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ORIGINAL ARTICLE

Characterization of mycotoxins produced by two *Fusarium* species responsible for postharvest rot of banana fruit

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Abstract
In an open-air market in southern Italy, we noticed 'Lady finger' banana fruit imported from Costa Rica showing a severe rot, whose symptoms consisted of necrotic peel lesions with variable shape and size. *Fusarium sacchari* and *F. proliferatum* were consistently isolated from symptomatic fruit. In pathogenicity tests on 'Lady finger' banana fruit, *F. proliferatum* was more virulent than *F. sacchari*. Quantitative Time-of-Flight Mass Spectrometric analysis of secondary metabolites produced by isolates of these two *Fusarium* species on three different matrices (banana peel, barley and maize kernels) identified 11 mycotoxins. Seven of them (Fusaproliferin, Fumonisins A_1, Fumonisins A_2 and Fumonisins B_1, Hydrolysed Fumonisin B_1, Fusarin C and Moniliformin) were detected in matrices contaminated by *F. proliferatum* isolates. Fumonisin A_1 was the prevalent mycotoxin in both maize kernels and banana peel, while Fumonisin A_2 prevailed in barley kernels. Similarly, seven mycotoxins (the cyclic hexadepsipeptides Enniatins B_2, B_3 and B_4, Fumonisins A_1 and B_2, Hydrolysed Fumonisin B_1 and Fusarin C) were detected in matrices contaminated by *F. sacchari* isolates, but they were only in part the same as those produced by *F. proliferatum* isolates. Fusarin C prevailed in all three matrices colonized by *F. sacchari*. Fumonisin A_1 was detected exclusively in maize kernels while Enniatins B_3 and B_4, Fumonisin B_2 and Hydrolysed Fumonisin B_1 were detected exclusively in barley kernels. Overall, *F. proliferatum* produced a higher amount of mycotoxins than *F. sacchari*. Moreover, in banana peel both species produced a lower number and amount of mycotoxins than in the other two matrices.

Keywords *Musa acuminata* hybrid · *Fusarium proliferatum* · *F. sacchari* · *F. fujikuroi* species complex · Quantitative Time-of-Flight Mass Spectrometry · Enniatins · Fumonisins · Fusarins · Pathogenicity

Introduction

Banana is one of the most important fruit crops worldwide. It is estimated that there are more than 1000 different banana varieties. According to the Food and Agriculture Organization of the United Nations (FAO), global banana production reached about 120 million tons in 2020 on a cultivated area of more than 5 million hectares. India, which is the largest producer of banana in the world, contributes with 29% of world production, followed by China and Indonesia, which account for 9.60% and 6.40% of world production, respectively (FAOSTAT 2020). *Fusarium* is known as one of the most economically damaging genera of fungal pathogens of agricultural and ornamental crops. The *Fusarium* taxonomy, which traditionally was based on phenotypic characters, has been continuously evolving. Over the past decades, molecular systematics studies, which have relied on multilocus DNA sequence data to determine species limits, have revolutionized the taxonomy of *Fusarium* and provided new insights into species diversity and phyletic relationships within this genus of fungi (O'Donnell et al. 2015). Despite this, the *Fusarium* taxonomy is still controversial (Crous et al. 2021; Geiser et al. 2021).

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According to Geiser et al. (2021), the genus includes 23 species complex and more than 300 phylogenetically distinct species that have been identified through molecular phylogenetics. Most of these species are soil-borne and, depending on the ecological context, may have different lifestyles, acting as saprophytes, endophytes, or plant pathogens (Aoki et al. 2014; O'Donnell et al. 2015). One of the most destructive disease of banana crops worldwide is Fusarium wilt, also known as Panama disease, caused by *F. oxysporum* f. sp. *cubense* (E.F. Smith) Synder and Hansen (Foc) in the *Fusarium oxysporum* species complex (FOSC) (Warman and Aitken 2018). It comprises three main races (1, 2 and 4), which are distinguished on the basis of the range of dessert banana cultivars they infect, as well as at least 24 vegetative compatibility groups (VCGs) (Mostert et al. 2017, 2022). Foc race 4 causes disease on most cultivars and has been divided into Foc subtropical race 4 (SR4) and Foc tropical race 4 (TR4), on the basis of its pathogenicity on bananas of the Cavendish group in different environmental conditions. Very probably, this large diversity of Foc depends on its polyphyletic nature (Maryani et al. 2019). One of the most important post-harvest fruit diseases of banana is crown rot, a disease complex caused by several fungi, including among others *F. pallidoroseum* and *F. incarnatum* (syn. *F. semitectum*), sometimes in association with bacteria (Knight et al. 1977; Snowdon 1990; Uribe-Palacio et al. 2022). The infections occur at harvest, but the symptoms appear later, after overseas shipment (Kamel et al. 2016). Several other species or species complex of *Fusarium* were reported to be associated with crown rot and other post-harvest fruit rots of bananas, including *F. camptoceras*, *F. concentricum*, *F. concolor*, *F. fujikuroi*, *F. equiseti*, *F. incarnatum*, *F. oxysporum*, *F. proliferatum*, *F. pseudocircinatum*, *F. sacchari*, *F. solani* and *F. verticillioides* (Abd Murad et al. 2017; Anthony et al. 2004; Calderón-Santoyo et al. 2022; Ewané et al. 2012; Hirata et al. 2001; Indrakeerthi and Adikaram 2011; John et al. 1996; Kamel et al. 2016; Mirete et al. 2004; Moretti et al. 2004; Xie et al. 2022; Zeng et al. 2013). A post-harvest rot of banana 'Lady Finger' caused by *F. sacchari* was recently reported, for the first time in Italy (Riolo et al. 2020). Symptoms consisted of dark brown to black necrotic lesions of the peel varying in shape and size. *Fusarium sacchari* was also identified as the causal agent of banana leaf blight (BLB), an emerging disease occurring in Guangdong, China (Cui et al. 2021). This species belongs to the *Fusarium fujikuroi* species complex (FFSC), which encompasses approximately 50 species (O'Donnell et al. 2015) and is phylogenetically related to FOSC (Maryani et al. 2019). Besides being a pathogen of banana, *F. sacchari* is a highly destructive pathogen of sugar cane and wheat (Bao et al. 2020; Viswanathan et al. 2017). It was also reported as causal agent of yellow leaf spot of an orchid species (Dekham and Kanchanawatee 2020). Beside *F. sacchari*, *F. proliferatum*, another species in the FFSC, was found associated to post-harvest rot of banana 'Lady Finger'in Italy (Cacciola et al. unpublished data). *Fusarium proliferatum* was already listed among the fungal species responsible for banana crown rot (Snowdon 1990; Kamel et al. 2016; Uribe-Palacio et al. 2022). It is an extremely polyphagous species, with a host range including both animals and plants. As a plant pathogen, *F. proliferatum* has been reported on several economically important crops, including beside banana, diverse species of succulent plants, maize, hemp, garlic, onion, peach, red sage and rice (Desjardins et al. 1997; Gwinn et al. 2022; Jerushalmi et al. 2020; Kamali-Sarvestani et al. 2022; Logrieco et al. 1995; Murad et al. 2017; Stankovic et al. 2007; Xie et al. 2018; Yang et al. 2020). Damages caused by infections of *Fusarium* species include besides yield losses also food contamination by mycotoxins (Bentivenga et al. 2020; Stracquadanio et al. 2021). Mycotoxins are defined as secondary fungal metabolites that exert a toxic action on higher vertebrates and other animals at low concentrations (Bennett 1987). They pose a threat to vertebrates through ingestion, inhalation or skin contact and can enter the food chain via contaminated plant food components or as a consequence of the growth of toxigenic fungi on food (Alshannaq and Yu 2017; Bennett 1987). Agricultural products face the risk of mycotoxin contamination during harvest, transport, warehouse processing and storage, due to improper practices promoting the colonization by opportunistic fungal pathogens (Marin et al. 2013; Williams et al. 2004). Given the heat-stability of many mycotoxins and their endurance of chemical and physical treatments, adopting good hygienic and prophylactic management practices is crucial to reduce contamination risks (Marin et al. 2013; Pitt 2000). *Fusarium* is known to be a genus of mycotoxigenic fungi (Nesic et al. 2013; Perincherry et al. 2019) and the ability to produce certain types of toxins has been also suggested as a criterion of taxonomic relevance in this genus (Crous et al. 2021; Gwinn et al. 2022; Nirmaladevi et al. 2016; Pasquali et al. 2016; Somma et al. 2014). In particular, members of the FFSC are known as mycotoxin producers (Gwinn et al. 2022). Some of the mycotoxins produced by *Fusarium* species are also pathogenicity determinants although a direct correlation has not always been found between the amount of toxins produced by the *Fusarium* isolates tested and their virulence (Alghuthaymi et al. 2020; He et al. 2019; López-Díaz et al. 2018). The ability of *Fusarium* species, including *F. proliferatum*, to produce mycotoxins in

