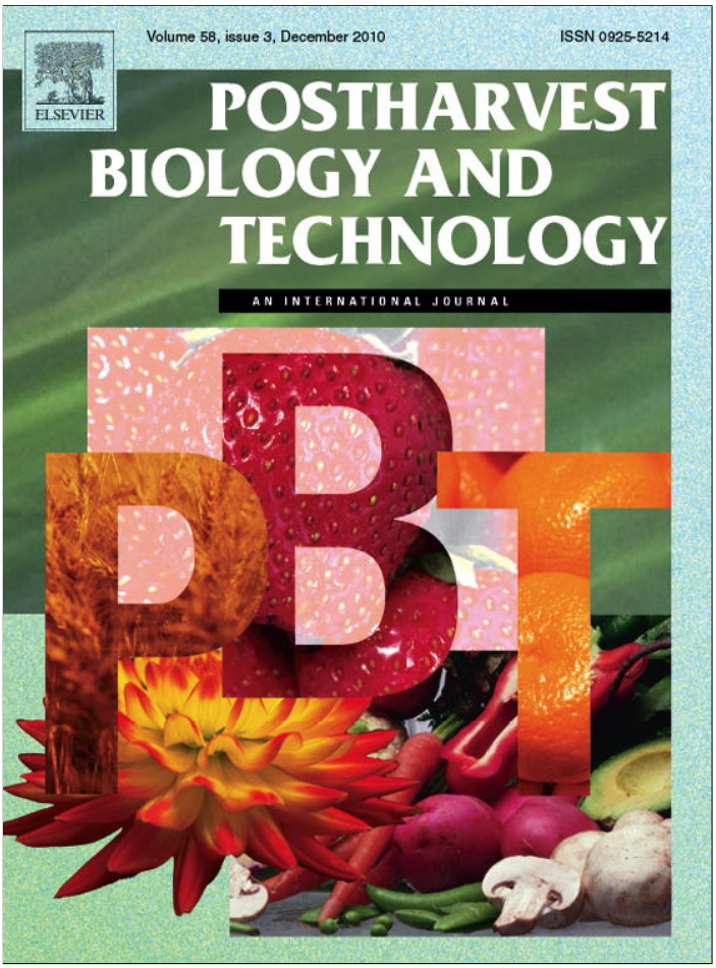

Postharvest Biology and Technology 58 (2010) 194–202

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Superoxide anion and hydrogen peroxide in the yeast antagonist–fruit interaction: A new role for reactive oxygen species in postharvest biocontrol?

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ARTICLE INFO

Article history:
Received 26 March 2010
Accepted 22 July 2010

Keywords:
Postharvest biological control
Yeast antagonists
Reactive oxygen species
Superoxide anion
Hydrogen peroxide

ABSTRACT

The importance of reactive oxygen species (ROS) in plant defense responses against certain pathogens is well documented. There is some evidence that microbial biocontrol agents also induce a transient production of ROS in a host plant which triggers local and systemic defense responses to pathogens. The ability of biocontrol agents used to control postharvest diseases to induce defense-related oxidative responses in fruits, however, has not been explored. Here we show that the yeast antagonists, *Metschnikowia fructicola* (strain 277) and *Candida oleophila* (strain 182) generate greater levels of super oxide anion (O_2^-) on intact fruit surfaces (poor in nutrients) then those applied on a nutrient-poor agar medium. Even though yeast antagonists show a high level of O_2^- on nutrient-rich media, when applied on fruits around wounds (areas abundant in nutrients) accumulation of O_2^-, as detected by nitro blue tetrazolium staining, occurred much more rapidly on the latter. Using laser scanning confocal microscopy we observed that the application of *M. fructicola* and *C. oleophila* into citrus and apple fruit wounds correlated with an increase in H_2O_2 accumulation in host tissue. In citrus fruit, the level of H_2O_2 around inoculated wounds increased by 4-fold compared to controls (wounds inoculated with water) as early as 18 h after inoculation. Yeast continued to stimulate H_2O_2 production in citrus fruit up to 66 h after inoculation and H_2O_2 levels were still 3-fold above the control. Living yeast cells were detected in fruit wounds at this time point indicating the ability of *M. fructicola* to tolerate host ROS, which has been reported to be an intrinsic characteristic of efficient yeast antagonists. Similar increase in H_2O_2 accumulation around yeast-inoculated wounds was observed in apple fruit exocarp. The present data, together with our earlier discovery of the importance of H_2O_2 production in the defense response of citrus flavedo to postharvest pathogens, indicate that the yeast-induced oxidative response in fruit exocarp may be associated with the ability of specific yeast species to serve as biocontrol agents for the management of postharvest diseases.

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1. Introduction

There are numerous studies demonstrating the production of reactive oxygen species (ROS) in plants as an initial response to microorganisms, both pathogenic and non-pathogenic (Bolwell and Wojtaszek, 1997; Bolwell et al., 1999, 2002). In the case of a non-compatible host–parasite interaction, an initial moderate increase in the production of ROS usually precedes a stronger oxidative burst, while in a compatible interaction no further increase in the level of reactive radicals in host tissue is observed (Able et al., 2000; Unger et al., 2005). Therefore, the presence of a biphasic oxidative burst or a general massive and prolonged production of oxidative molecules is considered to be critical for the development of host resistance in plant–microbial interactions (Baker and Orlandi, 1995). In contrast, lack of a strong oxidative burst has been linked with the ability of a pathogen to suppress directly or indirectly the accumulation of oxidative compounds in host cells (Cessna et al., 2000; Unger et al., 2005; Macarisin et al., 2007).

This observation is supported by studies showing that the application of exogenous ROS modulators or direct addition of hydrogen peroxide can increase host resistance even in compatible plant–fungal interactions (Shetty et al., 2007). Conversely, enzymatic or chemical removal of H_2O_2 increases host susceptibility to a pathogen (Mellersh et al., 2002; Shetty et al., 2007). In fruits, however, the role of ROS as mediators of host resistance against postharvest pathogens has not been well explored and limited to a few studies, done on apple (Torres et al., 2003), mango (Zeng et al.,

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0925-5214/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.postharvbio.2010.07.008

2006), citrus (Macarisin et al., 2007), strawberry (Horowitz et al., 2008), and peach (Jin et al., 2009).

The ability of microbial biocontrol agents to induce defense responses in plants has been the subject of numerous studies (Droby et al., 2009). Only recently, however, has the induction of defense responses in plants by a biocontrol microorganism been associated with a stimulation of ROS production in host tissue by the biocontrol agent. The biocontrol fungus, *Trichoderma virens*, was reported to secrete and deliver a proteinaceous effector into host tissue in both monocot (rice) and dicot (cotton) plants. This fungal effector is a 12.5 kDa polypeptide that triggers production of H_2O_2 in host tissue and induces the expression of defense-related genes both locally and systemically (Djonović et al., 2006). In plant defense against pathogens H_2O_2 can be involved in hypersensitive response mediated by a mitogen-activated protein cascade (Liu et al., 2007), lignin biosynthesis (Nicholson and Hammerschmidt, 1992), induction of specific heat shock transcription factors (Miller and Mittler, 2006; Shao et al., 2007), accumulation of PR genes (Hu et al., 2003), and antimicrobial compounds such as phytoalexins (Araceli et al., 2007).

