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Biological control of crown rot of bananas with *Pichia anomala* strain K and *Candida oleophila* strain O

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Abstract

The antagonistic activity of two yeast strains (*Pichia anomala* (E.C. Hansen) Kurtzman, strain K and *Candida oleophila* Montrocher, strain O) against the parasitic complex responsible for banana crown rot was evaluated. The strains were applied at three different concentrations (10^6, 10^7, 10^8 cfu/ml) and their efficacy tested *in vivo* on three separate fungi (*Colletotrichum musae* (Berk. & Curt.) Arx, *Fusarium moniliforme* Sheldon, and *Cephalosporium* sp.) and on a parasitic complex formed by association of these three fungi. At the concentrations used *C. musae* appeared to be the most pathogenic. The complex showed intermediate aggressiveness between *C. musae* and both other fungi.

Statistically significant antagonistic effects were observed on *C. musae*, *F. moniliforme*, and the fungal complex. The highest protection level (54.4%) was observed with strain O added at 10^8 cfu/ml on crowns previously inoculated with the fungal complex. The level was lower when the fungi were inoculated separately.

Furthermore, the antagonistic effect was strongly reinforced when strain O at 10^8 cfu/ml was applied 24 h before fungal complex inoculation (59.9%), as compared to its application 15 min (24.3%) or 3 h (27.3%) after fungal complex inoculation. Bananas showed increased susceptibility to the fungal complex from March to June, and this influenced the level of protection by yeast, which decreased over the same period. A strict negative correlation ($R^2 = 0.83$) was highlighted between susceptibility of banana to crown rot and protection provided by yeast.

Keywords: Banana; Biological control; *Candida oleophila*; *Cephalosporium* sp.; *Colletotrichum musae*; Crown rot; *Fusarium moniliforme*; Musa; *Pichia anomala*; Post-harvest

1. Introduction

Crown rot disease of bananas is widespread in producing countries and is considered the most important post-harvest disease of exported bananas, causing losses in consumer countries (Krauss and Johanson, 2000; Muirhead and Jones, 2000; Reyes et al., 1998). The symptoms of this post-harvest disease are not visible at packing stations in banana-growing countries. They develop later, during shipment, ripening, and storage in consumer countries. During the rainy season, losses of more than 10% have been recorded for Windward Islands bananas arriving in the UK (Krauss and Johanson, 2000). Losses as high as 86% have been observed in the case of bananas from the Philippines having undergone no chemical treatment (Alvindia et al., 2000).

This disease affects the crown, i.e. the tissues joining the fruit pedicels with each other. When infection is severe, the rot may reach the pedicel and ultimately the banana pulp. A wide range of organisms are involved in crown rot of bananas, and important variations of both the severity of the damage and the nature of the complex are observed, in particular according to the location and time of year (Hostachy et al., 1990; Slabaugh and Grove, 1982). The pathogens most frequently isolated include *Verticillium*

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theobromae (Turconi) Mason and Hughes, *Colletotrichum musae* (Berk. & Curt.) Arx. *Fusarium moniliforme* Sheldon, *Fusarium roseum* Snyder and Hansen, *Ceratocystis paradoxa* (Dade) C. Moreau, *Botryodiplodia theobromae* Pat., *Nigrospora sphaerica* (Sacc.) Mason *Cladosporium* sp., *Cephalosporium* sp., *Penicillium* sp., and *Aspergillus* sp. (Anthony et al., 2004; Greene and Goos, 1963; Griffee and Burden, 1976; Johanson and Blasquez, 1992; Lukezic et al., 1967; Marin et al., 1996; Ogundero, 1987; Shillingford, 1976; Wallbridge and Pinegar, 1975; Wallbridge, 1981). Although field infection cannot be excluded, contamination of the wounded crown occurs while hands are being harvested (Meredith, 1971).

In most banana-growing areas, crown rot is principally controlled by post-harvest fungicide treatments (de Lapeyre de Bellaire and Nolin, 1994; Johanson and Blasquez, 1992; Krauss et al., 1998), but alternative control methods, including biological control, are being sought because of (i) the emergence of resistance to some commonly used fungicides (de Lapeyre de Bellaire and Dubois, 1997; East and Kenyon, 1998; Hostachy et al., 1990; Johanson and Blasquez, 1992), (ii) environmental problems linked to dumping of fungicide mixtures used at packing stations, and (iii) consumer aversion to chemical residues in food. Post-harvest biological control is particularly promising, because the target area is limited to the fruits, environmental conditions are defined and stable during storage, and products for post-harvest treatment are a high-value market (Jijakli et al., 1999).

