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Managing and monitoring of *Aspergillus flavus* in corn using bioplastic-based formulations

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ABSTRACT

In this study, we evaluated the feasibility of bioplastic-based formulations for delivering a non-aflatoxigenic strain of *Aspergillus flavus* and for monitoring Aspergilli with the final objective of controlling aflatoxin contamination in corn. Field application of inoculated bioplastic granules showed a rapid shift in the composition of soil *A. flavus* population, with a significant decrease in relative abundance of indigenous aflatoxigenic isolates. Application of bioplastic granules at 30 kg ha^{-1} was more efficient in replacing aflatoxigenic isolates than a 15 kg ha^{-1} dosage. In the test plots evaluated, aflatoxin contamination levels at corn maturity were 4.4 and 28.9 ng g^{-1} for the 2009 and 2010 field seasons, respectively. However, the biocontrol formulation was effective in reducing aflatoxin contamination in both years. More precisely, soil application of 15 and 30 kg ha^{-1} of bioplastic granules reduced aflatoxin contamination by 59 and 86% in 2009, and 80 and 92% in 2010, respectively.

1. Introduction

Aflatoxins refer to a group of mycotoxins mainly produced by the filamentous fungi *Aspergillus flavus* and *Aspergillus parasiticus* (Yu et al., 2008). Since the discovery of aflatoxins in the early 1960s, numerous studies have focused on the ecology of *A. flavus* in the agro-ecosystem (Wicklow et al., 1998; Abbas et al., 2008; Accinelli et al., 2008). Some of the crops most susceptible to aflatoxin contamination include peanuts, cotton and corn (Scheidegger and Payne, 2003). The severity of aflatoxin contamination is largely determined by the environment, with pre-harvest contamination being favored under drought and temperature stress (Payne, 1992, 1998; McGee et al., 1996; Abbas et al., 2007). *A. flavus* is a ubiquitous fungus readily isolated from diverse environments, with soil and plant tissues or residues being the natural habitat of this fungus (Geiser et al., 2000; Horn, 2003; Abbas et al., 2009). Since soil serves as a reservoir for primary inoculum for the infection of susceptible crops, most studies have focused on the occurrence and quantification of Aspergilli propagules in soil (Horn and Dorner, 1998; Zablotowicz et al., 2007; Accinelli et al., 2008). During the last two decades, several pre-harvest strategies for reducing aflatoxin contamination have been proposed (Abbas et al., 2009). An innovative approach is a biocontrol strategy consisting in the use of non-aflatoxigenic isolates of *A. flavus* to competitively exclude indigenous aflatoxigenic Aspergilli (Brown et al., 1991; Abbas et al., 2006). A 4-year study conducted in the Mississippi Delta demonstrated that soil application of wheat grains inoculated with the non-aflatoxigenic strain *A. flavus* NRRL 30797 was successful in reducing aflatoxin contamination in corn (Abbas et al., 2006). Subsequent laboratory studies showed that grains can be efficiently replaced by granules made of the bioplastic Mater-Bi® (Accinelli et al., 2009). In addition to having a favorable environmental profile, the starch-based bioplastic matrix promotes vigorous growth and sporulation of the fungus, thus facilitating soil colonization. This property can also have other practical implications. For instance, it can be expected that the starch-based bioplastic would serve as a baiting material for isolation of Aspergilli. In this two-year study, we investigated the feasibility of bioplastic granules entrapping propagules of the non-aflatoxigenic isolate *A. flavus* NRRL 30797 for controlling aflatoxin contamination in corn. We also monitored the soil Aspergilli population and corn kernel infestation using conventional protocols and a novel bioplastic-based formulation specifically developed for facilitating recovery of Aspergilli from environmental samples.