Droby et al. (2009) have suggested that in addition to other documented mechanisms of action (competition for nutrients, secretion of hydrolytic enzymes, and mycoparasitism) ROS production by yeast antagonists may also be involved in triggering local and systemic resistance of host fruit against postharvest pathogens. In the present study we have begun to examine ROS production by yeast antagonists in harvested fruits in order to better understand the role of ROS in postharvest biocontrol systems. The ability of yeast to produce ROS on artificial media and on harvested fruits was examined as was the oxidative response of the fruit to inoculation with yeast antagonists.

2. Materials and methods

2.1. Plant material

Apple (cultivar Granny Smith) fruits were obtained from an experimental orchard at the USDA-ARS, Appalachian Fruit Research Station, Kearneysville, WV, USA. Citrus fruits were obtained from ARO, The Volcani Center, Bet Dagan, Israel. Shortly after harvest, fruits were either used immediately or stored at an optimal storage temperature for each fruit (grapefruit and lemons at 11 °C, oranges at 6 °C) until use. Prior to inoculation, fruits were washed thoroughly with tap water and sterilized by drenching in a water solution of 0.05% hypochlorite for 10 min.

2.2. Biocontrol agent cultivation and inoculation

Yeast antagonists *Candida oleophila* (Strain 182), and *Metschnikowia fructicola* (Strain 277) were grown on YM (DIFCO) liquid media for 48 h. Yeast cells were pelleted by centrifugation at 500 × *g* and suspended in sterile water. Cell suspensions were adjusted to 1.5×10^8 cells/mL. Viability of yeast cells was assayed prior to inoculation using a Cellometer (Nexcelom, Inc., Lawrence, MA USA). Cell viability of yeast suspensions ranged from 97.7% to 99.7%. A 1.5 mL eppendorf tube containing 200 μL yeast suspension was incubated for 5 min in boiling water to prepare heat-inactivated yeast suspension, which was used in addition to controls. Ten μL of either heat-inactivated or living yeast suspension was placed on an intact apple fruit surface, 20 μL into a fruit wound, and 5 μL of living yeast suspension on nutritional media (100%, 10% and 1% of YM Broth, DIFCO solidified with 2% agar), hence referred to as YMA. In experiments evaluating superoxide anion production controls represented fruit wounds inoculated with 20 μL YMA. All inoculations were performed in three replicates for each time point.

2.3. Detection of superoxide anion

Superoxide anion was detected using Nitrotetrazolium Blue chloride (NBT) staining as described by Dunand et al. (2007). Forty 40 μL of dye solution were applied directly on a yeast colony, either on fruit surface or on YMA. Dye infiltration and staining development were allowed to proceed for 1 h in the dark prior to image acquisition. Images were acquired using a LEICA MZFLIII (Heerbrugg, Switzerland) binocular microscope equipped with a CCD camera. Experiments were repeated twice with identical results.

2.4. Detection and quantification of H_2O_2 production in fruits

The fluorescent dye, 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCF-DA), was used to identify H_2O_2 production in host tissue. Laser scanning confocal microscopy (LSCM) was employed to localize and quantify H_2O_2 in citrus fruit as previously described (Macarisin et al., 2007). Briefly, the relative intensity of H_2DCF-DA fluorescence from every consecutive focal plane was estimated by calculating average pixel intensity for green channel. The final value of each measurement presented its average (±standard error) from three different slices with the total number of focal planes ranging from 10 to 30. Mean values were analyzed statistically by Student's *t* test at $P < 0.05$. Accumulation and quantification of H_2O_2 in apple fruit was performed using identical protocol except that LSCM analysis was conducted under the Zeiss™ 710 system. The images were observed using a Zeiss Axio Observer™ inverted microscope with 40 × 1.2 NA water immersion and 63 × 1.4 NA oil immersion plan apochromatic objectives. Both differential interference contrast (DIC) and confocal fluorescence images were acquired simultaneously. A photomultiplier tube captured the light emitted from a 488 nm argon laser with a pin-hole of 3.7 μm passing through a MBS 488 filter with limits set between 515 and 525 nm for detection of dichlorodihydrofluorescein diacetate fluorescence. Zeiss Zen™ 2008 software was used to obtain the composite (7 × 7) images each with 512 × 512 pixel resolution.

3. Results

3.1. Superoxide anion production by yeast antagonists

Yeast antagonists *C. oleophila* (Strain 182), and *M. fructicola* (Strain 277) growing on 1%, 10% and 100% YMA were examined. Fig. 1 shows the production of superoxide by *C. oleophila* over time (3, 6, 24, and 48 h after inoculation) on different concentrations of YMA to simulate nutrient-poor and nutrient-rich conditions. As determined by NBT staining, O_2^- produced by *M. fructicola* on the identical media and at the same time points is shown in Fig. 2. Both yeasts exhibited strongest production of O_2^- in 100% YMA approximately 24 h after inoculation.

The time-course of superoxide anion production by these yeasts on apple fruit was also monitored. *C. oleophila* applied on wounded and intact fruit surfaces exhibited an intense production of O_2^- about 3 h after inoculation (Fig. 3A and C). No accumulation of superoxide molecules was observed in control apple wounds (inoculated with YMA) (Fig. 3E and F) and those inoculated with heat-inactivated yeast (Fig. 3G and H). Similar to *C. oleophila*, *M. fructicola* also displayed elevated production of O_2^- as early as 3 h after inoculation on both the intact fruit surface and wounded areas (data not shown).

3.2. Production of H_2O_2 in fruit in response to inoculation with yeast antagonists

M. fructicola, caused an increase in H_2O_2 levels in host tissue when applied into fruit wounds (apple, lemon, orange, and

