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Wound age effect on the efficacy of *Candida oleophila* strain O against post-harvest decay of apple fruits

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Abstract: In the present work, wound age effect on the efficacy of *Candida oleophila* strain O against *Botrytis cinerea* was assessed on harvested apple fruits. To this end, a *C. oleophila* strain O suspension was applied to apple wounds 0, 30, 60, 120, 180 or 240 min. after wounding. One hour after treatment, wounds were inoculated with a conidial suspension of *B. cinerea* and the population of the antagonist at the wound site was recovered and quantified. After an incubation period of 7 d, the lesion diameter caused by *B. cinerea* was measured and the protective level of strain O estimated. There was no effect of the tested periods on the population size of strain O one hour after its application on apple wounds (*i.e.* moment of *B. cinerea* inoculation). In the absence of the antagonistic strain, the extent of the lesions depends on the freshness of the wound at the inoculation time. The lesion diameter of the infection was in fact smaller in fresh (0 and 30 min after wounding) than in old (60 to 240 min after wounding) wounds. In the presence of strain O, there was a significant reduction of the infection lesions regardless of the lag period between wounding and treatment. The protective level decreased with increasing time lapse between wounding and treatment, the highest protection being obtained when strain O was applied on fresh wounds. This suggests that *C. oleophila* strain O should be applied immediately after wounding. As postharvest decay of apples is mainly due to handling operations occurring between harvest and storage, an application of the antagonistic strain O as soon as possible after harvest is recommended for an optimal biocontrol of postharvest decay caused by *B.* cinerea on apple fruits.

Keywords: biological control, *Botrytis cinerea*

Introduction

Gray mould rot caused by *Botrytis cinerea* is one of the most devastating diseases of stored apple fruits. The yeast *Candida oleophila* strain O was isolated from the surface of Golden Delicious apples and selected for its greater effectiveness against this post-harvest pathogen (Jijakli et al., 1993).

Wounds inflicted to apple fruits are the primary site for infection by conidia of postharvest pathogens. Immediately after wounding, a massive and temporary generation of reactive oxygen species (ROS) was detected in apple wounds during the first four hours following wounding (Castoria et al., 2003). While moisture in fresh wounds is favourable for an optimal microbial growth, this parameter may rapidly be limiting as the wound surface dries (Mercier & Wilson, 1995). After wounding, apple fruits were found to exhibit healing by formation of wall thickenings (Lakshminarayana et al., 1987). These reported data suggest that the post-wounding prevailing conditions at the wound site are continually changing with increasing incubation periods.

In practical situations, the delays between wounding and biocontrol treatment may be of hours or even days. The behaviour of the antagonist agent after its application could thus

depend on the freshness (age) of the apple wound. Knowledge of the effect of this parameter on the efficacy of *C. oleophila* strain O against *B. cinerea* could help in the development of an effective biological control method against gray mould on postharvest apple fruits.

Materials and methods

The present work has required apple fruits (cv. Golden Delicious), a culture of the antagonist *Candida oleophila* strain O previously subcultured each day (3 times) on potato dextrose agar (PDA) at 25°C, and a 10- to 15-day-old PDA culture of the pathogen agent *Botrytis cinerea* incubated at 25°C.

Apple fruits were surface disinfected with 10% sodium hypochlorite for 2 min and rinsed with sterile water. Then, each apple was wounded 3 times, the wounds being 4 mm in diameter and about 3 to 4 mm deep. Wounds were after that treated with 40 µl of strain O suspension (10^7 cfu/ml) 0, 30, 60, 120, 180 or 240 min after wounding. For each period, the control received water only. One hour after strain O treatment, two different experiments were conducted. For the first one, a set of wounds was used for the estimation of the antagonist population (5 apples per period). Strain O cells were recovered by pipeting and then diluted-plating for a colony forming unit (CFU) assay. For the second experiment, wounds (15 apples per treatment) were inoculated with 40 µl of a conidial suspension of *B. cinerea* (10^6 conidia/ml). Fruits were stored under high humidity in enclosed plastic trays and incubated in the dark at 25°C for 7 d, after which the lesion diameter caused by *B. cinerea* infection was measured. The protective level (P) was estimated according to the formula (Dc-Da)/Dc x 100, where Dc and Da are respectively the diameter lesion of the control and treated apples.

Data were subjected to analysis of variance. Means were separated using the Duncan's multiple range test ($P \leq 0.05$) using the Statistical Analysis System (SAS) program.

Table 1. *C. oleophila* strain O population size (10^4 cfu/wound) after one hour of incubation in apple wounds

[Wounding - Strain O application] min	Strain O population (10^4 cfu/wound)
0	4.38 ± 0.20a
30	4.16 ± 0.60a
60	4.43 ± 0.50a
120	4.60 ± 0.26a
180	4.81 ± 0.64a
240	5.56 ± 0.24a

Strain O (40 µl of 10^7 cfu/ml) was applied after increasing periods following wounding (0 - 240 min). Five apples with three wounds each were used per period. Each value represents mean ± standard error. Values with the same letter are not significantly different ($P \leq 0.05$).