2. Materials and methods

2.1. Preparation of inoculated bioplastic granules

The non-aflatoxigenic strain *A. flavus* NRRL 30797, isolated in the Mississippi Delta in 2001, was selected for this study. Detailed

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properties of strain NRRL 30797 are described elsewhere (Abbas et al., 2006; Abbas et al., 2011). The fungus was grown and maintained on acidified potato dextrose agar (PDA). After incubation for two weeks at 37 °C, spores were removed by gently scraping PDA plates and suspended in aqueous 0.2% Tween 20. Density of spore suspensions was determined using a hemocytometer and adjusted as necessary. Spores were then entrapped into spherical granules (diameter 3 mm) made of the bioplastic Mater-Bi®(MB) type PE01S (Novamont S.p.A., Novara, Italy), following the procedure described in Accinelli et al. (2009). Briefly, bioplastic granules were equilibrated at room temperature with a concentrated spore suspension (1:1.25 w/v) with rotary shaking at 300 rpm. After 4-h shaking, spore suspensions were forced through granules by pressure (60 kPa) and granules were dried at 40 °C. Inoculated granules were stored at room temperature for no longer that one week. Potency of the final product (number of viable spores entrapped in granules) was determined by plate count. Granules were transferred to centrifuge tubes (3 granules/tube) containing 10 ml of phosphate buffer saline (PBS) and glass beads. After vortexing for 3 min, tubes were shaken at 300 rpm for 1 h. Suspensions were serially diluted in PBS and 100 μl aliquots were plated onto modified dichloronitroaniline rose bengal agar (MDRBA; Abbas et al., 2004b). Colonies were enumerated after 7–10 days of incubation at 37 °C.

2.2. Site description and experimental design

The study was carried out during two consecutive years (2009–2010) at the experimental farm of the University of Bologna (Bologna, Italy). For each year, experiments were conducted in single adjacent 1.5-ha corn fields. Physico-chemical properties of soil of the two selected fields have been reported elsewhere (Accinelli et al., 2002). Experiments were conducted using a completely randomized block design with three replicates. Each experimental unit consisted of a 600-m^2 area (30 m × 20 m) surrounded by a 10-m wide buffer zone. Experimental treatments were the following: inoculated bioplastic granules at the rate of 15 and 30 kg ha^{-1}, and an untreated control. A conventional corn hybrid (Pioneer Hi-Bred PR31K18) was planted on 16 April 2009 and 12 April 2010. Fields were managed according to ordinary practices of the region. Bioplastic granules were uniformly spread by hand on the ground surface of each plot at corn growth stage V4 (Ritchie and Hanway, 1982). Corn was harvested on 25 August 2009 and 27 August 2010. A total of 60 ears were randomly collected from each plot, shelled and dried at 50 °C for 72 h and ground (<1 mm) for chemical analysis.

2.3. A. flavus population

Size (propagule density) of the *A. flavus* soil population and relative abundance of non-aflatoxigenic isolates were measured over the two corn-growing seasons. At each sampling time, three surface (0–10 cm) soil samples were collected from each plot. Samples were sieved through a 4-mm sieve and stored at 4 °C until processed. Soil moisture was determined gravimetrically. Enumeration of *A. flavus* propagules was performed following the procedure described in Abbas et al. (2004b) with minor modifications. Briefly, 10 g of soil were suspended in a 90-ml water agar solution (0.2%), vortexed for 3 min, and shaken for 1 h at 300 rpm. Suspensions were used for preparing ten-fold serial dilutions in PBS, with 100-μl aliquots plated onto MDRBA and incubated at 37 °C for 7–10 days. Ten colonies were randomly selected and subculture on PDA at 28 °C. After seven days of incubation in the dark, aflatoxin-producing isolates were identified as colonies that displayed blue fluorescence during exposure to UV light (365 nm). At crop maturity, in addition to ears collected for chemical analysis, 10 more corn ears were collected as describe above and used for microbiological analysis. After drying at 50 °C for 72 h, kernels were removed from ears, and a randomly selected number of kernels were surface sterilized and plated onto MDRB agar. After incubation for 7–10 days at 37 °C, kernels showing *A. flavus* infection were recorded and a selected number of isolates were assessed for their potential to produce aflatoxins after UV-exposure (Abbas et al., 2004b).