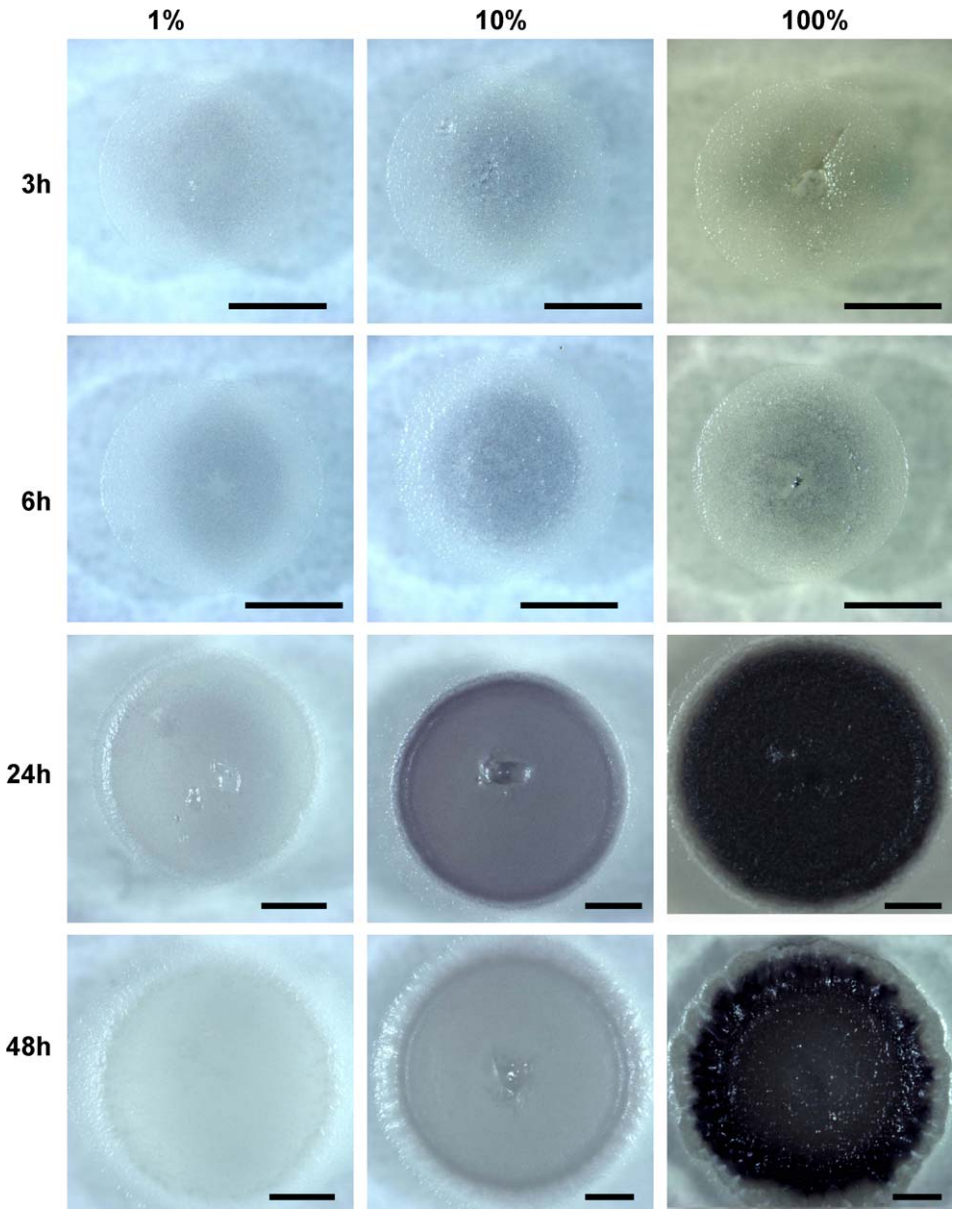

Fig. 1. NBT staining of superoxide anion by *Candida oleophila* growing on 1%, 10% and 100% Yeast maltose agar (YMA). Numbers on the left indicate hours after inoculation (3, 6, 24 and 48 h). Scale bar = 2000 μm.

grapefruit). Fig. 4 shows the time-course and spatial accumulation of H_2O_2 in lemon flavedo. In the case of non-inoculated, control wounds, production of H_2O_2 was observed to be concentrated at the periphery of the wound, while yeast triggered an H_2O_2 accumulation in areas distant from the actual wound. Hydrogen peroxide was initially detected predominantly around oil glands in the vicinity of the wound, and later (2–3 d after inoculation) became more widespread (Fig. 4).

Quantification of the fluorescent signal showed that the level of H_2O_2 around inoculated lemon wounds was 4-fold greater than control wounds within 18 h after inoculation (Fig. 5). H_2O_2 levels remained elevated in yeast-inoculated fruit wounds up to 66 h postinoculation. At that time, H_2O_2 levels were still 3-fold above the control. Importantly, despite high levels of H_2O_2 in the wound, living yeast could be observed even on the third day after inoculation (Fig. 6). The presence of living yeast cells was confirmed by the ability to re-culture them from the wound site. A similar induction of the oxidative response to inoculation with *M. fructicola* was observed on orange and grapefruit flavedo (data not shown).

In apple fruit (cv. Granny smith), both *M. fructicola* and *C. oleophila*-induced a stronger and more widespread oxidative response around inoculation sites than in control wounds. Forty eight hours after inoculation, levels of H_2O_2 in apple wounds inoculated with *M. fructicola* were about 2.5-fold higher than in those inoculated with water (Fig. 7). *C. oleophila* also caused an increased accumulation of H_2O_2 in apple exocarp around sites inoculated with yeast cells (Fig. 8). Importantly, in non-inoculated, control wounds of apple, H_2O_2 was localized to the periphery of the wound (Fig. 8A) as was observed in lemons (Fig. 4 upper panel). Comparatively, in response to yeast inoculation H_2O_2 production was more widespread (Fig. 8B), again similar to that found in lemons inoculated with yeast antagonists (Fig. 4, lower panel).

4. Discussion

Results of the current study demonstrate that yeasts antagonists used to control postharvest diseases have the ability to produce relatively high amounts of O_2^-. An increase in superoxide anion