association with post-harvest rot of banana fruit has been previously reported.(Alghuthaymi et al. 2020; Hirata et al. 2001; Moretti et al. 2004). However, to the best of our knowledge, no *F. sacchari* isolate from banana was included in these studies. Moreover, *F. sacchari* was demonstrated to be responsible for post-harvest rot of 'Lady Finger' bananas observed in Italy (Riolo et al. 2020), while the etiological role of *F. proliferatum*, also isolated from these fruit even though in a lower proportion than *F. sacchari* (38% against 62%, respectively) (Cacciola et al. unpublished), has not been proved.

The aim of this study was to compare the pathogenicity of *F. proliferatum* and *F. sacchari* isolates recovered from bananas in Italy and test their mycotoxigenic potential on banana and other food matrices.

Materials and methods

Fusarium isolates

Overall, 17 isolates of *Fusarium*, obtained from fruit of 'Lady Finger' banana (a diploid hybrid of *Musa acuminata* Colla) with rot symptoms, were included in this study (Table 1). Isolates were sourced in April 2019 from a fruit stock imported into Italy from Costa Rica (Riolo et al. 2020). They were purified by single-conidium subculture on water-agar (WA) in accordance with a standard protocol (Choi et al. 1999) and preserved in the collection of the laboratory of Molecular Plant Pathology at the Department of Agriculture, Food and Environment (Di3A) of the University of Catania, Italy. Reference strains of *F. sacchari*, *F. proliferatum* and other *Fusarium* species from CBS-KNAW and ARS Culture Collection (NRRL) were included for comparison (Table 2).

Morphological characterization of isolates

Isolates were grown in Petri dishes on Potato Dextrose Agar (PDA; Oxoid Ltd., Basingstoke, UK) and Malt Extract Agar (MEA; Sigma-Aldrich, Burlington, MA, USA). Dishes were incubated for 7 days at 25 ± 1 °C in the dark.

Molecular characterization of isolates

Isolates were grown on PDA for 7 days at 25 ± 1 °C, in the dark. Mycelium of each isolate was harvested with a sterile scalpel, and the genomic DNA (gDNA) was extracted using a PowerPlant® Pro DNA isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA), following the manufacturer's protocol. The DNA was preserved at −20 °C. A multilocus approach was adopted to characterize and determine the phylogenetic allocation of the isolates obtained from banana fruit. The β-tubulin (Tub 2) and translation elongation factor 1-α (EF-1α) genes of all isolates were amplified from gDNA. The primer pairs used for amplifying these gene regions were βt2α/βt2β (Glass and Donaldson 1995) and EF1/EF2 (O'Donnell et al. 1998), respectively.

PCR amplifications were performed on a GeneAmp PCR System 9700 (Applied Biosystems, Monza-Brianza, Italy). All PCR reactions were carried out by using Taq DNA polymerase recombinant (Invitrogen™) in a total volume of 25 µL containing PCR Buffer (1X), dNTP mix (0.2 mM), $MgCl_2$ (1.5 mM), forward and reverse primers

Table 1 Single-conidium *Fusarium* isolates obtained from 'Lady Finger' banana fruit characterized in this study, their putative geographical origin and respective GenBank accession numbers of translation elongation factor 1-α (EF-1α) and β-tubulin (Tub 2)