2.4. Recovering Aspergilli using a bioplastic-based bait formulation

In addition to the cultivation-based method a bioplastic-based baiting system was developed. For baiting Aspergilli, MB bioplastic was shaped as rods (diameter 2 mm, length 40 mm; MB type ZF03U/A) and granules (diameter 3 mm; MB type PE01S). Autoclaved rods and granules were infiltrated with sterile MDRB by suspending them in the broth for 3 h at 300 rpm and 40 °C, and drying under a laminar flow hood for 2 h. Rods and granules were then stored at 4 °C until use. The soil incubation study was carried out using soil collected at corn maturity from the field selected in 2010. Triplicate surface samples (0–10 cm) were collected from each plot, sieved at 4 mm and 25 g (air-dried weight equivalents) were weighed in 50-ml sterilized screw-top tubes and moisture adjusted to the gravimetric content at −33 kPa. Rods (4 rods per sample) were then inserted into the soil mass and samples incubated in the dark at 28 °C. After 14 days of incubation, rods were aseptically removed and processed to quantify Aspergilli DNA by quantitative PCR (qPCR). Rods were cut in four equal parts and processed as described in Accinelli et al. (2009). Briefly, rod parts were dried at 40 °C for 2 h under a laminar flow hood, transferred to 2-ml centrifuge tubes, vortexed for 5 min to remove adhering soil particles and air-flushed by high-pressure air. Each dried rod fragment was transferred to a 2-ml microcentrifuge tube containing 500 μl of CTAB buffer and glass beads. After vortexing for 2 min, tubes were incubated at 65 °C for 15 min, and an equivalent volume of chloroform:isoamyl alcohol (24:1, v:v) was added to tubes. Tubes were gently shaken and centrifuged at 10,000*g* for 5 min before the addition of 2/3 volume of isopropanol/7.5 M ammonium acetate to precipitate the DNA. The pellet was rinsed with 70% ethanol, air dried and resuspended in 100 μl of TE buffer.

Ten ears per plot were randomly collected at maturity from the same field and used for baiting Aspergilli from kernels. Twenty grams of dried kernels were weighed in 50-ml sterilized screw-top tubes containing 10 MRB-infiltrated bioplastic granules. After vortexing the tubes for 5 s, tubes were incubated at 28 °C in the dark for 10 days, granules were aseptically removed and used for DNA isolation to conduct qPCR analysis as described above. Remaining granules were used for assessing the potential of baited isolates to produce aflatoxin. A single granule was transferred to a test tube containing 2 ml of yeast extract sucrose broth and incubated at 30 °C. After incubation for seven days in the dark without shaking, cultures were extracted with chloroform. Chloroform was then evaporated to dryness under vacuum and residues redissolved in methanol/H_2O (70:30, v:v). Dry weight of mycelium was determined after drying the mycelia mats for 48 h at 70 °C. A selected number of isolates (42) recovered from the surface of bioplastic granules were transferred to PDA plates and used for DNA sequencing. The experiment was repeated using 25 samples of corn kernel provided by two private laboratories (AGER Bologna and Caip Bologna-Modena). Aflatoxin concentration of these samples was determined by using the same HPLC method described below.

2.5. DNA analysis

Amplification was carried out in a total volume of 25 μl containing 2 μl of DNA, 12.5 μl of 2× TaqMan Universal PCR Master Mix

(Applied Biosystems Inc., Foster City, CA), and 0.2 μM of each of the primer pair *omtB*-F and *omtB*-R (Kim et al., 2008). Thermocycling conditions were as follows: 2 min at 50 °C, 10 min at 95 °C, and 40 cycles of 15 s at 95 °C and 1 min at 60 °C. The resulting samples were analyzed using an ABI Prism 7700 Sequence Detection System (Applied Biosystem Inc.). After quantification, amplified fragment samples were subjected to melting-curve analysis. A standard curve was generated by plotting cycle threshold values (Ct) against logarithmic-transformed amounts of target DNA obtained from 10-fold dilutions of DNA isolated from soil treated with spore dispersions of *A. flavus* NRRL 30797.

As described above, a randomly selected number of fungal isolates recovered from the surface of rods and granules used to bait Aspergilli from soil and corn kernels, respectively, were used for sequencing of the internal transcribed spacer (ITS) region of rDNA. After subculturing on PDA, and extracting total DNA, the ITS region was amplified following the procedure described in Accinelli et al. (2008). After clean-up with ExoSAP-IT (USB Co., Cleveland, OH), PCR products were sequenced using the ABI Dye Terminator Cycle Sequencing Ready Reaction Kit and analyzed using an ABI 3730XL automated sequencer (Applied Biosystems Inc.). Sequences were aligned using the software DNAMAN (Lynnon Co., Quebec, Canada) and deposited in the NCBI GenBank with accession numbers from HQ844675 to HQ844716.