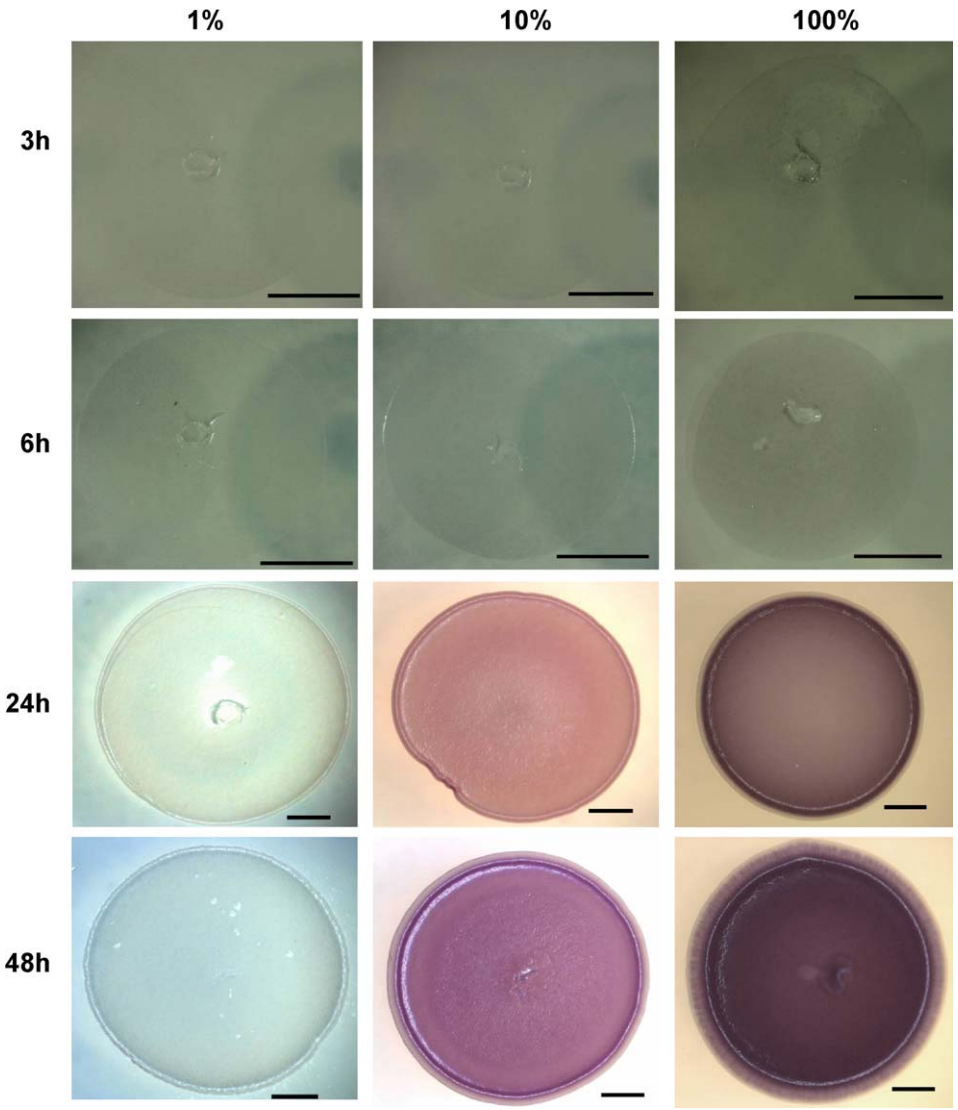

Fig. 2. NBT staining of superoxide anion production by *Metschnikowa fructicola* growing on 1%, 10% and 100% YMA. Numbers on the left indicate hours after inoculation (3, 6, 24 and 48 h). Scale bar = 2000 μm.

production can be detected as early as 6 h after application of yeasts on 100% YMA and the generation of the oxidant intensifies thereafter (Figs. 1 and 2). The large oxidative burst produced by the yeast reaches a maximum approximately 24 h after inoculation and does not cause substantial cell death as the yeast colonies continue to grow and expand (Figs. 1 and 2). These data are consistent with an earlier report by Derouet-Humbert et al. (2007) where they demonstrated that unlike mammalian cells, where intensive production of superoxide anion is strongly associated with programmed cell death, cells of the fission yeast, *Schizosaccharomyces pombe,* are highly robust in the presence of endogenous ROS and do not undergo apoptotic cell death.

C. olephila and *M. fructicola* applied on media containing a low level of nutrients (10% YMA) produced less O_2^-, while production of O_2^- by yeast on nutrient-poor media (1% YMA) was below the detection limit (Figs. 1 and 2).

In *Saccharomyces cerevisiae,* O_2^- production was suggested to be required for robust growth while low levels of O_2^- resulted in growth inhibition (Buetler et al., 2004). Our results are in agreement with this relationship in that yeasts grow rapidly and produce a high level of O_2^- while a lack of nutrients and limited growth resulted in the low production of O_2^-. Interestingly, when yeasts were applied to fruit wounds and intact fruit surfaces, the production of O_2^- occurred more rapidly than in yeast growing on artificial media (Fig. 3A–D). On the other hand, an absence on the purple NBT staining in and around fruit wounds inoculated with YMA and those with heat-treated yeasts (Fig. 3E–H) indicates that O_2^-, accumulating in and around the wounds treated with living yeast suspensions, is indeed produced by yeasts and not by host cells.

Intact fruit surfaces are known to be poor in nutrients and one would expect a reaction similar to that observed on nutrient-poor media (Figs. 1 and 2). *C. oleophila* (Fig. 3) and *M. fructicola* (data not shown), however, developed strong NBT staining on apple 3 h postinoculation. This observation indicates that the rapid burst in O_2^- production by yeast antagonists applied to an intact fruit surface was associated with some factor(s) other than just the availability of nutrients. The hydrophobicity of fruit peel, fruit volatiles, or other host signals, are all potential candidates involved in the observed induction of the oxidative response in yeasts applied on fruits. In the case of wounded apples, the strong O_2^- production by these biocontrol yeasts may act as a signal to stimulate rapid multiplication in fruit wounds (abundant in nutrients) to assure maximal spread within a favorable biological niche. This suggestion would