Isolate	Species	Origin	Accession number	
			Tub 2	EF-1α
CBS 145950	*Fusarium proliferatum*	Costa Rica	MN255817	MN255819
FBA-1	*F. proliferatum*	Costa Rica	PP105546	PP125310
FBA-2	*F. proliferatum*	Costa Rica	PP105547	PP125311
FBA-4	*F. proliferatum*	Costa Rica	PP105548	PP125312
FBA-5	*F. proliferatum*	Costa Rica	PP105549	PP125313
FBA-6	*F. proliferatum*	Costa Rica	PP105550	PP125314
FBA-7	*F. proliferatum*	Costa Rica	PP105551	PP125315
FBA-8	*F. proliferatum*	Costa Rica	PP105552	PP125316
FBA-9	*F. proliferatum*	Costa Rica	PP105553	PP125317
CBS 145949	*Fusarium sacchari*	Costa Rica	MN255816	MN255818
FBA-B	*F. sacchari*	Costa Rica	PP105539	PP105554
FBA-C	*F. sacchari*	Costa Rica	PP105540	PP105555
FBA-D	*F. sacchari*	Costa Rica	PP105541	PP105556
FBA-E	*F. sacchari*	Costa Rica	PP105542	PP105557
FBA-F	*F. sacchari*	Costa Rica	PP105543	PP105558
FBA-G	*F. sacchari*	Costa Rica	PP105544	PP105559
FBA-H	*F. sacchari*	Costa Rica	PP105545	PP105560

Table 2 GenBank accession numbers for sequences of *Fusarium* isolates belonging to various species, sourced from different geographical regions and hosts, used as references in phylogenetic analyses

Species	Isolate	Country	Host	Source	Accession numbers	
					Tub2	Tef 1
F. acutatum	NRRL 13308	India	Environment	Scauflaire et al. (2011)	U34431	AF160276
F. andiyazi	CBS 119857	South Africa	*Sorghum bicolor*	Yilmaz et al. (2021)	KP662894	KP662901
F. begoniae	NRRL 25300	Germany	*Begonia elatior*	O'Donnell et al. (1998)	U61543	AF160293
F. circinatum	NRRL 25331	USA	*Pinus radiata*	Yilmaz et al. (2021)	U61547	AF160295
F. ficicrescens	CBS 125178	Iran	Environment	Yilmaz et al. (2021)	KP662896	KP662899
F. fujikuroi	NRRL 13566	China	*Oryza sativa*	Yilmaz et al. (2021)	U34415	AF160279
F. globosum	NRRL 26131	South Africa	*Zea mays*	Yilmaz et al. (2021)	U61557	AF160285
F. guttiforme	NRRL 22945	England	*Ananas comosus*	Yilmaz et al. (2021)	U34420	AF160297
F. marasasianum	CMW 25261	Colombia	*Pinus tecunumanii*	Yilmaz et al. (2021)	KJ541054	KJ541063
F. proliferatum	NRRL 66451	USA	*Vitis vinifera*	Bolton et al. (2016)	MH398240	KX656221
F. proliferatum	NRRL 22944	Germany	*Cattleya* pseudobulb	Yilmaz et al. (2021)	U34416	AF160280
F. proliferatum	NRRL 66417	USA	*V. vinifera*	Bolton et al. (2016)	MH398182	KX656215
F. proliferatum	NNRL 31860	USA	*V. vinifera*	Bolton et al. (2016)	MH398153	KX656208
F. sacchari	NRRL 13999	India	*Saccharum officinarum*	Yilmaz et al. (2021)	U34414	AF160278
F. sacchari	CBS 201.37	Unknown	Unknown	Yilmaz et al. (2021)	MW402310	MW402112
F. sacchari	CBS 185.33	India	*S. officinarum*	Yilmaz et al. (2021)	MW402304	MW402106
F.sacchari	CBS 223.76	India	*S. officinarum*	O'Donnell et al. (2000)	KU603910	KU604430

(0.5 µM each), Taq DNA Polymerase (1 U) and 100 ng of genomic DNA. The reaction protocol for Tub2 included an initial preheat at 94 °C for 2 min; followed by 35 cycles of denaturation at 94 °C for 15 s, annealing at 58 °C for 15 s, and extension at 72 °C for 45 s; and final extension at 72 °C for 10 min. The Tef 1 included an initial denaturation at 95 °C for 8 min; followed by 35 cycles of 95, 58, and 72 °C for 15, 20, and 60 s, respectively; and a final extension at 72 °C for 10 min. The amplicons were detected in 1% agarose gel and purified products were sequenced by Macrogen Europe (Amsterdam, The Netherlands). For molecular identification, sequences were aligned using MUSCLE and introduced to MEGA6 for phylogenetic analysis with the Maximum Likelihood method using the Tamura–Nei model (Tamura and Nei 1993). Analyses were performed with 1000 bootstrap replications. In order to maximize the effectiveness of the investigation into the genetic diversity among isolates obtained in the present study, the phylogenetic analysis was conducted using a combined dataset of sequenced markers (EF-1α and Tub 2).

Pathogenicity test

A conidial suspension of each isolate in sterile distilled water (sdw) was prepared by gently scraping 10-day old cultures grown on Potato Dextrose Agar (PDA; Oxoid Ltd., Basingstoke, UK) at 25 °C and used as inoculum. 'Lady finger' bananas were surface disinfected with 70% ethanol for 2 min, rinsed with sdw and placed on filter paper for drying. After disinfection bananas were wounded with a sterile needle in an equatorial position (two wounds, 5 cm apart from each other, on the same side of the fruit) and a 20 µl droplet of conidial suspension (10^4 conidia mL^{-1} sdw) was pipetted on the surface of the two wounds (Parra et al. 2022; Purwati and Hidayah 2008).

Five isolates for each *Fusarium* species and 10 fruit per isolate were used in each trial. Ten wounded fruit served as non-inoculated control. Control fruit received a 20 µL droplet of sdw. After inoculation, fruit were incubated in a humid chamber at 23 ± 1 °C, with 80% relative humidity and a photoperiod of 16 h of light and 8 h of darkness. They were regularly monitored up to 10 days post inoculation (dpi) and the external area of the fruit peel with typical brown lesions was measured. The trial was replicated three times.

Preparation of samples to produce mycotoxins

The extraction of mycotoxins from 'Lady Finger' banana peel was performed according to Fanelli et al. (2012) with some modifications (Fig. 1). Pieces of banana peel were collected from symptomatic fruit 10 dpi (see section "Pathogenicity test"). Pieces were excised from necrotic lesions around the inoculation site and placed into 15 mL Eppendorf tubes. Each tube contained three fragments from three distinct fruit of the same batch, with three replicates for each batch.