The identification of biological antagonists for crown rot control is recent, and their use has not been extensively studied. Yet there is evidence suggesting that it is feasible to use molds (East and Kenyon, 1998; Kanapathipillai et al., 1987; Krauss et al., 1998; Magan and Baxter, 1993; Postmaster et al., 1997; Ragazzi and Turco, 1997), yeasts (Postmaster et al., 1997), and even bacteria (De Costa and Subasinghe, 1998; De Costa and Erabadupitiya, 2005; East and Kenyon, 1998; Gunasinghe et al., 2004) for the biological control of banana post-harvest diseases caused by *C. musae*. Efficacy tests have been performed *in vitro* (De Costa and Subasinghe, 1998; Kanapathipillai et al., 1987; Krauss et al., 1998; Magan and Baxter, 1993; Postmaster et al., 1997; Ragazzi and Turco, 1997), on banana leaf discs (Postmaster et al., 1997) or peel discs (East and Kenyon, 1998), or *in vivo* on banana crown (De Costa and Erabadupitiya, 2005; Gunasinghe et al., 2004).

The micro-organisms selected for their antagonistic activity in the above-cited papers were isolated from banana material, except for the two bacteria tested by Gunasinghe et al. (2004), which were isolated from rice flour paste. In the present investigation, the antagonistic ability of two yeasts strains, *Pichia anomala* (E.C. Hansen) Kurtzman, strain K and *Candida oleophila* Montrocher, strain O, to control crown rot has been investigated. These strains were previously selected for their high and reliable antagonistic activity against post-harvest diseases of apples caused by wound pathogens (Jijakli and Lepoivre, 1993). These strains must be further characterized before considering a practical application by the apple growers.

The mechanisms of action of yeasts used as biocontrol agent have been recently reviewed by El-Tarabily and Sivasithamparam (2006). They include competition for space and nutrients, antibiosis, mycoparasitism (including the action of cell wall degrading enzymes), and finally induction of host resistance. The modes of action of *P. anomala* strain K against *Botrytis cinerea* Pers. on apple have been investigated. Competition for nutrients and implication of exo-ß-1,3-glucanases have been highlighted (Friel et al., 2007). Furthermore several genes are also suspected to play a role in the antagonistic activity of strain K (Massart and Jijakli, 2006). Competition for nutrients is the main mode of action for strain O.

The characterization of these strains include also the development of a monitoring method (Massart et al., 2005) and the study of their ecological behavior (Lahlali et al., 2008). As strain K and strain O were already extensively characterized, it is easier to asses their efficacy against crown rot disease of banana than to select novel uncharacterized micro-organisms. Conducting efficacy tests under controlled conditions is the first crucial stage of this evaluation.

The aim of the present study was to evaluate the *in vivo* antagonistic activity of these yeast strains against some individual pathogens and against a parasitic complex responsible for crown rot disease of bananas. *In vivo* tests were preferred to *in vitro* tests because they better represent what happens under real conditions (Postmaster et al., 1997; Ragazzi and Turco, 1997). The influence of yeast concentration was assessed, as was the influence of the timing of yeast treatment in relation to inoculation of crowns with the fungal complex.

2. Materials and methods

2.1. Plant material and fruit sampling

The banana cultivar used was Grande Naine (*Musa acuminata* AAA, Cavendish group). Bananas were harvested from March to June 2003 at the CIRAD experimental station (Neufchâteau) in Guadeloupe, France. As susceptibility to some post-harvest diseases depends on banana physiological age (Chillet et al., 2006), all fruits were harvested at the same physiological age of 900 °C day according to the method described by Ganry (1978). Five homogenous bunches were harvested in the morning on the day each experiment began. The 2nd and 3rd hands of these bunches were separated into clusters of four bananas, excluding the fruits at the edge. These two hands were selected for having more than 20 fruits per hand, and no observable defect. Each bunch constituted one replicate for the different treatments studied.