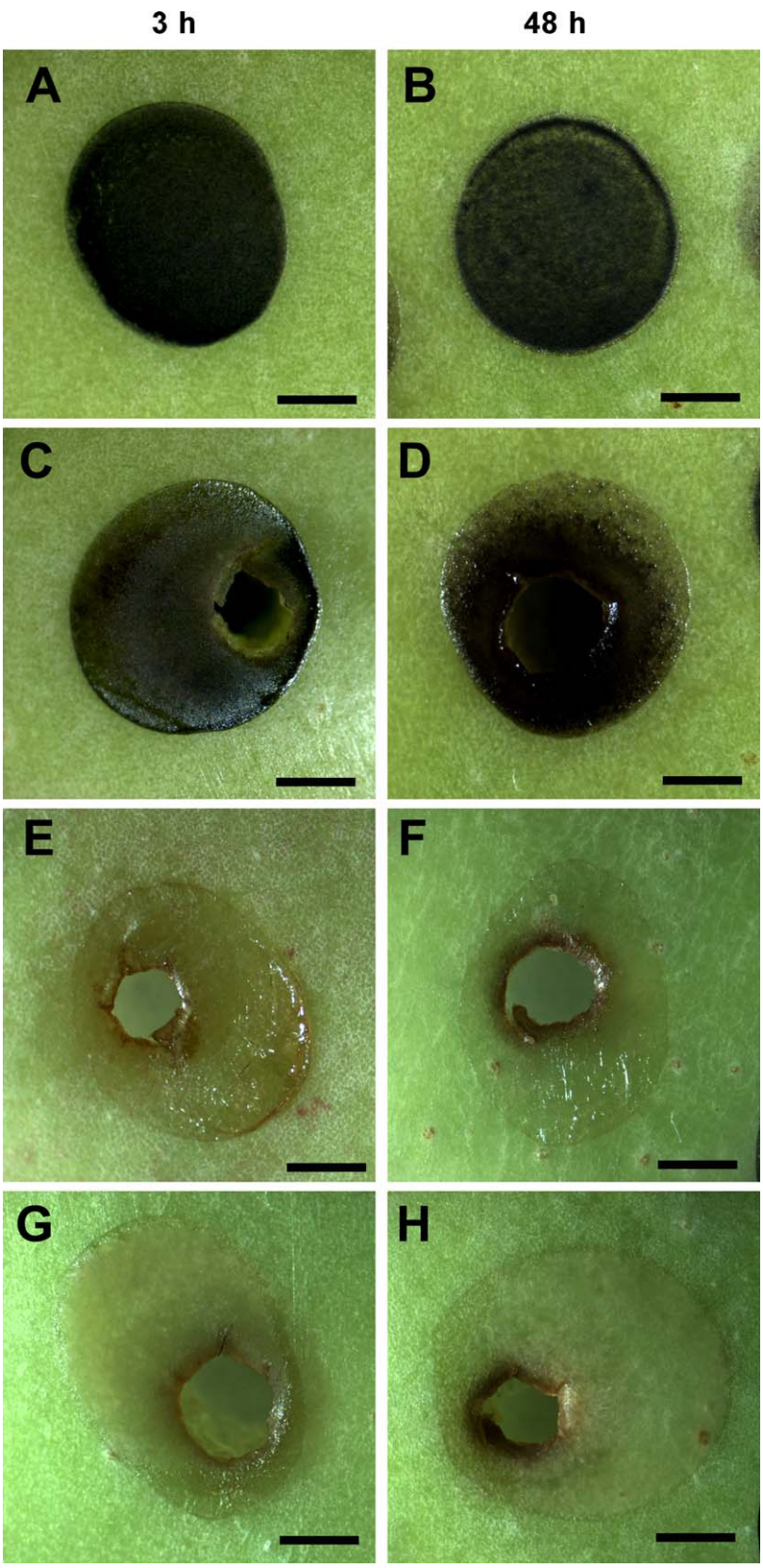

Fig. 3. NBT staining of superoxide anion production by *Candida oleophila* on intact (A and B) and wounded (C and D) apple fruit surface at 3 and 48 h after inoculation. Wounded apple tissue inoculated with YMA and heat-inactivated *C. oleophila*, and stained with NTB at 3 and 48 h after inoculation is shown in panels E, F and G, H respectively. Scale bar = 1 mm.

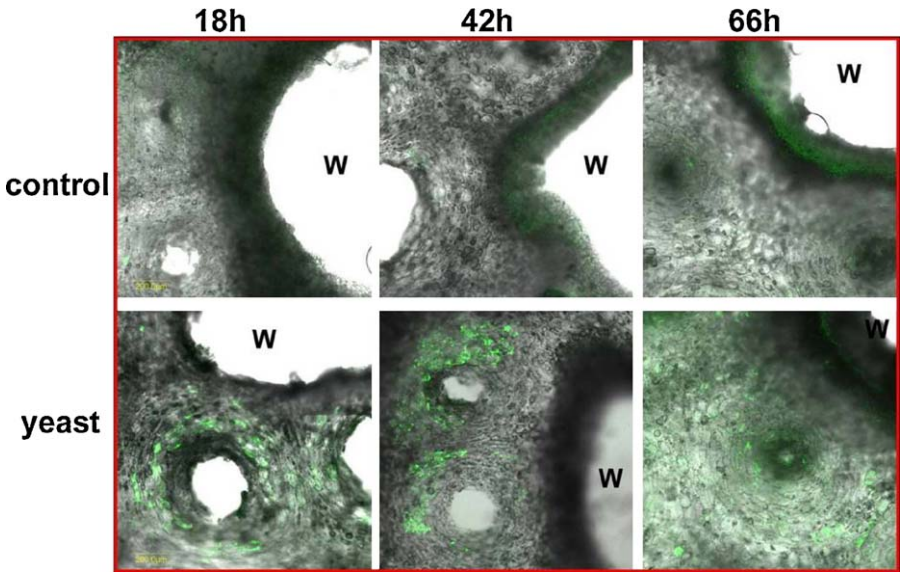

Figure 4. Laser scanning confocal fluorescence images, combined with bright field images, showing H_2O_2 production in flavedo tissue of lemon fruit in response to wounding (upper panel) and inoculation with *Metschnikowia fructicola* (bottom panel). Micrographs illustrate the production and spatial localization of H_2O_2 (green fluorescence) in flavedo tissue surrounding control and inoculated wounds at 18, 42 and 66 h after inoculation. W = wound site. Scale bar = 200 μm. (For interpretation of references to color, in this figure legend the reader is referred to the web version of this article.)

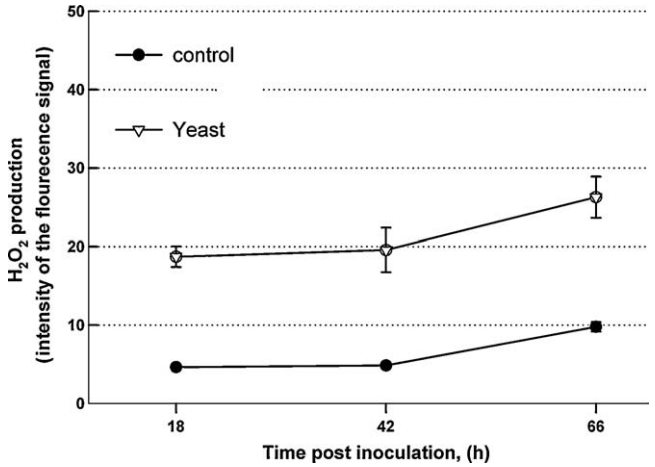

Fig. 5. Time-course production of H_2O_2 in lemon flavedo in response to wounding and inoculation with *Metschnikowia fructicola.* Concentration of H_2O_2 is expressed as relative pixel intensity. Values represent the mean ± standard error, n = 30–90.

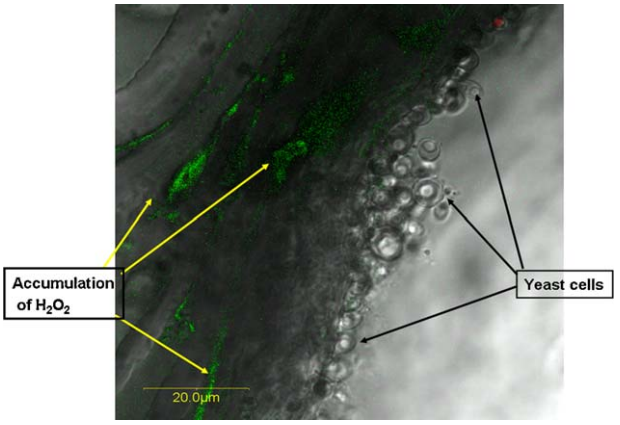

Fig. 6. Laser scanning confocal fluorescence image, combined with bright field image, showing individual yeasts cells in close vicinity to the sites of H_2O_2 accumulation (green fluorescence) in flavedo tissue of lemon fruit at 66 h after inoculation with *Metschnikowia fructicola.* Scale bar = 20 μm. (For interpretation of references to color, in this figure legend the reader is referred to the web version of this article.)

be in agreement with the hypothesis proposed by Buetler et al. (2004) for growth of *S. cerevisiae*.

Superoxide anion production on the intact fruit surface could also serve as a *quorum-sensing* signal to trigger aggregation into a biofilm which would increase yeast attachment and improve survival on the fruit surface by providing a microenvironment resistant to environmental stress. Bloomfield and Pears (2003) found that strong production of O_2^- by solitary growing *Dictyostelium discoideum* cells served as a signal for transition to a multicellular form, more resistant to environmental changes, while enzymatic or chemical scavenging of O_2^- disrupted aggregation, suggesting an important role for O_2^- in intercellular communication in unicellular eukaryotes. Another hypothesis to explain the role of high levels of O_2^- production in yeasts has been proposed by Fabrizio et al. (2004). They have suggested that in a natural environment, yeasts undergo accelerated aging mediated by O_2^- and that this oxidative-driven aging is associated with a release of nutrients and an increase in mutation frequency that may represent a major adaptative advantage for yeast under changing environmental conditions (Fabrizio et al., 2004). While the role of O_2^- in yeast cell multiplication, intercellular communication, or as an adaptive response to an unstable environment remains to be elucidated, our results clearly show that once sensing host tissue, yeasts are able to produce and apparently tolerate high levels of O_2^-, regardless of the availability of nutrients.