The banana peel pieces were weighted and a 1:5 (w/v) ratio of methanol (MeOH) was added. The samples were incubated at room temperature and continuous stirring (150 rpm) for 30 min. After the incubation period, the supernatant was collected and filtered through a 13 mm/0.22 µm nylon syringe filter (Membrane Solutions) into a 2 mL amber vial for chromatographic analysis. Several dilutions

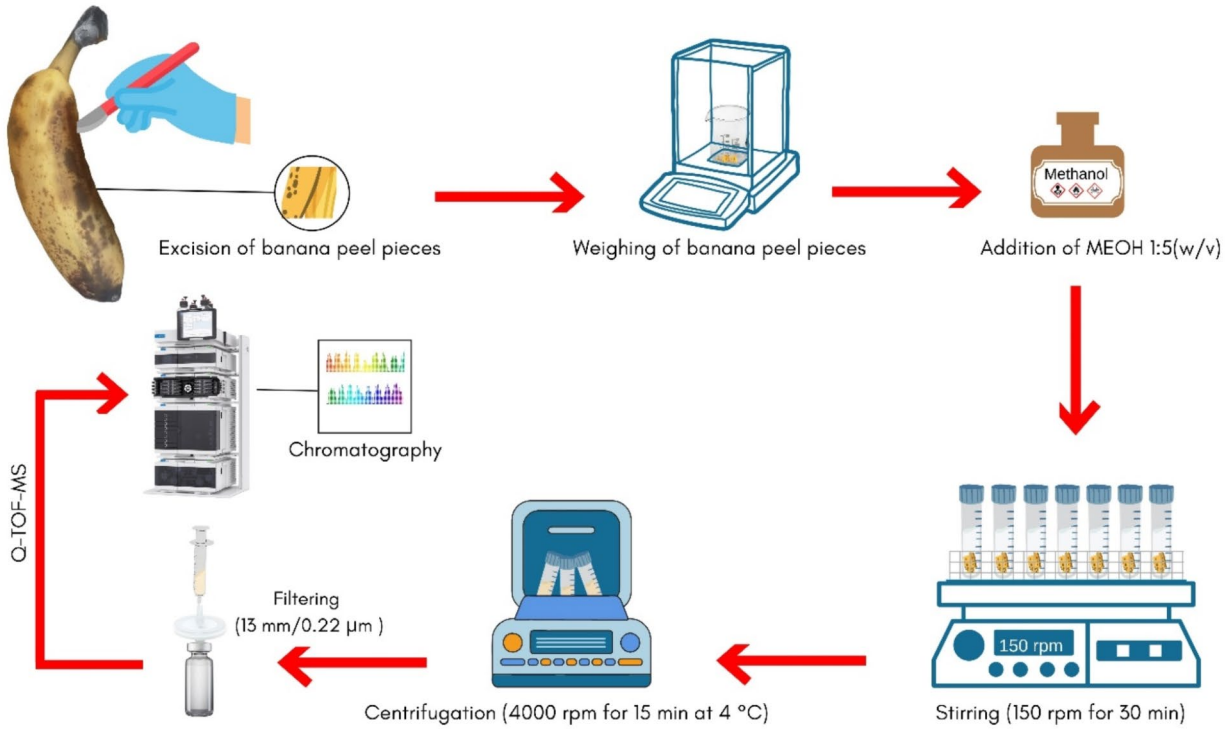

Fig. 1 Mycotoxin extraction and analysis: graphical summary

were performed to fit the concentrations among the calibration curves.

To assess the mycotoxigenic potential of *Fusarium* species isolated from 'Lady Finger' bananas, autoclaved maize and barley kernels (10 g each) were inoculated. A 5 mm mycelial plug from each strain was added to 50 mL Falcon tubes containing each matrix, followed by incubation at 30 °C for four weeks in the dark. Control samples of non-inoculated maize and barley kernels underwent the same treatment. This protocol is the same outlined by Serrano et al. (2013) with few modifications. Each cereal sample was homogenized using a grinder (Oster Classic Grinder 220–240 V, 50/60 Hz, 600 W, Oster, Valencia, Spain). Three 5-gram aliquots were transferred to 50 mL plastic Falcon tubes. In each tube, 25 mL of methanol were added, and the samples were homogenized for 3 min using an Ultra Turrax Ultra Ika T18 device (VWR, Staufen, Germany). Then the extracts were centrifuged at 10,000 rpm for five minutes at 5 °C, and the supernatant was transferred to a plastic flask and evaporated to dryness using a Rotavapor R-200 (Büchi Labortechnik AG, Flawil, Switzerland). The remaining material was re-suspended in 5 mL of methanol, transferred to a 15 mL plastic Falcon tube, and evaporated using a Turbovap LV multicampione evaporator (Zymark, Hopkinton, MA, USA) with the assistance of a nitrogen flow. Subsequently, the residue was reconstituted in 1 mL of methanol, filtered through a 13 mm/0.22 µm filter, and transferred to a 1 mL glass chromatography vial for analysis.

Quantitative Time-of-Flight Mass Spectrometry (Q-TOF-MS) analysis

The HPLC system used for the chromatographic determination was an Agilent 1290 (Agilent Technologies, Santa Clara, CA, USA) equipped with a vacuum degasser, autosampler and binary pump. The column was Agilent Zorbax RRHD SB-C18, 2.1 × 50 mm, 1.8 µm column. The mobile phase A was composed of Milli-Q water and acetonitrile was used for mobile phase B (both phases were acidified with 0.1% of formic acid), with gradient elution, as follows: 0 min, 2% B; 22 min 95% B; 25 min, 5% B. The flow rate was 0.4 mL /min, and 5 µL of sample was injected.

Mass spectrometry (MS) analysis was conducted using a Q-TOF-MS (6540 Agilent Ultra High Definition Accurate Mass), equipped with an Agilent Dual Jet Stream electrospray ionization (Dual AJS ESI) interface in positive ionization mode under the following conditions: gas temperature: 325 °C; gas flow: 10 L/min; nebulizer pressure: 40 psig; sheath gas temperature: 295 °C; sheath gas flow: 12 L/min; capillary voltage: 4000 V; nozzle voltage: 500 V; Fragmentor: 120 V; skimmer: 70 V; product ion scan range: 100–1500 Da; MS scan rate: 5 spectra/s; MS/MS scan rate: 3 spectra/s; maximum pre-cursors per cycle: 2; collision energy: 10,

20, 40 eV. The analysis of the metabolites was carried out in triplicate. Untargeted LC/Q-TOF based metabolomics approach was used to identify the differential metabolic profiles of *Fusarium* species growing on each batch. Integration, data elaboration and identification of metabolites were managed using MassHunter Qualitative Analysis Software B.08.00 and library PCDL Manager B.08.00.