2.6. Chemical analysis for quantifying aflatoxin concentration

Samples of 30 g of ground corn were extracted with 100 ml of methanol/water (70:30). After shaking overnight, samples were centrifuged at 5000 g for 10 min and a 5 ml aliquot of the supernatant was evaporated under N_2 followed by reconstitution of the residue in methanol/water (70:30). Sample clean-up was performed using a modification of Sobolev and Dorner (2002). Briefly, an aliquot (800-μl) of reconstituted sample was cleaned using a 1.5 ml extract-clean reservoir minicolumn packed with aluminum oxide (Alltech Co., Deerfield, IL). After elution by gravity, 20 μl of the eluate was injected on a HPLC system equipped with a Nova-Pak C18 column (150 × 3.9 mm, 4 μm) and a 2475 multi-wavelength fluorescence detector (Waters Inc., Macclesfield, UK). Separation was carried out at 30 °C, with a mobile phase consisting of water:methanol:1-butanol (60:25:1) and a flow rate of 0.9 ml min^{-1}. Detection of aflatoxins was achieved by setting the detector wavelength at 365 nm (excitation) and 440 nm (emission). Data are expressed as total of aflatoxins (aflatoxin B1, B2, G1 and G2).

2.7. Statistical analysis

Data were subjected to analysis of variance. Mean values were compared using Tukey's HSD test and significant differences were detected at the $P = 0.05$ level. Soil *A. flavus* populations are presented as log (10) transformed colony forming units (cfu) g^{-1} dry weight.

3. Results and discussion

3.1. A. flavus population

Size and dynamic of the soil *A. flavus* population during the two crop seasons is summarized in Table 1. Plate-count data indicate that size of the indigenous *A. flavus* population remained stable in untreated soil of the field selected in 2009, with an average value of 3.1 log cfu g^{-1}. Similar values were observed in 2010 in the nearest field, during the first two months after application of inoculated granules. However, contrary to what observed in 2009, *A. flavus* propagules significantly increased during the last month of the 2010 corn-growing season. In both fields, approximately half of the indigenous *A. flavus* isolates were capable of producing aflatoxin, with no significant changes during the corn-growing season (Table 1). Although aflatoxin contamination of corn is becoming a problem in the Mediterranean basin, surprisingly information on the abundance of aflatoxigenic isolates in agricultural soils of Italy is scarce (Piva et al., 2006; Giorni et al., 2007). Research has shown that the soil population level of *A. flavus* under corn cultivation can vary from 2.3 to >5.5 log cfu g^{-1} soil (Abbas et al., 2004a, 2009; Zablotowicz et al., 2007). Our findings are somewhat lower than those found in warm areas of the USA (Abbas et al., 2006; Accinelli et al., 2008). It is widely recognized that soil is a primary reservoir of *A. flavus* (Abbas et al., 2009). In addition, McGee et al. (1996) demonstrated that the size of the soil *A. flavus* population increases during epidemic years, which are characterized by high air temperature and drought conditions (Walker and White, 2001; Scheidegger and Payne, 2003; Abbas et al., 2007). As shown in Fig. 1, temperatures and rainfall recorded during the 2009 corn-growing season were not conducive to intense growth of the fungus. In contrast, rains that occurred in mid August 2010 likely stimulated mold growth. This is consistent with the nearly constant level of *A. flavus* propagules observed in untreated soil during the first year and with the increasing number of propagules recovered at corn maturity in 2010.

Table 1
Soil *Aspergillus flavus* population and percentage of aflatoxigenic isolates recovered from untreated plots and plots receiving 15 and 30 kg ha^{-1} of inoculated bioplastic granules.

	Propagule density (log 10 cfu g^{-1})				Aflatoxigenic isolates (%)			
	May[a]	June	July	August	May[a]	June	July	August
2009								
Untreated	3.11a[b]	3.13a	3.10a	3.17a	38a	43a	45a	47a
15 kg ha^{-1}	3.09a	3.35b	3.64b	3.69b	41a	35b	27b	20b
30 kg ha^{-1}	3.12a	3.38b	3.46c	3.58c	43a	37b	25b	12c
2010								
Untreated	3.10a[b]	3.09a	3.11a	3.41b	38a	41a	39a	37a
15 kg ha^{-1}	3.12a	3.39b	3.42b	3.67b	43a	26b	23b	16
30 kg ha^{-1}	3.18a	3.41b	3.65c	3.79c	36a	29b	21b	9c

[a] Soil samples were collected one day before granules application.
[b] Values within a column followed by the same letter are not significantly different ($P > 0.05$).