Based on the present study and our previous research (Macarisin et al., 2007), we hypothesize that the production of superoxide anions by yeasts antagonists at the site of inoculation may serve as a global regulator that can shift redox homeostasis in the host and as a result activate defense mechanisms in host tissue. Indeed, several reports have indicated a significant increase in peroxidase (POD) activity in harvested commodities, such as apple (Yu and Zheng, 2007), peach (Yao and Tian, 2005), sweet cherry (Chan and Tian, 2006) and pear (Zheng et al., 2007) in response to application of yeast antagonists used to control postharvest diseases. The function of POD as a generator of and the principal source of hydrogen peroxide (H_2O_2) in the initial stages of a host–pathogen interaction (basal resistance), has been well demonstrated (Bolwell and Wojtaszek, 1997; Bolwell et al., 1999, 2002; Bindschedler et al., 2006; Davies et al., 2006). Furthermore, the overexpression of a

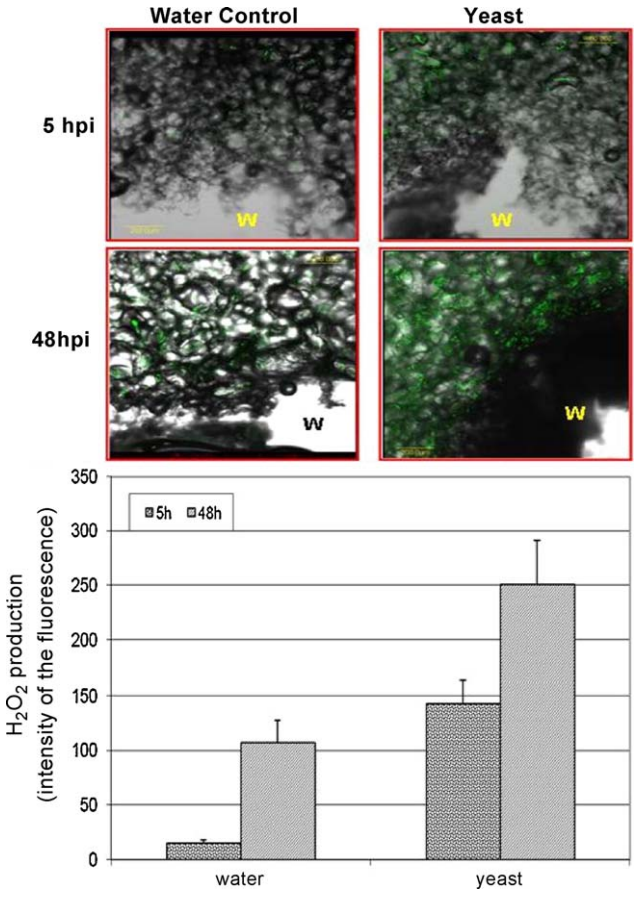

Fig. 7. (top panel) Laser scanning confocal fluorescence images, combined with bright field images, showing H_2O_2 production in the apple fruit in response to wounding (left panel) and inoculation with *Metschnikowia fructicola* (right panel). Micrographs illustrate the production and spatial localization of H_2O_2 (green fluorescence) in the exocarp of the apple fruit surrounding control and inoculated wounds at 5 and 48 h after inoculation. W = wound site. Scale bar = 200 μm. (bottom panel) Quantification of H_2O_2 in exocarp of the apple fruit in response to wounding and inoculation with *Metschnikowia fructicola* at 5 and 48 h after inoculation. Concentration of H_2O_2 is expressed in relative pixel intensity. Histogram represents the mean ± standard error, $n = 30–90$. (For interpretation of references to color, in this figure legend the reader is referred to the web version of this article.)

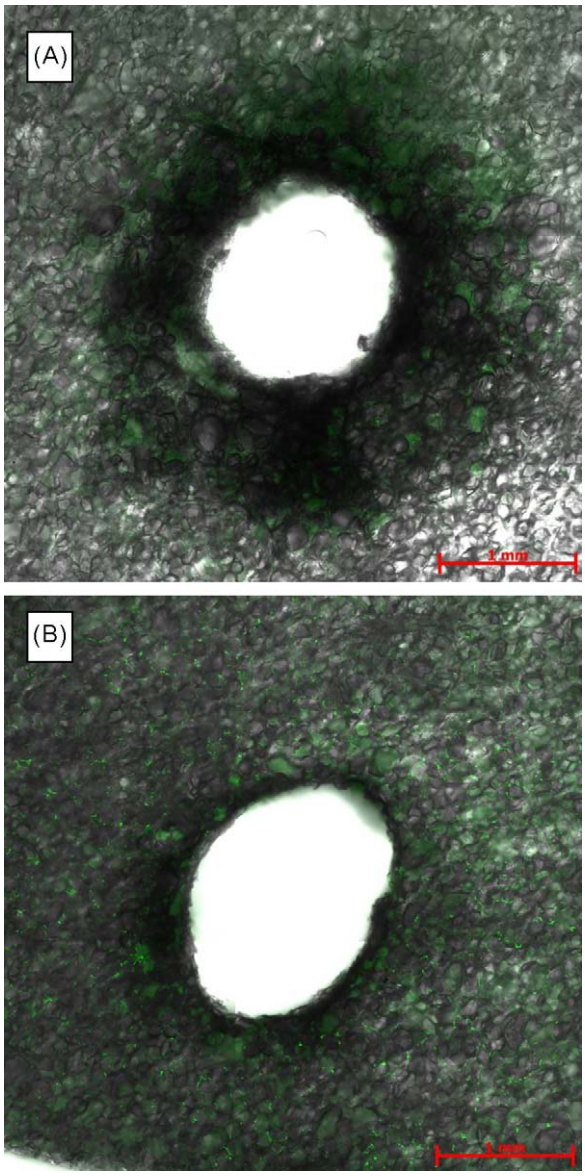

Fig. 8. Laser scanning confocal fluorescence images, combined with difference interference contrast images, showing H_2O_2 production in apple exocarp in response to wounding (A) and inoculation with *C. oleophila* (B). Micrographs illustrate the detection of H_2O_2 around the wound (bright area in the center of the image) in flavedo tissue surrounding control and inoculated wounds at 48 h after inoculation. Scale bar = 1 mm.

cationic peroxidase in *Arabidopsis* significantly increased resistance to the necrotrophic pathogens, *Botrytis cinerea* and *Plectosphaerella cucumerina* (Coego et al., 2005). Thus, the reported activation of POD in harvested fruits in response to postharvest biocontrol agents may also have been associated with an increase in host levels of H_2O_2 and concomitantly host defense response.