Method validation

The method used in this study was evaluated according to Tamura et al. (2015), with modifications. The linearity, recovery, and detection limit (LOD) of this method were evaluated to validate the analysis. These validations were performed using non-contaminated samples of banana peel, barley kernels, and maize kernels. To measure the linearity of the assay, the samples were spiked with increasing concentrations of the mycotoxins (FUS, FA1, FA2, FB1, HFB1, FC, MON, ENB2, ENB3, and ENB4). The concentrations were 4, 16, 80, 400, and 2000 µg/kg, matching the concentration of the analyte used in the extraction method. To evaluate the recovery of the method, 100 µg/kg of each mycotoxin was added to the analytes, and the extraction procedure was performed on the samples for analysis. The FB1, FB2, MON, and ENB2 used for calibration were obtained from Sigma-Aldrich (Burlington, MA, USA). HFB1 was obtained from Romer Labs (Getzersdorf, Austria). FC, ENB3, and ENB4 were obtained from BenchChem (Austin, TX, USA). FUS was obtained according to Meca et al. (2009). FA1 and FA2 were obtained as described in Tamura et al. (2015).

Statistical analysis of data

Data from pathogenicity tests were normalized by square root transformation and then subjected to analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test as a post hoc test (R software). Differences at $p \leq 0.05$ were considered significant.

Results

Identification of fungal isolates

On both PDA and MEA, all *Fusarium* isolates, obtained from infected 'Lady Finger' banana fruit (Fig. 2A), produced a fast-growing mycelium, which covered the Petri dishes (9 cm diam.) after 7 d incubation at 25 °C. On PDA, *F. sacchari* isolates produced aerial mycelium, white at the beginning and turning violet after 7 d at 25 °C, while the mycelium of *F. proliferatum* isolates was white at the beginning and subsequently turned light purple (Fig. 2B and C).

The phylogenetic analysis of the combined data set of sequences from EF-1α and Tub 2 regions of all single-conidium *Fusarium* isolates sourced in Italy from 'Lady Finger' bananas imported from Costa Rica (Table 1), along with sequences of the *F. proliferatum* and *F. sacchari* isolates used as references and the reference sequences of other species in the FFSC, produced a phylogenetic tree (Fig. 3) with a similar topology and high concordance with those reported by the authors who revised the systematics of this species complex using multigene sequence analysis (Herron et al. 2015). Eight single-conidium isolates from 'Lady Finger' banana were identified as *F. sacchari* because they clustered (bootstrap values 100%) with the CBS reference isolate of this species (CBS 185.33 and CBS 201.37). Conversely, all remaining single-conidium isolates from 'Lady Finger' banana, that showed also a distinct morphotype, clustered (bootstrap values 100%) with the sequences of EF-1α and Tub 2 regions of reference *F. proliferatum* isolates, including the isolates NRRL 31860, NRRL 66417 and NRRL 22944.

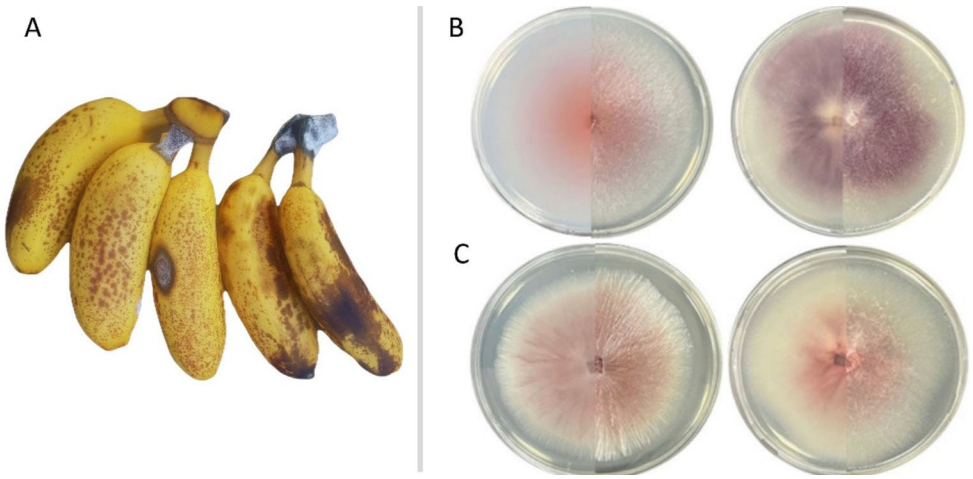

Fig. 2 Necrotic spots on peel and crown rot incited by *Fusarium sacchari* and *F. proliferatum* on 'Lady Finger'banana fruit; (**A**) Colony morphology (front and back) of *Fusarium sacchari* and *Fusarium proliferatum* (from left to right) after 7 days growth on MEA (**B**) and PDA (**C**) at 25 ± 1°C in the dark