Application of bioplastic granules entrapping spores of the non-aflatoxigenic strain *A. flavus* NRRL 30797 (potency ~ log 7.0 cfu g^{-1}) led to a rapid increase in the number of soil *A. flavus* propagules (Table 1). Specifically, one month after granule application, significantly more *A. flavus* propagules were recovered from soil in both fields. By July, and through the course of the experiment, *A. flavus* propagules remained significantly higher in plots treated with 30 kg ha^{-1} compared to the lower application rate (15 kg ha^{-1}). This tendency was more evident in 2010.

Other than promoting and facilitating soil colonization of the biocontrol fungus, application of bioplastic granules decreased the numbers of aflatoxigenic isolates. In both years, the displacement of aflatoxigenic isolates was significantly influenced by the application rate. At corn harvest, the mean level of soil aflatoxigenic isolates from plots that received 15 and 30 kg ha^{-1} was 20 and 12% of the total recovered *A. flavus* propagules in 2009, respectively, and 16 and 9% in 2010 (Table 1). The feasibility of bioplastic granules to efficiently deliver and promotes oil colonization of the strain NRRL 30797 has been previously demonstrated in a series of laboratory experiments (Accinelli et al., 2009). Vigorous growth and sporulation of *A. flavus* on granules was clearly visible after approximately one week from their application to the field (Fig. 2).

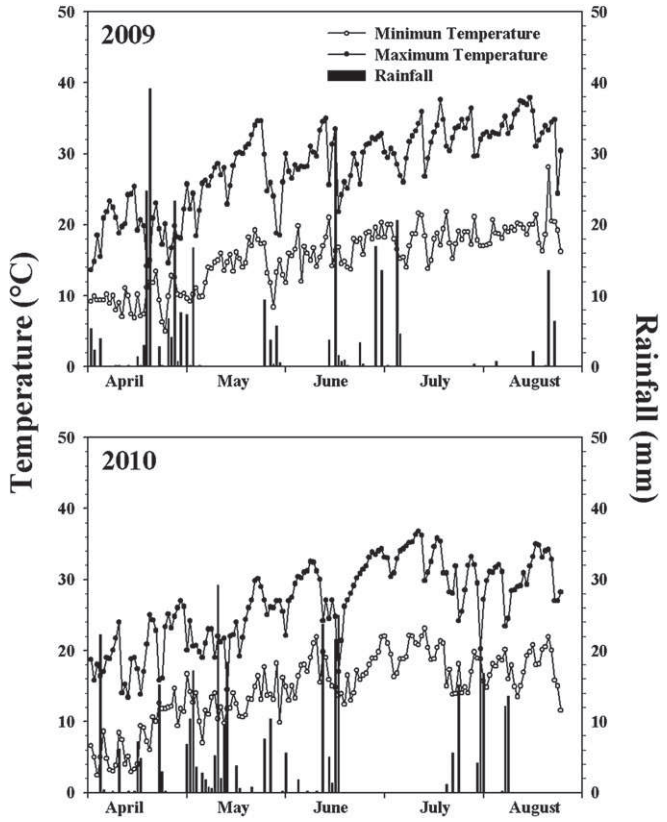

Fig. 1. Meteorological data at the experimental field during the 2009 and 2010 corn-growing seasons.