2.2. Artificial inoculation of crown rot agents

The clusters of four bananas were placed in tap water for 20 min, for latex elimination, before refreshing the crown surface with a knife. These cuttings were square, with regular and clean-cut sections in order to obtain similar crowns. The crowns were surface-sterilized by immersion in 50% ethanol. Three fungal pathogens frequently observed in the complex from Guadeloupe were used separately or together to inoculate the clusters, namely *C. musae*, *F. moniliforme*, and *Cephalosporium* sp. These pathogens had been isolated in Guadeloupe from different organs of the banana plant (crown rots, floral remnants) and identified as being frequently involved in the development of crown rot. They were conserved at −80 °C in glycerol solution (50%). Before use, they were grown at 25 °C on Potato Dextrose Agar (PDA) (BioMérieux, Lyon, France) for 7–10 days. Conidia were removed by flooding the plates with sterile distilled water and filtered through a 40-μm sieve. Conidia concentrations were determined with a Mallassez cell and adjusted to 10^4 conidia/ml for *F. moniliforme* and *Cephalosporium* sp. and to only 10^3 conidia/ml for *C. musae* because of its strong pathogenicity. For the parasitic complex, *F. moniliforme*, *Cephalosporium* sp., and *C. musae* were mixed and the respective final concentrations of these species were 10^4, 10^4, and 10^3 conidia/ml. One-hundred microliters of conidial suspension (containing one of the three pathogens or the fungal complex) was applied to the center of the freshly exposed crown tissue and covered with a small filter paper, which was withdrawn 15 min later. The five clusters of four bananas of the same treatment modality were packed in punched polyfilms normally used in the industry and placed in a small cardboard box (24 * 23 * 23 cm) in order to simulate commercial packing. There were as many boxes as treatment modalities. To simulate shipment, the boxes were stored on shelves in a conditioned room (15 m^3) at 13 °C for 10 days. Then artificial ripening was initiated by exposing the bananas to 1000 ppm ethylene (Azethyl, AIR LIQUIDE, France) for 24 h at 20 °C. After strong ventilation they remained at 20 °C for another 2 days before the assessment of crown rot.

2.3. Post-harvest biological treatments

Yeast strains *C. oleophila* strain O and *P. anomala* strain K were isolated by the Plant Pathology Unit of the Agricultural University of Gembloux (FUSAGx), Belgium, and stored at −80 °C in glycerol solution (50%). Before use, the yeast strains were successively subcultured on PDA at 20 °C and incubated for 24 h. Cells of the third generation were removed by flooding the plates with sterile isotonic solution (NaCl 8.5 g/l). Yeast concentration was determined by counting with a Mallassez cell. Banana crowns were immersed for 10 s in the yeast suspension. The untreated control crowns were immersed for 10 s in sterile distilled water.

2.4. Influence of yeast concentration on biological control of crown rot

Antagonistic effects of both strains (K and O) were evaluated at three different concentrations (10^6, 10^7, 10^8 cfu/ml) against each pathogen separately and against the fungal complex (four tests). Thus, for each pathogen and for the complex, eight treatments were performed on five clusters of four bananas from five different bunches. The treatments were: UC, untreated control clusters (just inoculated with the pathogens); FC, fungicide control clusters (treated with 500 mg/l thiabendazole (Mertec 20S, SYNGENTA) by submerging fruits in a bath for 1 min); K6, fruits treated with strain K at 10^6 cfu/ml; K7, fruits treated with strain K at 10^7 cfu/ml; K8, fruits treated with strain K at 10^8 cfu/ml; O6, fruits treated with strain O at 10^6 cfu/ml; O7, fruits treated with strain O at 10^7 cfu/ml; O8, fruits treated with strain O at 10^8 cfu/ml.

This experiment was repeated 6 times for each pathogen and for the complex. Yeast treatments were applied 3 h after inoculation of the clusters with the pathogens.

2.5. Influence of a yeast incubation period before inoculation of crowns

Secondly, the impact of the timing of yeast treatment on the antagonistic effect of strain O at 10^8 cfu/ml against the fungal complex was evaluated. In each experiment, five treatments were performed on five clusters of four bananas from five different bunches. The treatments were: UC, untreated control clusters (only inoculated with the pathogens); FC, fungicide control clusters (treated with thiabendazole 500 mg/l (Mertec 20S, SYNGENTA) by submerging fruits in a bath for 1 min); O-15, strain O at 10^8 cfu/ml applied 15 min after inoculation of the clusters with the complex; O-3, O strain at 10^8 cfu/ml applied 3 h after inoculation of the clusters with the complex; O-24, O strain at 10^8 cfu/ml applied 24 h before inoculation of the clusters with the complex.