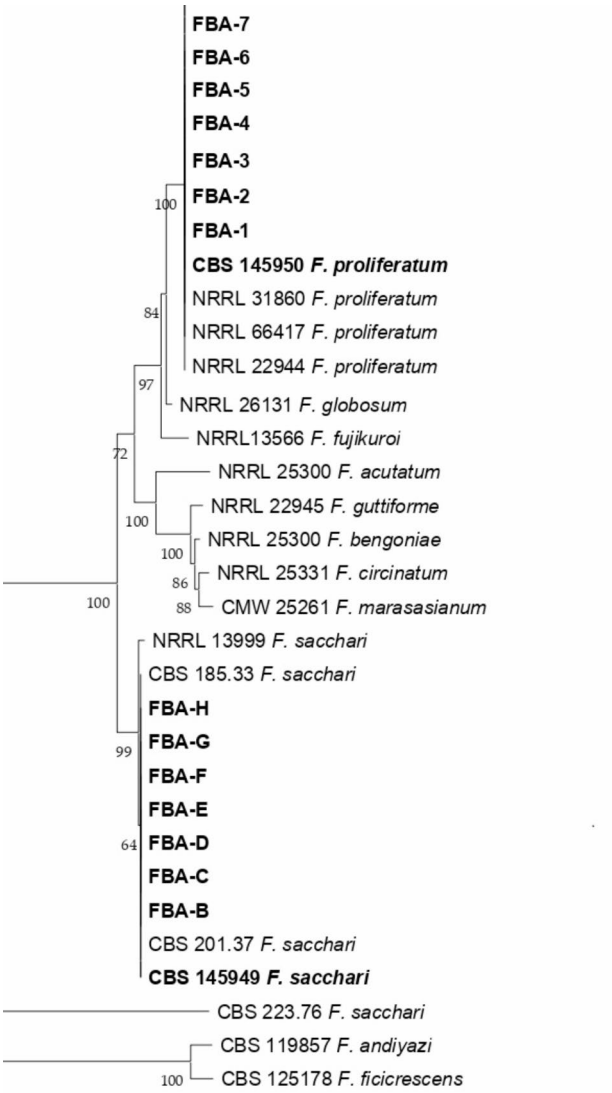

Fig. 3 Phylogenetic tree based on the translation elongation factor 1-α (EF-1α) and β-tubulin (Tub 2) regions developed using the Maximum Likelihood Method, based on the Tamura–Nei model. The tree with the greatest log likelihood (-3906.13) is shown. Relationships between the isolates from 'Lady Finger' banana and the CBS and NRRL reference isolates of *Fusarium fujikuroi* species complex. In bold, isolates characterized in this study

All isolates of both *F. proliferatum* and *F. sacchari* were pathogenic on 'Lady Finger' bananas. However, overall *F. proliferatum* isolates were more aggressive than *F. sacchari* isolates. No significant difference in pathogenicity was observed among isolates of the same species (Fig. 4). Symptoms induced by both these two *Fusarium* species were identical to those observed on banana fruit with natural infections and consisted in necrotic, brown spots of various size and shape (from lenticular to circular), with a regular margin.

All *Fusarium* isolates were re-isolated from the lesions and identified on the basis of colony morphology and sequencing of EF-1α, and TUB2 regions, thus fulfilling Koch's postulates.

Detection of mycotoxins produced by *F. proliferatum* and *F. sacchari* in different matrices

The mycotxin profiles of the two *Fusarium* species as determined by Q-TOF-MS were distinct. Q-TOF-MS analysis identified seven diverse mycotoxins in matrices colonized by *F. proliferatum* isolates, including Fusaproliferin (hereafter referred to as FUS), Fumonisin A_1 (FA1), Fumonisin A_2 (FA2), Fumonisin B_1 (FB1), Hydrolysed Fumonisin B_1 (HFB1), Fusarin C (FC) and Moniliformin (MON) (Table 3). Among these mycotoxins, FUS, FA1, FA2, FB1, and FC were consistently detected in all matrices, while FHB1 and MON were detected exclusively in barley kernels. The primary mycotoxin identified in maize kernels and banana peel was FA1, with maximum concentrations of 1757.35 mg/kg and 832.59 mg/kg, respectively. The highest levels of FA2 were detected in barley kernels, with a maximum concentration value of 494.93 mg/kg. In all three matrices, the nine isolates of this species consistently produced FA1, FA2, and FC. Notably, FBA-4 isolate exhibited the highest cumulative mycotoxin production in maize kernels, FBA-2 isolate in barley kernels, and FBA-1 isolate in banana peel, indicating an isolate/matrix interaction. Overall, the highest mycotoxin content was detected in maize kernels, while the lowest mycotoxin content was detected in banana peel.

Overall, seven diverse mycotoxins were detected in matrices colonized by *F. sacchari* isolates, including the cyclic hexadepsipeptides Enniatins B_2, B_3 and B_4 (ENB2, ENB3 and ENB4, respectively), Fumonisins A_1 and B_2 (FA1 and FB2, respectively), Hydrolysed Fumonisin B_1 (HFB1) and Fusarin C (FC) (Table 4). ENB2 and FC were present in all three matrices, while the remaining five mycotoxins were detected exclusively in maize (FA1) or barley kernels (ENB3, ENB4, FB2, and HFB1). FC was the prevalent mycotoxin in all matrices, reaching the highest concentration in barley kernels with a concentration value of 276.34 mg/kg, followed by maize kernels with a concentration value of 172.01 mg/kg and banana peel with 80.21 mg/kg. In maize kernels, all isolates produced FC, whereas in barley kernel, only FAB-C, FAB-E, and FAB-F isolates and in banana peel only FAB-E isolate produced this toxin. The FBA-C isolate produced the highest amount of FC in maize kernels, while FBA-E isolate produced the highest amount of this toxin in barley kernels and banana peel. Similarly to the samples colonized by *F. proliferatum*, the mycotoxin production in the banana peel was lower compared to the other two matrices. In fact, only ENB2 and FC were detected