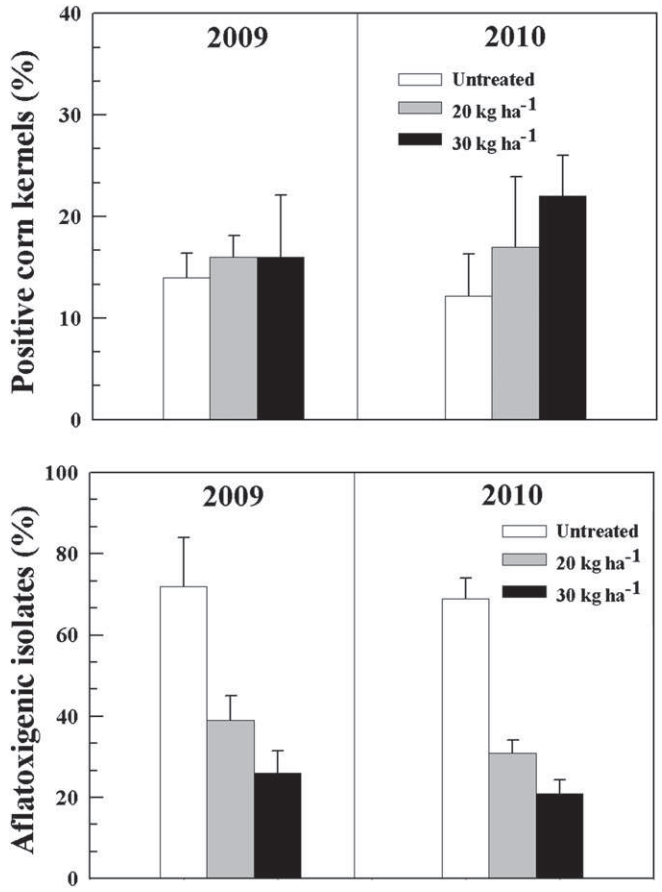

Fig. 3. Percentage of corn kernels infected by *Aspergillus flavus* in 2009 and 2010 (top) and relative abundance of isolates capable to produce aflatoxins (bottom). Each point represents mean ± STD.

A selected number of corn ears were evaluated for assessing the frequency of kernels infected by *A. flavus*. More than 10% of kernels from control plots were contaminated with the fungus in both years (Fig. 3). Studies have shown that the percentage of infected kernels is strongly variable, depends on weather conditions and geographical areas, and varies from 0 to more than 90% (Rambo et al., 1974; Hesseltine et al., 1976). In a survey conducted in representative corn-growing areas of Northern Italy, Giorni et al. (2007) reported that approximately 70% of the *A. flavus* isolates recovered from corn kernels were capable of producing aflatoxin. In the present study, approximately 75% of *A. flavus* isolates from kernels of untreated plots had the potential to produce aflatoxins, with no differences in the two years (Fig. 3). This value decreased down to 39 and 26% in kernels from plots receiving 15 and 30 kg ha^{-1} of bioplastic granules in 2009, and down to 31 and 21% in 2010, respectively.

3.2. Monitoring of Aspergilli using bioplastic-based baits

Results from the conventional cultivation-based method were confirmed by using the novel baiting system proposed here in this study. QPCR analysis showed that more *A. flavus* DNA was recovered from the surface of bioplastic rods which were inserted in treated soil than untreated control (Fig. 4). As shown in Fig. 2, bioplastic rods impregnated with MDRB were highly selective in recovering Aspergilli. For the biocontrol fungus delivering, we chose a rapidly biodegradable matrix, whereas a more persistent bioplastic grade, the MB bioplastic ZF03U/A, was used for baiting Aspergilli from soil. These granules maintained their physical integrity for the 14-day

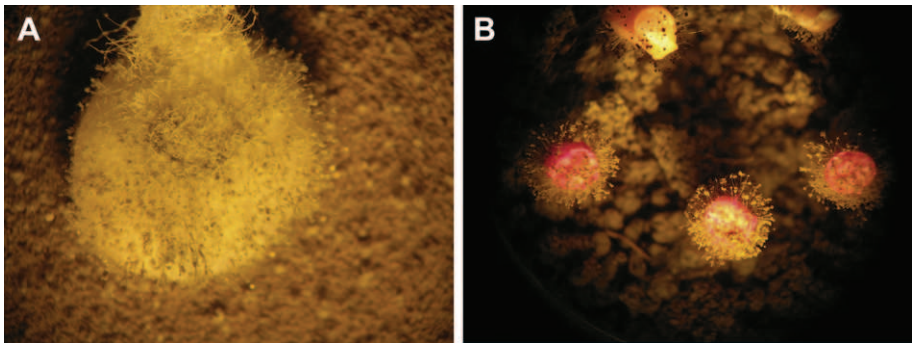

Fig. 2. Bioplastic granule inoculated with the non-aflatoxigenic isolate *Aspergillus flavus* NRRL 30797 after one week from field application (A). Growth of *A. flavus* isolates on the surface of bioplastic baits inserted in soil (B).