Results

Table 1 displays that, after one hour of incubation in apple wounds, the size of the recovered population of the applied strain O was not affected by the wounding to treatment interval time. Fig. 1 shows that the extent of the lesions caused by *B. cinerea* depends on the presence or the absence of strain O as well as on the freshness of the wounds at the treatment/inoculation time. In the controls (without strain O), the lesion diameter of the

infection caused by *B. cinerea* was significantly lower in fresh (0 and 30 min after wounding) than in old (60, 120 and 240 min after wounding) wounds. In the presence of strain O, there was a significant reduction of the infection lesions regardless of the lag period between wounding and treatment (Fig. 1).

The highest protective level was obtained when strain O was applied either immediately (0 min) or 30 min after wounding (Fig. 2). In case of wounds treated 60 to 240 min after wounding, the protection was reduced in comparison with freshly-made wounds. As the population size of strain O in apple wounds was the same whatever the moment *B. cinerea* was inoculated (Table 1), the reduction of the protective level in old wounds (60 – 240 min after wounding) may be ascribed to the important development of the infection in these conditions (Fig. 1).

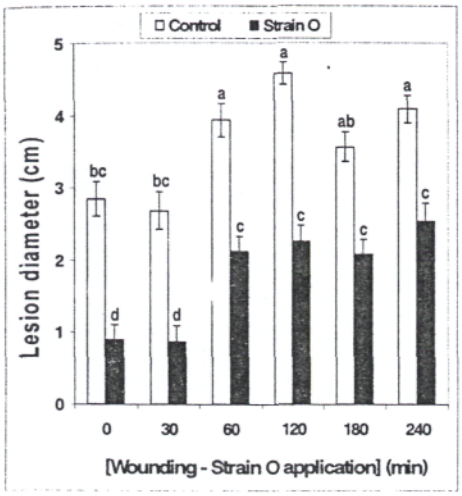

Figure 1. Lesion diameter (cm) on wounded apple fruits after an incubation period of 7 d. Each wound was first treated with 40 µl of a suspension of *C. oleophila* strain O (10^7 cfu/ ml) 0, 30, 60, 120, 180 and 240 min after wounding and then inoculation with 40 µl of a *B. cinerea* suspension at 10^6 spores/ml. Each value represents mean ± standard error. Histograms with the same letter(s) are not significantly different ($P \leq 0.05$).

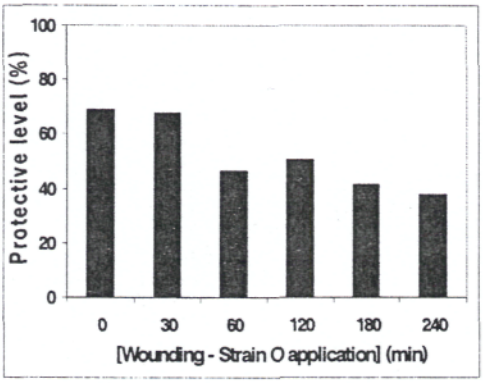

Figure 2. Protective level (%) of *C. oleophila* strain O against *B. cinerea* on wounded apple fruits after an incubation period of 7 d. Each wound was first treated with 40 µl of a suspension of *C. oleophila* strain O (10^7 cfu/ml) 0, 30, 60, 120, 180 and 240 min after wounding and then inoculation with 40 µl of a *B. cinerea* suspension at 10^6 conidia/ml.

Discussion

In our previous works dealing with the use of the yeast *C. oleophila* strain O as a potential biocontrol agent against different postharvest pathogens on apples, citrus and tropical fruits (*e.g.* banana), the most studied factors controlling the level of protection were the antagonist and the pathogen concentrations as well as the time elapsing between the application of the antagonist and the inoculation of the pathogen (Jijakli et al., 1993, 2004; Lahlali et al., 2004, 2005). In the present work, the effect of another important factor, which is the lag period between wounding and the application of the antagonistic strain O (*i.e.* wound age), on the control of gray mold caused by *B.* cinerea on apples was evaluated.

Wound age may have an impact on the resistance level of apple fruits against wound pathogens (Lakshminarayana et al., 1987) and on the wound water and oxidative status (Mercier & Wilson, 1995; Castoria et al., 2003). In our case, strain O development was not affected by the wound freshness (Table 1). However, lesions caused by *B. cinerea* were more important in old than in fresh wounds. The highest level of protection was obtained when strain O was applied immediately after wounding. In general, postharvest decay is mainly due to wounds resulting from handling operations during harvest of apple fruits. For this reason, it is recommended to apply the antagonistic strain O as soon as possible after harvest for an optimal biological control of gray mold disease caused by *B. cinerea* on apple fruits.

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