This experiment was repeated 6 times.

2.6. Assessment of crown rot development and statistical analysis

Assessment of rot progression was carried out 13 days after inoculation (i.e. 3 days after ethylene treatment). The internal progression of rot from the original inoculation point was determined by cutting the crown longitudinally and measuring the surface of rot in the crown. This "internal necrotic surface" (INS) was measured and expressed in mm^2. From this result a percentage of protection was calculated according to the formula:

$$\text{Percentage of protection} = [(INS_C - INS_O)/INS_C] * 100,$$

where, INS_C = mean INS of the untreated control; INS_O = mean INS of the treatment evaluated.

In each experiment, each treatment modality was applied to five clusters from five different bunches. Each experiment was repeated 6 times, and the mean INS for these six replicates was subjected to three-way ANOVA performed by MINITAB. Mean separations were calculated using the Newman and Keuls test at 5% probability level.

A linear regression was performed between the average severity of symptoms in the untreated control and the average protection level afforded by yeast.

3. Results and discussion

3.1. Evaluation of the pathogenic capacity of the three pathogens and the complex

The agents of the complex do not have the same pathogenic capacity. Although inoculated at 10-fold lower concentration (10^3 conidia/ml), *C. musae* was by far more aggressive than *F. moniliforme* and *Cephalosporium* sp. (Fig. 1A–C). Krauss (1996), Finlay and Brown (1993), and East and Kenyon (1998) likewise report that *C. musae* is more pathogenic than *Fusarium* sp. Finlay and Brown (1993) have found *C. musae* to be 20 times more pathogenic than *Fusarium* spp. Other authors have highlighted that *C. musae* can initiate infection from a low inoculum concentration (Finlay and Brown, 1993; Greene and Goos, 1963; Lukezic et al., 1967; Shillingford, 1976) and that other pathogens require much higher inoculum concentration to induce symptoms (Finlay and Brown, 1993; Griffee, 1971; Krauss, 1996; Krauss et al., 1998). Interestingly, the rotting effect induced by *C. musae* in our untreated control was greater when the pathogen was inoculated alone than in mixture with other fungi at the same final concentration. This suggests that antagonism might occur between these three fungi. Anthony et al. (2004) found that the severity of the disease was greater when the three fungi *Lasiodiplodia theobromae, Fusarium proliferatum*, and *C. musae* were inoculated in combination on the crowns, as compared to the severity of each fungus inoculated separately. This indicates that the disease severity is a function of its complex composition and existing interactions.

3.2. Evaluation of the antagonistic effects of P. anomala strain K and C. oleophila strain O

Crown rot severity was consistently higher on the untreated control, with an average INS ranging from 81.1 mm^2 to 340.8 mm^2, than on the fungicide control whose present an average INS from 1.1 mm^2 to 14.4 mm^2. This indicates that the chosen experimental con-