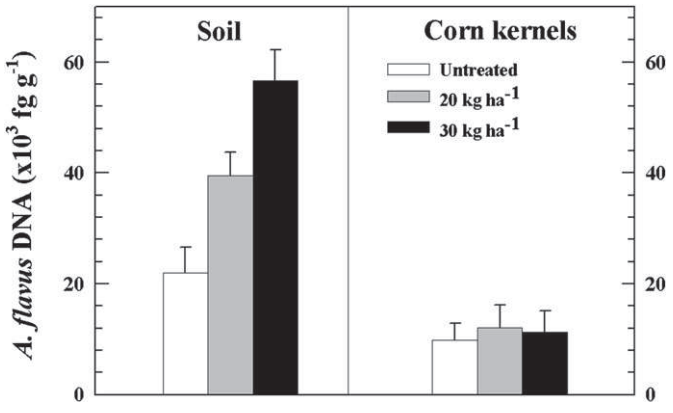

Fig. 4. *Aspergillus flavus* DNA recovered from the surface of bioplastic rods and granules incubated with soil (left) and corn kernel samples (right) collected on August 2010. Each point represents mean ± STD.

incubation period, thus facilitating surface cleaning, handling and DNA isolation. This bioplastic-based bait approach was also effective in selectively isolating Aspergilli from corn kernel samples (Fig. 4). This was also supported by DNA sequences from these fungal isolates (GenBank accession number from HQ844675 to HQ844716). More than 80% of the total isolates showed high identity (>98%) to *A. flavus*, after basic local alignment search tool (BLAST). Although total *A. flavus* DNA recovered from granules and aflatoxin contamination of corn were poorly correlated ($r = 0.68$; $P = 0.05$; data not shown), we found a significant correlation ($r = 0.89$; $P = 0.05$) between the amount of aflatoxin produced by baited fungi and aflatoxin contamination of corn kernels (Fig. 5). These preliminary results showed that the simple and cost-effective bait system would help with monitoring Aspergilli from soil and corn kernels. It is widely recognized that in developed countries, because of controlled storage conditions, aflatoxin level of corn remains approximately unchanged during storage. Conversely, corn storage under poorly controlled and far from ideal conditions, as often seen in developing countries, can result in additional fungal proliferation and aflatoxin production (Shier et al., 2006). In these cases, the availability of a rapid tool for monitoring this risk would be helpful.

A variety of baiting approaches have been proposed in the past decades for tracking plant fungal pathogens in soil or other plant-growing substrates (Paulitz and Schroeder, 2005;Eguchi et al., 2009). Most of these methods are based on tracking the target fungus and enumerating fungal propagules by conventional plate counting methods. More recently, attempts to use DNA-based approaches have been proposed (Nechwatal et al., 2001; Matsumoto, 2003). However, these methods are based on endpoint PCR approaches, which exclude quantification of a specific DNA sequence. Considering the practical importance of having information on the level of a specific plant pathogen fungus, in this experiment we focused on the development of a DNA quantification protocol. Unlike baiting materials that have been previously proposed by other authors (autoclaved seeds, etc.), our bioplastic rods or granules are composed of DNA-free matrices. After extrusion at high temperatures (>160 °C), rods and granules were subjected to a prolonged autoclaving process thus further reducing the occurrence of accidental contamination. It is widely demonstrated that other materials, such as autoclaved cereal seeds, are commonly contaminated by a large number of microorganisms which can result in false-positive samples.

3.3. Aflatoxin contamination of corn

The capability of this novel bioplastic-based formulation in promoting soil colonization of the biocontrol strain *A. flavus* NRRL 30797 and to compete with indigenous aflatoxigenic isolates resulted in reducing aflatoxin contamination of corn. As indicated in Fig. 6, aflatoxin contamination of untreated corn was widely different in the two years, with values of 4.4 and 28.9 ng g^{-1} in 2009 and 2010, respectively. In the first year, average aflatoxin contamination of corn kernels from plots received 15 and 30 kg ha^{-1} of bioplastic granules were 1.8 and 0.6 ng g^{-1}, respectively. As discussed above, weather conditions were not conducive to mold growth (Fig. 1). This likely explains the low level of aflatoxin contamination. The positive effect of inoculated bioplastic granules was more pronounced in 2010. Contamination of aflatoxin in corn treated with the biocontrol formulation at the lowest and highest dosages was 5.7 and 2.3 ng g^{-1}, respectively. Although, information on aflatoxin pre-harvest contamination of corn cultivated in Italy is scarce, high variability among years has been reported (Pietri et al., 2004; Piva et al., 2006). This field experiment was designed to obtain practical information on the feasibility of bioplastic granules to deliver the biocontrol isolates NRRL 30797, with the final purpose being assessing this technology for controlling aflatoxin in corn. Based on the results from this two-