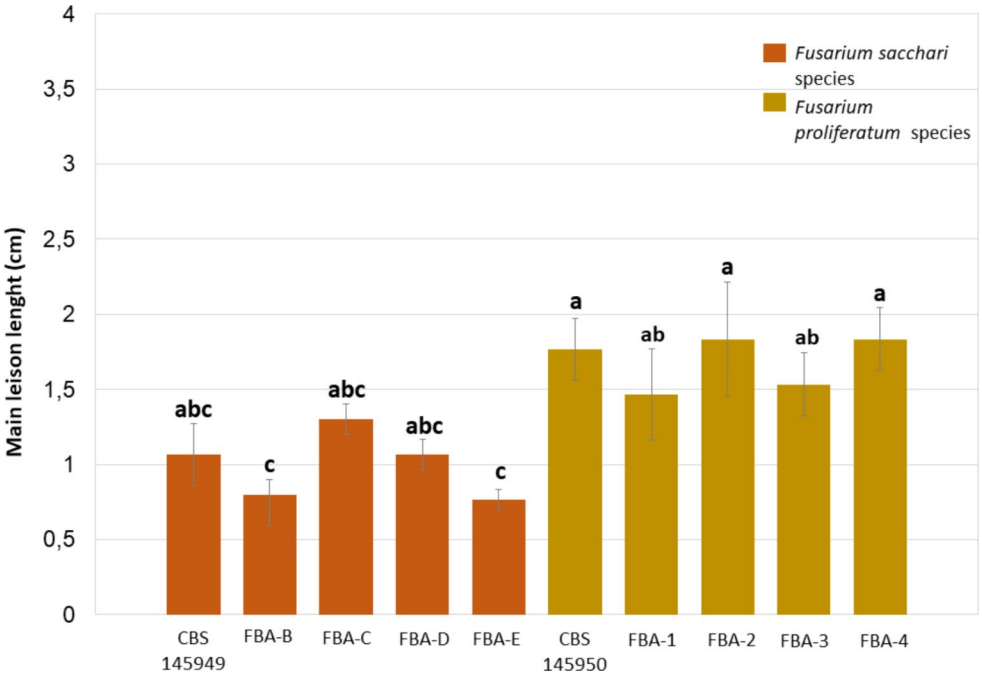

Fig. 4 Mean area (± SD) of necrotic lesions (mm^2) induced by *Fusarium sacchari* and *Fusarium proliferatum* isolates on wound inoculated 'Lady Finger' bananas, 10 dpi. Values sharing the same letters are not statistically different according to Tukey's honestly significant difference (HSD) test ($p \leq 0.05$)

in banana peel. The most distinctive qualitative characteristics between the mycotoxin profiles of the two *Fusarium* species were the presence of Enniatins (mainly ENB1) only in the profile of *F. sacchari* isolates and the exclusive presence of FA2, FB1 and FUS in the profile of *F. proliferatum* isolates. In particular, all isolates of the latter species produced FA2 in all three matrices, with mean concentration values in banana peel varying from 48.4 to 416.3 mg/kg (Tables 4 and 5). As for the mycotoxins that were present in the profile of both *F. proliferatum* and *F. sacchari* (FA1, FC and HFB1), relevant quantitative differences were observed between the two fungal species. In particular, all isolates of *F. proliferatum* produced FA2 in all three matrices, with mean concentration values in banana peel varying from 322.16 to 832.59 mg/kg (Table 4). Conversely, only two isolates of *F. sacchari* produced these mycotoxins, but only in maize kernels and in traces.

Accuracy of the mycotoxin determination method in different matrices

The validation of the method was performed by the analysis of the linearity, recovery, LOD and LOQ and the results are evidenced in the Table 5. The determination of the linearity coefficient evidenced values above 0.99, recovery values were observed to range percentages from 97.3 to 102.8%. Therefore, it can be accepted that the method used in this article evidenced an optimal performance for obtaining the results. The LOD was determined to be 0.71 to 2.11 µg/kg. Detections below these limits were marked as n.d.

Discussion

Results of this study confirm that both *F. proliferatum* and *F. sacchari* are able to infect banana fruit. Although the former *Fusarium* species was isolated in a lower proportion than the latter from symptomatic 'Finger Lady' bananas sampled in southern Italy, it was proved to be more virulent in pathogenicity tests, confirming previous findings of other scholars (Abd Murad et al. 2017). No significant intraspecific difference in pathogenicity was observed among diverse isolates of these two species. In line with previous studies investigating post-harvest rots of banana fruit caused by *Fusarium* species, *F. proliferatum* and *F. sacchari* showed significant activity as opportunistic wound pathogens in pathogenicity tests. (Abd Murad et al. 2017; Anthony et al. 2004; Kamel et al. 2016; Riolo et al. 2020). Koch's postulates were fulfilled for both species, which can be consequently regarded as the causal agents of post-harvest rot of 'Finger Lady' banana fruit reported in Italy.

Overall, Q-TOF-MS analysis identified 11 diverse mycotoxins produced by *F. proliferatum* and *F. sacchari* in artificially inoculated banana peel, barley and maize kernels. These toxins included Fumonisins A_1, A_2, B_1 and B_2, Enniatins B_2, B_3 and B_4, as well as Hydrolysed Fumonisin B_1, Fusaproliferin, Fusarin C and Moniliformin.

The fumonisins, a family of carcinogenic mycotoxins, were first isolated from cultures of *F. verticillioides* (formerly *F. moniliforme*) on maize kernels (Mostrom 2016; Ostry et al. 2017). Successively, they were shown to be produced by other *Fusarium* species (Rheeder et al. 2002). *Fusarium proliferatum* is well known as a producer of

Table 3 Presence of mycotoxins on banana peel, barley kernels and maize kernels contaminated with the different isolates of *Fusarium proliferatum* (FUS = fusaproliferin, FA_1 = fumonisin A_1, FA_2 = fumonisin A_2, FB_1 = fumonisin B_1, HFB_1 = hydrolysed fumonisin B_1, FC = Fusarin C, MON = moniliformin)