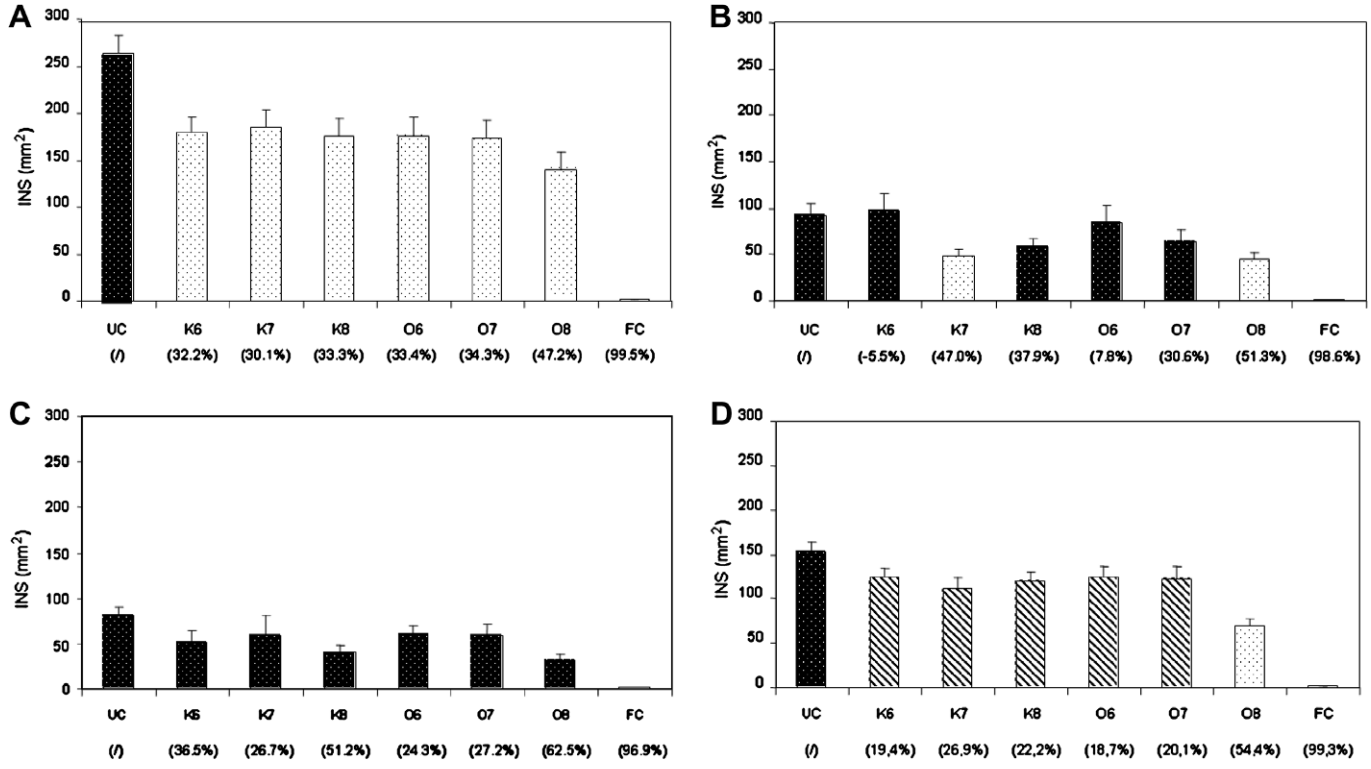

Fig. 1. Internal necrotic surface (INS) assessed on banana clusters inoculated with (A) *Colletotrichum musae* (10^3 conidia/ml), (B) *Fusarium moniliforme* (10^4 conidia/ml), (C) *Cephalosporium* sp. (10^4 conidia/ml), and (D) an artificial complex composed of these three pathogens, after treatment with *Candida oleophila* strain O or *Pichia anomala* strain K at various concentrations. Results with no statistical difference ($P > 0.05$) are represented in the same color. Values are expressed as means of six replicates, vertical bars represent standard errors. O6, O7, O8, treatment with strain O applied, respectively, at 10^6, 10^7, 10^8 cfu/ml; K6, K7, K8, treatment with strain K applied, respectively, at 10^6, 10^7, 10^8 cfu/ml; UC, untreated control; FC, fungicide control: bananas dipped in thiabendazole (500 mg/l) for 1 min. Values in parentheses represent the percentage of protection offered by each treatment.

ditions provided (i) reliable disease symptoms and (ii) sufficient resolution to detect effective control of the disease.

As compared to the untreated control, all strain-concentration combinations showed a significant and similar antagonistic effect against *C. musae*, with a percentage of protection ranging between 30.1% and 47.2% (Fig. 1A). Only strain K at 10^7 cfu/ml and strain O at 10^8 cfu/ml caused a significant reduction of INS caused by *F. moniliforme*, the respective protection levels being 47.0% and 51.3% (Fig. 1B). Against *Cephalosporium* sp., a reduced necrotic area was observed, especially at the highest concentration, but this effect was not significant (Fig. 1C). All strain-concentration combinations had an antagonistic effect against the fungal complex, particularly strain O at 10^8 cfu/ml. This treatment offered a significantly higher percentage of protection (54.4%) than the other biological treatments (Fig. 1D). None of the biological treatments controlled any of the three pathogens or their combination as much as the fungicide treatment (percentage of protection between 96.9% and 99.5%) (Fig. 1A–D).