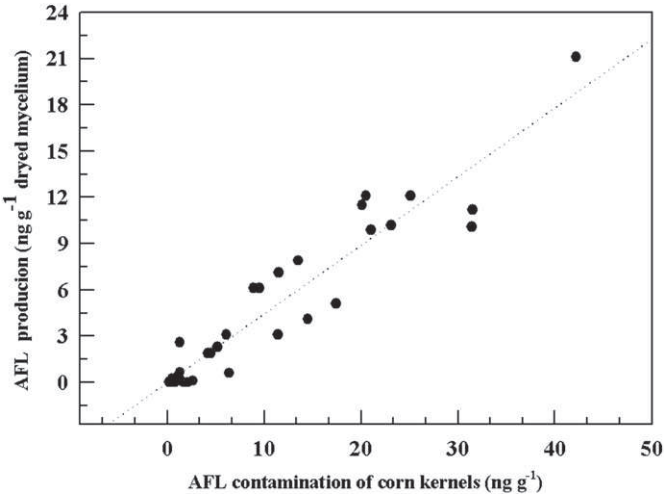

Fig. 5. Scatter plot showing the linear relationship between aflatoxin (AFL) contamination of corn kernels and aflatoxin produced by *A. flavus* isolates baited on bioplastic granules. The linear regression gives a correlation coefficient of 0.89.

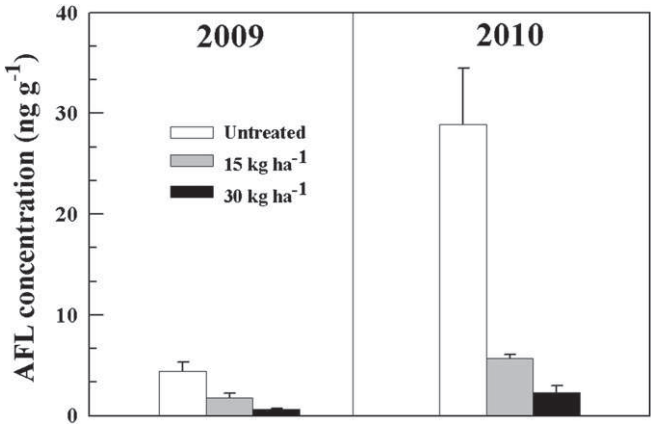

Fig. 6. Aflatoxin (AFL) contamination of corn collected from untreated control plots and plots receiving a ground application of inoculated bioplastic granules at the rate of 15 and 30 kg ha^{-1}. Each point represents mean ± STD.

year trial, inoculated bioplastic granules should be included in the list of available technologies for reducing aflatoxin contamination in corn. A number of field studies have demonstrated the efficiency of the use of grain seed inoculated with non-aflatoxigenic strains in reducing aflatoxin contamination of corn and other crops (Brown et al., 1991; Abbas et al., 2006). Considering the economical impact of aflatoxin contamination on corn production, other strategies are under evaluation (Abbas et al., 2009). More recently, Lyn et al. (2009) proposed a liquid formulation for direct spray application of non-toxigenic *A. flavus* strains. The study described here demonstrated that bioplastic granules can serve as a modern formulation for field application of this *A. flavus* biocontrol isolate. Bioplastic granules are industrially produced from renewable sources (mainly corn starch). Beside the favorable environmental profile, bioplastic granules offer a series of practical advantages, including easy handling and field application (Accinelli et al., 2009).

4. Conclusions

This study demonstrated the capability of the novel bioplastic-based formulation entrapping propagules of the non-aflatoxigenic A. flavus NRRL 30796 to replace indigenous toxigenic *A. flavus* isolate to control aflatoxin contamination of corn. The favorable environmental profile of this biodegradable formulation and other practical characteristics (easily handling and application) make this formulation an alternative solution for delivering and facilitating soil colonization of the biocontrol aflatoxigenic strain with respect to other traditional formulations (e.g. autoclaved grains, alginate prills, etc.). We also showed than this capability can be exploited for selective isolation of Aspergilli form soil and corn kernel samples.

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