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Preharvest application of beneficial fungi as a strategy to prevent postharvest mycotoxin contamination: A review

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ABSTRACT

The continuous pursuit of food quality, the need to feed an increasing global population and the legislative requirement to reduce the input of chemicals in the environment and in agriculture are seen as a very pressing “sword of Damocles”, all highlighting the importance of the discovery and development of alternative strategies to guarantee food security. Beneficial fungi, both filamentous fungi and yeasts, are well-known as potential biocontrol agents for use in crop protection, as part of integrated or biological strategies, and represent one of the possible solutions to these needs.

Among all the possible plant pathogens potentially affecting crops, mycotoxigenic fungi are considered as the main threat to food security, as they are present as contaminants in the main sources of nutrition for the population worldwide. Reducing mycotoxin contamination in produce during postharvest is one of the greatest challenges. The prevention of mycotoxigenic plant pathogens, mostly during preharvest, is considered as a valid strategy to reduce the risks associated with the mycotoxin contamination of processed food and feed.

We present an overview of the possible applications of beneficial fungi in the preharvest of cereals, grapes and apples, in order to control the attack of these crops by important mycotoxigenic plant pathogens such as *Fusarium*, *Aspergillus* and *Penicillium* spp., respectively. We also examine the effects of these applications in terms of reduction of the risk of mycotoxin contamination such as trichothecenes (*Fusarium*), aflatoxins and ochratoxins (*Aspergillus*) and patulin (*Penicillium*), at a postharvest stage. Finally, the use of modern technologies, such as Next Generation Sequencing (NGS), is also discussed in terms of improving the success of beneficial fungi in preventing mycotoxin contamination.

1. Beneficial fungi and biocontrol strategy: an introduction

Through the EU Directive 2009/128/EC, the European Commission recently set rules for the sustainable use of pesticides to reduce the risks and impacts of pesticide use on people's health and on the environment. One of the key features of the Directive is that “each Member State should develop and adopt its National Action Plan and set up quantitative objectives, targets, measures and timetables to reduce risks and impacts of pesticide use on human health and the environment, and to encourage the development and introduction of integrated pest management and of alternative approaches or techniques in order to reduce dependency on the use of pesticides”. There is a strong signal also from agro-industries. Many companies, whose activities have historically focused on the development, registration and commercialization of chemical pesticides are now acquiring small companies that are already developing or have the potential to develop new biopesticides based on beneficial microorganisms as active principles (Jensen et al., 2016).

1.1. Beneficial fungi

Beneficial fungi (including both filamentous fungi and yeasts) represent a large group of “good” organisms which, due to their ability to interfere with the growth of plant pathogen and their positive effects on plants, are considered as potential bioactive principles of biopesticides (Sarrocco et al., 2015a, 2015b; Sarrocco, 2016). Several fungal species interact with plant pathogens using mechanisms such as mycoparasitism, competition, antibiosis or the induction of resistance. Mycoparasitism is the direct attack of one fungus by another. This is a very complex process frequently involving three sequential events: recognition, attack and subsequent penetration and killing of the host (Fig. 1). Competition occurs when two or more organisms require the same resource which, when used by one of the two organisms, reduces its availability for the other. In addition to nutrients, competition can also occur for space or infection sites, and it is well known that competition between pathogens and beneficial organisms is an important issue in biocontrol. Antibiosis, which consists in the production and

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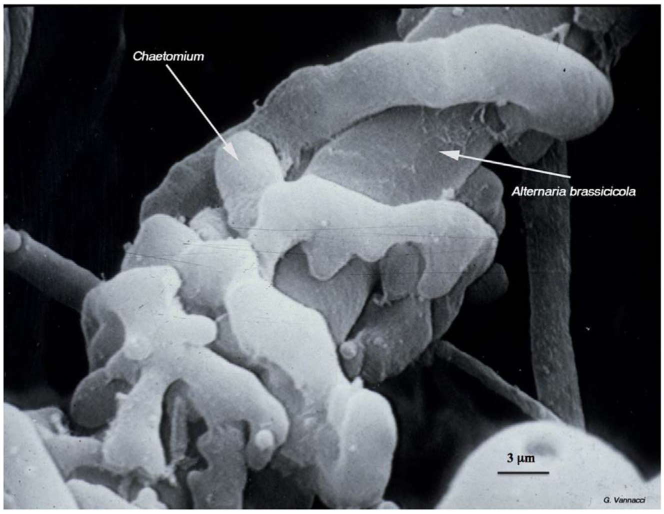

Fig. 1. Coilings of *Chaetomium* sp. around *Alternaria brassicicola* hyphae. Coilings are one of the typical signs of mycoparasitism.

release of molecules that can poison or kill other microorganisms, can be effective in the control of plant pathogens. Fungi produce a wide variety of these toxic substances, which can often help to colonize and maintain the possession of a substrate. Finally, beneficial fungi actively colonize plants, thus stimulating host defense responses and enhancing resistance against plant pathogens.