Because in all experiments strain O at the highest concentration had the best antagonistic effect, this treatment was selected for subsequent experiments. This antagonistic concentration of 10^8 cfu/ml is frequently reported for biological control even for the biological control of crown rot in *vivo* (Gunasinghe et al., 2004). Chuang and Yang (1993) noticed that conidial germination of *C. musae* was not inhibited at an antagonist concentration below 10^8 conidia/ml. Lastly, the most effective concentration of *Burkholderia cepacia* for crown rot control was determined to be 10^{10} cfu/ml (De Costa and Erabadupitiya, 2005).

Although the optimal treatment conditions (yeast strain and concentration) varied according to the pathogen, all protection levels were close to 50%. This is consistent with the antagonistic effects against post-harvest pathogens of bananas reported by other authors. Gunasinghe et al. (2004) obtained similar percentages of protection with *Pantoea agglomerans* and *Flavobacterium* sp. at 10^{10} cfu/ml for biological control of crown rot caused by *C. musae*. De Costa and Subasinghe (1998) report, respectively, 42% and 39% inhibition of *in vitro* growth of *Fusarium* sp. and *C. musae* by bacterial antagonists. Postmaster et al. (1997), who focused on necrotic lesions caused by *C. musae* on banana leaves, report significant but less than 35% protection by three yeast strains: *Rhodotorula glutinis, Cryptococcus albidus*, and *Cryptococcus laurentii*, which were applied 48 h before the pathogen.

3.3. Influence of a yeast incubation period before inoculation of crowns

The antagonistic effect was strongly reinforced when strain O at 10^8 cfu/ml was applied 24 h before fungal complex inoculation (59.9%), as compared to its application 15 min (24.3%) or 3 h (27.3%) after inoculation (Fig. 2). In keeping with this observation, the antagonistic effect of *C. oleophila* strain O against post-harvest diseases of

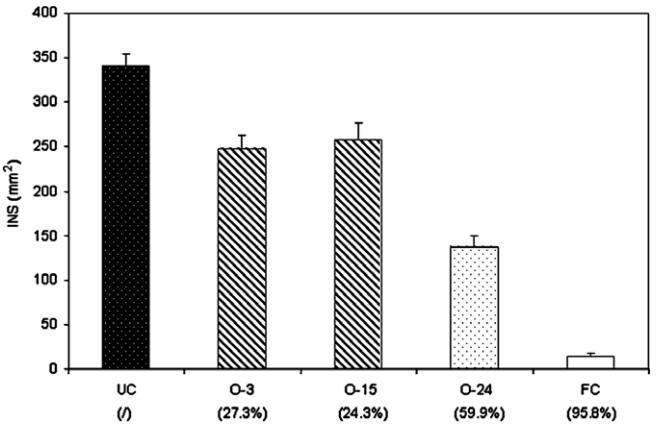

Fig. 2. Internal necrotic surface (INS) assessed on banana clusters inoculated with the fungal complex (*Colletotrichum musae* (10^3 conidia/ml), *Fusarium moniliforme* (10^4 conidia/ml) and *Cephalosporium* sp. (10^4 conidia/ml)) and treated with *Candida oleophila* strain O (10^8 cfu/ml). Statistically similar values of INS are represented in the same color. The mean INS is the result of six replicates, and standard errors are represented by vertical bars. O-24, strain O applied 24 h before the fungal complex inoculation; O-15 and O-3, strain O, respectively, applied 15 min and 3 h after the fungal complex inoculation; UC, untreated control; FC, fungicide control: bananas dipped in thiabendazole (500 mg/l) for 1 min, 3 h after the fungal complex inoculation. Values in parentheses represent the percentage of protection offered by each treatment.

apples was reinforced when this strain was applied before pathogen inoculation (Jijakli and Lepoivre, 1993). Likewise, Postmaster et al. (1997) found inhibition of *C. musae* on banana leaves to be stronger when the pathogen was introduced at least 48 h after the application of the antagonistic yeasts. In crown rot disease, it is assumed that most of the contamination occurs at harvest (Meredith, 1971), either by transfer of conidia from the stalk region via the dehanding knife into the fresh wound (Finlay et al., 1992; Greene and Goos, 1963; Stover, 1972; Wallbridge, 1981) or by contamination of the clusters in the water tanks (Shillingford, 1977). Practically, taking into account this fact, it seems difficult to apply the yeast on the crown 24 h before its contamination by the complex. However, this result indicated that the application time should be considered when devising biocontrol strategies against crown rot disease of bananas, and that the antagonist should be applied as soon as possible during the harvest process. Furthermore, the post-harvest application of the yeast treatment would reinforce the protection towards subsequent contaminations during packaging, transportation or ripening that cannot be disregarded.