The induction of H_2O_2 accumulation in harvested fruit commodities by methyl jasmonate (JM) and salicylic acid (SA) has been shown to enhance their resistance to postharvest pathogens. A recent study by Jin et al. (2009) showed that the increased resistance in peach to *B. cinerea*, *Penicillium expansum* and *Rhizopus stolonifer* after application of methyl jasmonate was associated with a significant increase in H_2O_2 levels in the fruit exocarp. An increase in H_2O_2 in mango fruit, as result of SA treatment, was found to enhance resistance to anthracnose rot (Zeng et al., 2006). In this regard, our results show a significantly (Student's *t*-test, $P < 0.05$) higher level of H_2O_2 in climacteric and non-climacteric fruits inoculated with yeasts compared to non-inoculated fruits (Figs. 4, 5, 7 and 8). Based on these observations, we suggest that an increase in POD activity, detected after the application of yeast antagonists to apple, peach, sweet cherry and pear fruit (Yao and Tian, 2005; Chan and Tian, 2006; Yu and Zheng, 2007; Zheng et al., 2007) may be associated with an accumulation of oxidants around inoculation sites, rather than with an increase in antioxidant activity in host tissue as was suggested by these various authors. A high level of yeast O_2^- at inoculation sites, however, may also induce host-generated superoxide dismutase (SOD) to neutralize these highly toxic molecules. This raises the question as to whether or not H_2O_2 can accumulate in fruit and activate host defense response if there is an elevation in host SOD activity. An earlier study by Torres et al. (2003), however, supports this possibility. These authors clearly demonstrated that increased levels of both H_2O_2 and SOD in apple fruit were associated with postharvest disease resistance.

Results of the current study show that after coming in contact with a fruit, yeast antagonists begin to generate significant amounts of O_2^- which is followed by the accumulation of H_2O_2 in host tissue. Importantly, despite high levels of H_2O_2 in the inoculated wound sites, living yeast cells were observed even on the

third day after inoculation (Fig. 6), indicating that the biocontrol yeast, *M. fructicola*, is able to tolerate high levels of host H_2O_2. This data supports the earlier work by Castoria et al. (2003), where they suggested that H_2O_2 – tolerance is an intrinsic characteristic of efficient yeast antagonists, while weak antagonists cannot cope with host-generated ROS and therefore exhibit poor biocontrol ability.

Apart from O_2^-, other yeast-secreted small molecules can be potential triggers for ROS production in host cells. For instance, a quorum-sensing isoprenoid, farnesol, secreted by *C. albicans*, was also shown to be employed in the pathogenicity through induction of oxidative stress in host cells (Abe et al., 2009). Furthermore, farnesol was show to be used by *C. albicans* in antagonistic competition against other microorganisms in mammalian host environment. Semighini et al. (2006) showed that *C. albicans* inhibits the growth and development of *Aspergillus nidulans* via farnesol, by inducing ROS-mediated apoptosis in this filamentous fungus. More recently, Semighini et al. (2008) found that *C. albicans* can also induce oxidative damage in phytopathegenic fungus *Fusarium graminearum. C. albicans*-produced extracellular farnesol affected germination and impaired development in *F. graminearum,* suggesting that this isoprenoid and its derivates also may have value as antifungal agents in agriculture (Semighini et al., 2008). The secretome of the biocontrol yeast is largely unknown; however, the possibility for the role of small molecules secreted by yeast antagonists in postharvest biocontrol environment, similar to that of *C. albicans* virulence and antagonism, cannot be ruled out.

In summary, the role of ROS in fruit resistance to postharvest pathogens is complex and poorly understood. Current data support the hypothesis of a beneficial role for both superoxide anion and hydrogen peroxide on the adaptative re-growth of yeasts in changing environments (Fabrizio et al., 2004) and together with earlier reports demonstrating the importance of H_2O_2 in fruit defense mechanisms (Torres et al., 2003; Macarisin et al., 2007), indicate that the ability of yeast antagonists to self-generate and possibly stimulate an oxidative response in host tissue could be one of the major factors underlying the performance of a biocontrol agent. The induction of H_2O_2 accumulation in fruit tissue may be in response to yeast-produced O_2^- or a result of a more complex interaction, involving specific effectors/elicitors such as in the case of *T. virens*–host interaction (Djonović et al., 2006) or *C. albicans*/mammalian cells interaction (Abe et al., 2009). Nevertheless, the current study provides intriguing information that may represent a potentially new mechanism of action for yeast antagonists used to control postharvest diseases of fruits and demonstrates the need for a more detailed investigation of the role of ROS in postharvest biocontrol systems.

Acknowledgements

This research was partially supported by a grant (IS-4268-09) received from the U.S.-Israel Binational Agricultural Research and Development (BARD) Fund.

References

Abe, S., Tsunashima, R., Iijima, R., Yamada, T., Maruyama, N., Hisajima, T., Abe, Y., Oshima, H., Yamazaki, M., 2009. Suppression of anti-candida activity of macrophages by a quorum-sensing molecule, farnesol, through induction of oxidative stress. Microbiol. Immunol. 53, 323–330.

Able, A., Guest, D., Sutherland, M., 2000. Hydrogen peroxide yields during the incompatible interaction of tobacco suspension cells inoculated with *Phytophthora nicotianae*. Plant Physiol. 124, 899–910.

Araceli, A.C., Elda, C.M., Edmundo, L.G., Ernesto, G.P., 2007. Capsidiol production in pepper fruits (*Capsicum annuum* L.) induced by arachidonic acid dependent of an oxidative burst. Physiol. Mol. Plant Pathol. 70, 69–76.

Baker, C.J., Orlandi, E.W., 1995. Active oxygen in plant pathogenesis. Annu. Rev. Phytopathol. 33, 299–321.

Bindschedler, L., Dewdney, J., Blee, K., Stone, J., Asai, T., Plotnikov, J., Denoux, C., Hayes, T., Gerrish, C., Davies, D., Ausubel, F., Bolwell, G., 2006. Peroxidase-dependent apoplastic oxidative burst in Arabidopsis required for pathogen resistance. Plant J. 47, 851–863.

Bloomfield, G., Pears, C., 2003. Superoxide signaling required for development of *Dictyostelium*. J. Cell Sci. 116, 3387–3397.

Bolwell, P.G., Bindschedler, L.V., Blee, K.A., Butt, V.S., Davies, D.R., Gardner, S.L., Gerrish, C., Minibayeva, F., 2002. The apoplastic oxidative burst in response to biotic stress in plants: a three component system. J. Exp. Bot. 53, 1367–1376.

Bolwell, P.G., Blee, K.A., Butt, S.V., Davies, D.R., Gardner, S.L., Gerrish, C., Minibayeva, F., Rowntree, E.G., Wojtaszek, P., 1999. Recent advances in understanding the origin of the apoplastic oxidative burst in plant cells. Free Radical Res. 31, 137–145.

Bolwell, P.G., Wojtaszek, P., 1997. Mechanism for the generation of reactive oxygen species in plant defense––a broad perspective. Physiol. Mol. Plant. Pathol. 51, 347–366.