Several microbial pesticides are commercially available globally and are included both in biological crop protection programs and in Integrated Pest Management (IPM) strategies, the latter in combination with reduced rates of chemical products (Fravel, 2005). Resistance to chemical fungicides in a key feature for the practical use of a beneficial isolate. The combined use of a resistant organism with a fungicide should prove useful, and could limit competition derived from the fungal population naturally living in the substrate (Cristani et al., 1995). The use of beneficial fungi in combination with compatible fungicides can also have an additive effect if the fungicide and the organism both target the same pathogen(s) (Jensen et al., 2016). The application of tolerant or resistant beneficial fungi in combination with fungicides can be a winning strategy for the treatment of seeds, by providing high protection early by the chemical and subsequently by the biological action during seedling development (Whipps and Lumsden, 2001).

Trichoderma is historically one of the most studied beneficial fungi (Sarrocco et al., 2009; Lorito et al., 2010; Shoresh et al., 2010; Baroncelli et al., 2015; Fiorini et al., 2016; Sarrocco et al., 2017), representing one of the main used active principles of commercial biopesticides (Whipps and Lumsden, 2001) (Table 1). These fungi seem to be well suited for IPM as they are resistant to many chemical pesticides.

In addition to *Trichoderma*, which are mainly present in soil, researchers are investigating underexplored ecological niches to find new species and genera or to identify novel isolates able to act against plant pathogens (Doveri et al., 2010, 2012). The "novel" beneficial fungi include *Piriformospora indica*, which promotes adventitious root formation in cuttings, increases drought tolerance and induces the production of gibberellins which modulate plant defense in the roots (Deshmukh et al., 2006; Schafer et al., 2009).

1.2. Control of mycotoxigenic plant pathogens

Within the wide scenario of plant pathogens, controlling mycotoxigenic fungi is critical, since they are considered as among the most dangerous threat in terms of food safety. Mycotoxins are low-molecular-weight toxic compounds, resulting from fungal secondary metabolism, naturally occurring in approximately 25% of the global food and feed crop (FAO, 2003; Moretti and Sarrocco, 2015). At least 300–400 mycotoxins - characterized by a wide range of biological activities - are known, with aflatoxins, deoxynivalenol, zearalenone, fumonisins and ochratoxin A as the most common ones (Berthiller et al., 2007). These compounds are mainly produced by the *Aspergillus*, *Penicillium* or *Fusarium* species (Sweeney and Dobson, 1998). Contamination can occur in the field before harvest, as it is heavily dependent on environmental factors, and also during postharvest as a result of improper storage (Bryden, 2012; Battilani et al., 2009).

Many different chemical, physical and biological methods have been developed to control mycotoxigenic pathogens and to prevent the risks associated with mycotoxin contamination, both at pre- and post-harvest stage, or to decontaminate or detoxify mycotoxins accumulated in food and feed (Tsitsigiannis et al., 2012).

This paper concerns the use of beneficial fungi at the preharvest stage as a strategy to prevent mycotoxin contamination during post-harvest, mainly focusing on cereals and fruit trees. Table 2 summarizes some of the organisms described in the text together with their beneficial effects.

2. Prevention of mycotoxins in cereals

A staple food constitutes the dominant part of the diet of the world's population and supplies a major proportion of energy and nutrient needs. Of the more than 50,000 edible plant species in the world, just 15 crop plants provide 90% of the world's food energy intake, with three - rice, corn and wheat - making up two-thirds of this 90%. These cereals are the staple foods of over 4000 million people. In Western Europe cereals represent 26% (in terms of energy) of the main staple foods, while in the African diet, cereals represent 46% (FAO, 2017).

Cereals are very often targets for mycotoxigenic fungi, which cause severe crop losses in the field and a quality reduction in harvested crops due to the accumulation of mycotoxins. However, mycotoxin production continues after harvest, thanks to the growth of the inoculum present in caryopses and improper storage conditions (Magan et al., 2010). We discuss examples of how beneficial fungi can be applied during cultivation in order to reduce mycotoxin accumulation in the resulting food and feed commodities. We focus on Fusarium Head Blight (FHB) on wheat, with trichothecene contamination as the most serious consequence, and *Aspergillus* infection of maize connected with aflatoxin production.

2.1. Fusarium Head Blight on wheat

Wheat provides 20% of all calories consumed by people worldwide and also provides a significant contribution to animal feed. Demand for wheat is predicted to increase in line with the global population increase, and production will have a crucial bearing on food security and the global economy. The World Bank estimated that global wheat production would need to increase by 60% between 2000 and 2050. In addition, farmers around the world will need to increase wheat production using constrained resources such as fertilizers, pesticides and water.

Fusarium Head Blight is a worldwide re-emerging disease of wheat and other small grains caused by several fungal species, with *Fusarium graminearum* Schwabe and *Fusarium culmorum* (W. G. Smith) Saccardo as the most prevalent causal agents (Parry et al., 1995; Champei et al., 2004; Walter et al., 2010). Infection of wheat heads by *Fusarium* spp (Fig. 2). alters both the grain yield and the quality, whereas trichothecenes, particularly deoxynivalenol (DON) and its acetylated derivatives, 3- and 15-acetyl-DON, are the most frequently encountered mycotoxins associated with this disease (Bottalico and Perrone, 2002; Desjardins, 2006).

The early detection and control of trichothecene-producing *Fusarium* spp. is crucial to prevent toxins from entering the food chain (Wagacha and Muthomi, 2007). There are various strategies for