3.4. Variation of the susceptibility of bananas to crown rot disease and efficiency of biocontrol

The percentage of protection offered by strain O at 10^8 cfu/ml against the complex is variable. Indeed, it was 54.4% in test 3.2 and only 27.3% in test 3.3 under the same experimental conditions. This may be related to the fluctuation of the severity of the disease on the untreated control.

As shown in Fig. 3, the INS of the untreated control inoculated with the complex increased regularly from 112.6 mm^2 to 426.6 mm^2 in the course of all experiments. These values represent, respectively, 31% and 100% of the total crown surface.

Seasonal fluctuations in crown rot severity have already been reported for situations of natural contamination (Griffee, 1971; Lukezic et al., 1967; Shillingford, 1978), but the present investigation is the first report of such fluctuations in the case of artificial contaminations.

Such seasonal fluctuations of the severity of banana anthracnose caused by *C. musae* have been linked to a parameter called the "fruit quality potential" (Chillet and de Lapeyre de Bellaire, 1996a), defined as a combination of (i) a physiological component, which determines fruit susceptibility to banana anthracnose, and (ii) a parasitic component, e.g. the level of fruit contamination by the pathogen *C. musae*. Variations of these parameters depend on climatic and edaphic conditions and on cultural practices. Taking into account these two components in the crown rot case, the present results show that the development of crown rot at the commercial level does not depend solely on the parasitic component, but also on the physiological state of the fruit at harvest, which determines its susceptibility to the fungal complex. In this work, the parasitic component was constant and controlled, while the edaphic conditions can be assumed to be reasonably constant although not actively controlled. So, different climate conditions were probably involved in the fluctuations of fruit susceptibility to crown rot. These experiments show that the effectiveness of *C. oleophila* strain O in controlling crown rot of bananas depends on the severity of the disease. From March to June 2003, a strong negative correlation ($R^2 = 0.83$) (DF:11; $p < 0.001$) was observed between the severity of symptoms in the untreated control and the protection level afforded by strain O, used at 10^8 cfu/ml (Fig. 4). The more susceptible bananas to crown rot, the more severe symptoms and the lower the protection level provided by yeast (expressed as a percentage of protection).

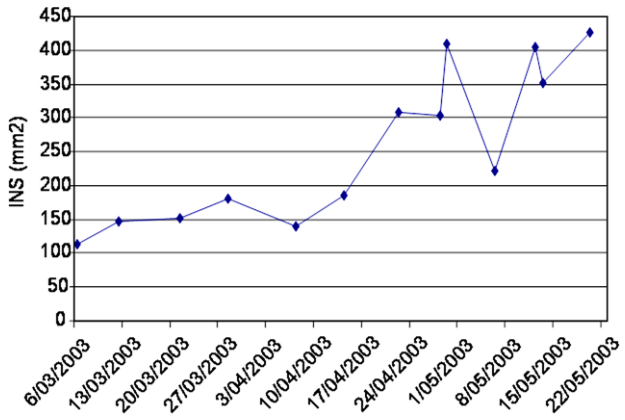

Fig. 3. Variation of the internal necrotic surface (INS) assessed on untreated clusters inoculated with the fungal complex composed by *Colletotrichum musae* (10^3 conidia/ml) *Fusarium moniliforme* (10^4 conidia/ml) and *Cephalosporium* sp. (10^4 conidia/ml) in the course of all experiments carried out between 06/03/03 and 22/05/03.

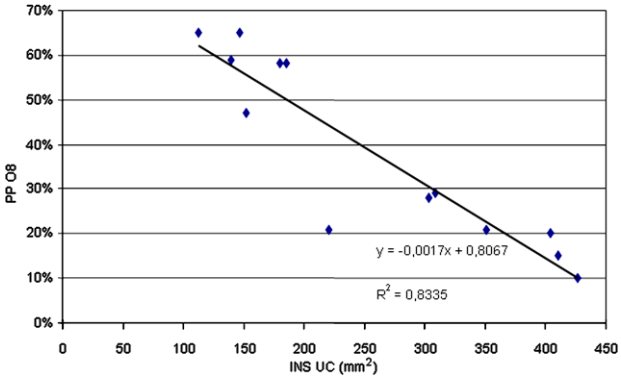

Fig. 4. Protection level (PP O8) afforded by *Candida oleophila* strain O, used at 10^8 cfu/ml, as a function of the internal necrotic surface (INS) measured in untreated clusters (UC) inoculated with the artificial fungal complex composed by *Colletotrichum musae* (10^3 conidia/ml) *Fusarium moniliforme* (10^4 conidia/ml) and *Cephalosporium* sp. (10^4 conidia/ml).

