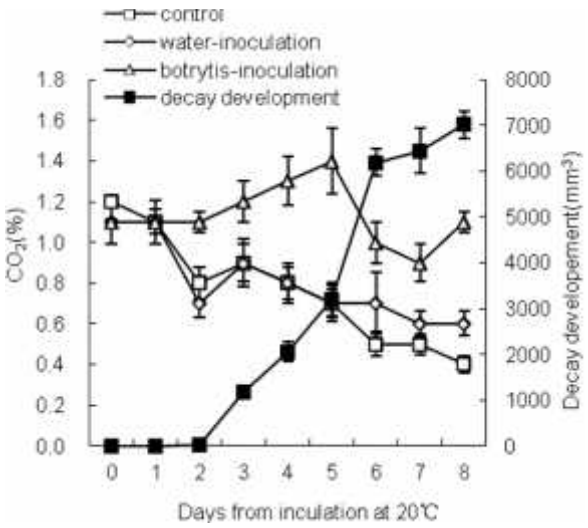

Fig.3. CO_2 production in *Botrytis*-inoculated 'Red Globe' grape in comparison with control (wounded, not inoculated) and water-inoculated fruit, and in correlation with decay development of *Botrytis* inoculated fruit during 8 days of post-inoculation incubation at 20 °C.

Acetaldehyde (one of the natural flavor compounds) along with ethanol, is often detected in fruit during storage and ripening (Pesis and Marinansky, 1990). However we observed no acetaldehyde production in any condition in this experiment.

DISCUSSION AND CONCLUSION

We studied the effect of *B. cinerea* inoculation into 'Red Globe' grape on production of several simple volatile compounds intended to identify infection before obvious decay occurred. Once *B. cinerea* is active in a host tissue, it can, if detected, be arrested.

Ethylene is involved in plant senescence, ripening and physiological disorders of common fruit, vegetables and ornamental crops. *B. cinerea* infection of tomato and table grapes can also induce ethylene production at significantly higher rates than uninfected fruit (Cristescu *et al*., 2002). Our results showed that, under the conditions tested, grey mould became visible on grapes between 2 and 3 days after *B. cinerea* innoculation. Among the four volatile compounds we measured, only an increase in ethylene generation in the inoculated fruit could be detected 24 h before visible decay occurred. It seems that *Botrytis*-inoculated fruit exhibited stress-related transient ethylene production similar to that induced by wounding. Rivka *et al*. (1989) found that the dimension of the wound in mature green tomatoes affected ethylene production. Needle-size wound in red tomato did not induce significant production of ethylene (Fallik *et al*. 1993). More research

is therefore needed to establish non-pathogen related sources of ethylene from plant tissue.

In general, the respiration rate of plant tissue increases shortly after pathogen infection. Further increases in respiration rate will depend on the progress of the disease. Normally, respiration continues to increase as the pathogen multiplies and breaks down plant tissues. Increased respiration in diseased plants has been reported in response to fungal attack. It was reported that the growth and the formation of mycelia and spores on the deteriorating orange peel contributed to the increase in CO_2 in *Botrytis*-inoculated fruits (Pesis and Marinansky 1990). The high amount of ethanol, a well-known anaerobic metabolite, in apples, pears and oranges inoculated with pathogens compared to the controls can also help to discriminate diseased from non-diseased fruit(Luisa *et al*., 2015). In this study, we also found that both CO_2 and ethanol produced by grapes inoculated with *B. cinerea* were apparently higher than that produced by non-inoculated grapes. However, unlike ethylene, increased CO_2 and ethanol emission were measured in parallel to or after visible grape berry decay had been observed.

Traditional ways for the detection of postharvest decay-causing agents has relied on isolation and molecular identification of the pathogens or observation of the symptoms they induce in the susceptible host (Simona *et al*., 2012). However, isolation or molecular identification of microorganisms is a time-consuming process, and observable symptoms become evident at a more mature stage of infection when treatment is more difficult. Thus, more sensitive, rapid and non-destructive instrumental methods for sensing the presence of infected fruit are needed to mark the beginning of infection and enhance quality control when treatment can be more time- and cost-effective. Application of electronic nose (E-nose) and gas chromatography-mass spectrometry (GC-MS) detection of a variety of volatiles produced by fungal infected fruits and vegetables has been used for early detection of fungal disease of many fruits and vegetables in recent years (Stijn *et al*., 2004; Antihus *et al*., 2006; Pan *et al*., 2014; Luisa *et al*., 2015; Li *et al*., 2010; Magan and Evans 2003). The success of these methods will be affected by the emanation rate of volatiles and the ability to detect low quantities of volatiles rapidly (Stijn *et al*., 2004). At the present time, identification of easily detected volatiles for prediction of postharvest disease severity is a potential and practical way to reduce and control postharvest loss during storage and transportation of fresh fruits and vegetables.

In summary, we conclude that ethylene could serve as an early indicator of grey mould infection in 'Red Globe' grape. Because there are several types of industrialized sensor for ethylene detection, this study underscores the potential feasibility of using ethylene sensor arrays for early grey mould disease detection of 'Red Globe' grape during storage. However, many factors such as types of grapes, storage conditions, and the interaction with other pathogens can influence the types and amounts of volatile compounds generated. Further studies may be conducted to investigate additional volatile compounds and develop simple instruments that can detect fruit infection during storage and transit.

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