Buetler, T., Krauskopf, A., Ruegg, U., 2004. Role of superoxide as a signaling molecule. News Physiol. Sci. 19, 120–123.

Castoria, R., Caputo, L., De Curtis, F., De Cicco, V., 2003. Resistance of postharvest biocontrol yeasts to oxidative stress: a possible new mechanism of action. Phytopathology 93, 564–572.

Cessna, S., Sears, V., Dickman, M., Low, P., 2000. Oxalic acid, a pathogenicity factor for *Sclerotinia sclerotiorum*, suppresses the oxidative burst of the host plant. Plant Cell 12, 2191–2199.

Chan, Z., Tian, S., 2006. Induction of H_2O_2-metabolizing enzymes and total protein synthesis by antagonistic yeast and salicylic acid in harvested sweet cherry fruit. Postharvest Biol. Technol. 39, 314–320.

Coego, A., Ramirez, V., Gil, M., Flors, V., Mauch-Mani, B., Vera, P., 2005. An arabidopsis homeodomain transcription factor, overexpressor of cationic peroxidase 3, mediates resistance to infection by necrotrophic pathogens. Plant Cell 17, 2123–2137.

Davies, D., Bindschedler, L., Strickland, T., Bolwell, G., 2006. Production of reactive oxygen species in *Arabidopsis thaliana* cell suspension cultures in response to an elicitor from *Fusarium oxysporum*: implications for basal resistance. J. Exp. Bot. 57, 1817–1827.

Derouet-Humbert, E., Drăgan, C., Hakki, T., Bureik, M., 2007. ROS production by adrenodoxin does not cause apoptosis in fission yeast. Apoptosis 12, 2135–2142.

Djonović, S., Pozo, M.J., Dangott, L.J., Howell, C.R., Kenerley, C.M., 2006. Sm1, a proteinaceous elicitor secreted by the biocontrol fungus *Trichoderma virens* induces plant defense responses and systemic resistance. MPMI 19, 838–853.

Droby, S., Wisniewski, M., Macarisin, D., Wilson, C., 2009. Twenty years of postharvest biocontrol research: Is it time for a new paradigm? Postharvest. Biol. Technol. 52, 137–145.

Dunand, C., Crèvecoeur, M., Penel, C., 2007. Distribution of superoxide and hydrogen peroxide in arabidopsis root and their influence on root development: possible interaction with peroxidases. New Phytol. 174, 332–341.

Fabrizio, P., Battistella, L., Vardavas, R., Gattazzo, C., Liou, L., Diaspro, A., Dossen, J., Gralla, E., Longo, V., 2004. Superoxide is a mediator of an altruistic aging program in *Saccharomyces cerevisiae*. J. Cell Biol. 166, 1055–1067.

Horowitz, S., Yarden, O., Gollop, N., Chen, S., Zveibil, A., Belausov, E., Freeman, S., 2008. Differential protein expression in *Colletotrichum acutatum*: changes associated with reactive oxygen species and nitrogen starvation implicated in pathogenicity on strawberry. Mol. Plant Pathol. 9, 171–190.

Hu, X., Bidney, D., Yalpani, N., Duvick, J., Crasta, O., Folkerts, O., Lu, G., 2003. Overexpression of a gene encoding hydrogen peroxide-generating oxalate oxidase evokes defense responses in sunflower. Plant Physiol. 133, 170–181.

Jin, P., Zheng, Y., Tang, S., Ruia, H., Wang, C., 2009. Enhancing disease resistance in peach fruit with methyl jasmonate. J. Sci. Food Agric. 89, 802–808.

Liu, Y., Ren, D., Pike, S., Pallardy, S., Gassmann, W., Zhang, S., 2007. Chloroplast-generated reactive oxygen species are involved in hypersensitive response-like cell death mediated by a mitogen-activated protein kinase cascade. Plant J. 51, 941–954.

Macarisin, D., Cohen, L., Eick, A., Rafael, G., Belausov, E., Wisniewski, M.E., Droby, S., 2007. *Penicillium digitatum* suppresses production of hydrogen peroxide in host tissue during infection of citrus fruit. Phytopathology 97, 1491–1500.

Mellersh, D., Foulds, I., Higgins, V., Heath, M., 2002. H_2O_2 plays different roles in determining penetration in three diverse plant–fungal interactions. Plant J. 29, 257–268.

Miller, G., Mittler, R., 2006. Could heat shock transcription factors function as hydrogen peroxide sensors in plants? Ann. Bot. 98, 279–288.

Nicholson, R., Hammerschmidt, R., 1992. Phenolic compounds and their role in disease resistance. Annu. Rev. Phytopathol. 30, 369–389.

Semighini, C.P., Hornby, J.M., Dumitru, R., Nickerson, K.W., Harris, S.D., 2006. Farnesol-induced apoptosis in *Aspergillus nidulans* reveals a possible mechanism for antagonistic interactions between fungi. Mol. Microbiol. 59, 753–764.

Semighini, C.P., Murray, N., Harris, S.D., 2008. Inhibition of *Fusarium graminearum* growth and development by Farnesol. FEMS Microbiol. Lett. 279, 259–264.

Shao, N., Krieger-Liszkay, A., Schroda, M., Beck, C., 2007. A reporter system for the individual detection of hydrogen peroxide and singlet oxygen: its use for the assay of reactive oxygen species produced in vivo. Plant J. 50, 475–487.

Shetty, N., Mehrabi, R., Lütken, H., Haldrup, A., Kema, G., Collinge, D., Jørgensen, H., 2007. Role of hydrogen peroxide during the interaction between the hemibiotrophic fungal pathogen *Septoria tritici* and wheat. New Phytol. 174, 637–647.

Torres, R., Valentines, M., Usall, J., Vinas, I., Larrigaudiere, C., 2003. Possible involvement of hydrogen peroxide in the development of resistance mechanisms in 'Golden Delicious' apple fruit. Postharvest Biol. Technol. 27, 235–242.

Unger, Ch., Kleta, S., Jandl, G., Tiedemann, A., 2005. Suppression of the defence-related oxidative burst in bean leaf tissue and bean suspension cells by the necrotrophic pathogen *Botrytis cinerea*. J. Phytopathol. 153, 15–26.

Yao, H., Tian, S., 2005. Effects of a biocontrol agent and methyl jasmonate on postharvest diseases of peach fruit and the possible mechanisms involved. J. Appl. Microbiol. 98, 941–950.

Yu, T., Zheng, X., 2007. An integrated strategy to control postharvest blue and grey mould rots of apple fruit by combining biocontrol yeast with gibberellic acid. Int. J. Food Sci. Technol. 42, 977–984.

Zeng, K.F, Cao, J.K., Jiang, W.B., 2006. Enhancing disease resistance in harvested mango (*Mangifera indica* L. cv. 'Matisu') fruit by salicylic acid. J. Sci. Food Agric. 86, 694–698.

Zheng, X., Yu, T., Chena, R., Huanga, B., Wu, V., 2007. Inhibiting *Penicillium expansum* infection on pear fruit by *Cryptococcus laurentii* and cytokinin. Postharvest Biol. Technol. 45, 221–227.