4. Conclusion

Although the fungal complex used in these experiments was artificial and included only three pathogens, the observed levels of protection against *C. musae*, and *F. moniliforme* suggest that the use of *C. oleophila* strain O for the biological control of crown rot disease of bananas is feasible but must be improved. In our study, the level of protection obtained was variable. It did not reach the efficacy of fungicide control, and a concentration of 10^8 cfu/ml was required to achieve an average 50% protection. In order to enhance further the protection level of yeast treatments, other additional techniques might be considered and combined.

The use of antagonist mixtures is likely to increase the protection level (Janisiewicz and Korsten, 2002; Krauss and Johanson, 2000; Krauss et al., 2001). This could be particularly interesting in the case of crown rot disease control, because the composition of the complex varies considerably, and a single strain cannot be expected to perform equally against all possible compositions of the fungal complex.

The yeast formulation may also be improved in order to reinforce the efficacy of treatment and the stability of the antagonistic effects. For the antagonist used in this study, *C. oleophila* strain O, it has been showed that addition of calcium gluconate can reinforce the protective effect against *Penicillium expansum* on apples (Jijakli and Creemers, 2006). Other additives such as nutritional components (Janisiewicz et al., 1992), an emulsion of a film-forming antitranspirant (Jijakli et al., 1993), chitosan (Bautista-Banos et al., 2003, 2006), glycolchitosan (El-Ghaouth et al., 2000b), calcium in different forms (Ippolito et al., 2005), sodium bicarbonate (Droby et al., 2003; Ippolito et al., 2005), or 2-deoxy-D-glucose (El-Ghaouth et al., 2000a) are also useful in order to improve the efficacy of

biological control of post-harvest diseases with yeast antagonists. For example, the use of sodium bicarbonate incorporated into a wax coating with *C. oleophila* (2×10^8 cfu/ml) is a commercially acceptable alternative to chemicals for post-harvest control of anthracnose of papaya during storage (Gamagae et al., 2004). Biological control of post-harvest diseases of bananas with natural plant compounds (Anthony et al., 2004; Demerutis et al., in press; Ranasinghe et al., 2002, 2005; Thangavelu et al., 2004), antioxidants (Khan et al., 2001), and inorganic salts alone (Alvindia et al., 2004) or in combination with surfactant (Alvindia and Natsuaki, 2007) has also been studied. The combination of such compounds with the yeast treatment could also enable an improvement of the biological control.

Physical methods, such as a modified atmosphere (Karabulut et al., 2001; Karabulut and Baykal, 2004) or a hot water treatment (De Costa and Erabadupitiya, 2005; Karabulut and Baykal, 2004; Reyes et al., 1998), can also be combined with an antagonist. De Costa and Erabadupitiya (2005) have even gained nearly 100% control by combining antagonistic bacteria, at 10^{10} cfu/ ml, with hot water treatment. It has been shown that modified atmosphere packaging has an inhibitory effect on anthracnose lesions due to *C. musae* (Chillet and de Lapeyre de Bellaire, 1996b) and that modified atmosphere has already been used to enhance *Candida* spp. efficacy (Karabulut et al., 2001). Then, since bananas are commonly shipped in modified atmosphere or controlled atmosphere, it would be reasonable to combine the yeast with this treatment.

Once the antagonistic activity has been reinforced in controlled conditions, it is essential to perform tests under natural conditions of infestation and through real exportation to assess the real protection afforded by the biocontrol strategy. Our experiments were carried out under severe conditions, through artificial inoculations especially with *C. musae* which is the most pathogenic species. Such situations are seldom encountered in practical conditions and the protection level could be better under natural infestation. Nevertheless, how natural conditions will affect the "Banana-Strain O-Parasitic complex" system and the protection efficacy of the yeast remain until now unpredictable.

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