Isolate code	Substrate	mg/kg						
		FUS	FA_1	FA_2	FB_1	HFB_1	FC	MON
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
FBA-1	Banana peel	n.d.*	801.43 ± 32.71	398.32 ± 50.19	5.10 ± 1.56	n.d.*	89.49 ± 5.10	n.d.*
FBA-2		n.d.	322.16 ± 18.99	416.30 ± 29.54	4.19 ± 0.23	n.d.	72.12 ± 8.15	n.d.
CBS 145950		4.12 ± 1.47	763.24 ± 13.61	180.45 ± 20.46	7.18 ± 2.40	n.d.	23.81 ± 2.80	n.d.
FBA-4		n.d.	688.15 ± 18.68	219.35 ± 19.74	4.18 ± 1.12	n.d.	58.13 ± 3.17	n.d.
FBA-5		n.d.	720.37 ± 29.15	142.79 ± 43.81	10.54 ± 2.99	n.d.	59.87 ± 5.32	n.d.
FBA-6		n.d.	436.91 ± 9.66	187.92 ± 54.91	7.23 ± 2.00	n.d.	97.88 ± 9.45	n.d.
FBA-7		n.d.	832.59 ± 36.51	204.00 ± 12.98	4.25 ± 1.52	n.d.	63.02 ± 4.31	n.d.
FBA-8		n.d.	768.26 ± 21.43	173.98 ± 31.00	4.69 ± 1.34	n.d.	46.34 ± 1.26	n.d.
FBA-9		n.d.	659.56 ± 6.87	48.40 ± 6.97	4.00 ± 2.36	n.d.	75.92 ± 4.76	n.d.
FBA-1	Barley kernels	n.d.	85.91 ± 27.11	240.16 ± 34.32	2.97 ± 0.60	8.29 ± 2.86	100.68 ± 7.80	13.50 ± 5.90
FBA-2		10.22 ± 3.16	111.68 ± 22.69	494.93 ± 21.76	2.68 ± 0.53	n.d.	138.02 ± 14.60	n.d.
CBS 145950		n.d.	218.76 ± 10.88	303.40 ± 15.31	5.09 ± 0.70	n.d.	99.92 ± 12.95	n.d.
FBA-4		10.25 ± 2.56	211.24 ± 16.99	410.41 ± 28.32	n.d.	3.04 ± 1.95	69.83 ± 9.17	n.d.
FBA-5		4.43 ± 2.14	203.36 ± 27.65	332.65 ± 31.63	n.d.	n.d.	68.56 ± 9.40	n.d.
FBA-6		9.22 ± 3.96	51.79 ± 7.61	213.46 ± 16.07	n.d.	8.09 ± 1.32	112.18 ± 17.29	2.82 ± 1.59
FBA-7		6.17 ± 2.41	109.20 ± 22.15	316.47 ± 40.01	3.69 ± 0.59	n.d.	80.37 ± 8.58	n.d.
FBA-8		n.d.	144.86 ± 19.43	218.33 ± 28.39	n.d.	n.d.	58.05 ± 8.00	n.d.
FBA-9		11.98 ± 3.58	138.90 ± 9.36	0.34 ± 1.22	4.12 ± 0.76	3.41 ± 0.72	62.02 ± 7.50	n.d.
FBA-1	Maize kernels	n.d.	494.50 ± 37.31	603.80 ± 19.15	9.46 ± 4.22	n.d.	164.57 ± 14.82	n.d.
FBA-2		8.06 ± 3.97	103.68 ± 25.37	677.85 ± 36.27	n.d.	n.d.	15.87 ± 4.83	n.d.
CBS 145950		n.d.	1298.63 ± 58.06	318,44 ± 35.27	9.12 ± 3.47	n.d.	258.62 ± 17.73	n.d.
FBA-4		n.d.	1757.35 ± 42.25	265.94 ± 28.94	3.79 ± 2.23	n.d.	191.02 ± 14.73	n.d.
FBA-5		n.d.	213.54 ± 22.21	305.61 ± 14.16	10.07 ± 1.31	n.d.	44.09 ± 16.10	n.d.
FBA-6		n.d.	413.43 ± 36.78	329.72 ± 27.69	11.95 ± 1.47	n.d.	135.62 ± 13.81	n.d.
FBA-7		n.d.	131.78 ± 26.62	244.78 ± 26.84	9.75 ± 0.63	n.d.	174.89 ± 14.01	n.d.
FBA-8		n.d.	144.37 ± 28.49	276.21 ± 13.29	10.68 ± 0.72	n.d.	171.45 ± 13.48	n.d.
FBA-9		n.d.	188.31 ± 39.77	250.36 ± 26.19	10.44 ± 1.90	n.d.	89.35 ± 10.64	n.d.

**n.d.* not detected

fumonisins (Alghuthaymi and Bahkali 2015; Gu et al. 2019; Moretti and Susca 2017; Shi et al. 2017). Shi et al. (2017) classified this species in the group of *Fusarium* species producing both Fuminosins and Fusaric acid based on the mycotoxigenic profile on three different matrices (PDA, rice and maize), while they classified *F. sacchari* in the group of *Fusarium* species producing only Fusaric acid. Indeed, in the present study only three out of eight isolates of *F. sacchari* produced fumonisins, but exclusively on barley and maize kernels and only in traces. Consistently with the result of this study, Rheeder et al. (2002) listed *F. sacchari* among the *Fusarium* species producing Fumonisins. All *F. proliferatum* isolates produced high amounts of Fuminosins on all three matrices, including the banana peel. Fumonisins, in particular FB1, was shown to exert phytotoxic activity on diverse host plants (Abbas et al. 1991, 1998; Abbas and Boyette 1992; Al Abboud et al. 2012; Doehlert et al. 1994; Kritzinger et al. 2006). In a previous study aimed at characterizing seven *Fusarium* species, including *F. proliferatum*, it was found that the virulence of isolates on banana fruit did not correlate with the amount of Fumonisin they produced (Alghuthaymi et al. 2020). Moreover it can not be excluded that diverse mycotoxins produced by this *Fusarium* species act synergistically as virulence factors.

Xie et al. (2021) demonstrated that FB1 treatment increases the aggressiveness of *F. proliferatum* on banana fruits by suppressing the fruit's defense mechanisms through the reduction of key enzyme activities such as phenylalanine ammonia-lyase (PAL), β-1,3-glucanase (GLU), and chitinase (CHI). Additionally, FB1 accelerates cell death in banana fruits, as indicated by increased relative conductivity, malondialdehyde (MDA) content, and transcripts of cell death-related genes. The increased hydrogen peroxide (H2O2) content, likely due to the induction of MaRBOHs, further contributes to reactive oxygen species (ROS)-dependent cell death, thereby reducing the fruit's resistance to disease (Xie et al. 2021).

Table 4 Amount of mycotoxins on banana peel, barley kernels and maize kernels contaminated with different isolates of *Fusarium Sacchari* (ENB_2 = enniatin B_2, ENB_3 = enniatin B_3, ENB_4 = enniatin B_4, FA_1 = fumonisin A_1, FB_2 = fumonisin B_2, HFB_1 = hydrolysed fumonisin B_1, FC = Fusarin C)

Isolate code	Substrate	mg/kg						
		ENB_2	ENB_3	ENB_4	FA_1	FB_2	HFB_1	FC
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
CBS 145949	Banana peel	n.d.*	n.d.*	n.d.*	n.d.*	n.d.*	n.d.*	n.d.*
FBA-B		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
FBA-C		13.82 ± 1.52	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
FBA-D		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
FBA-E		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	80.21 ± 5.52
FBA-F		3.49 ± 1.60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
FBA-G		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
FBA-H		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
CBS 145949	Barley kernels	17.86 ± 2.80	2.19 ± 0.31	n.d.	n.d.	n.d.	n.d.	n.d.
FBA-B		18.38 ± 2.53	9.73 ± 0.60	n.d.	n.d.	n.d.	n.d.	n.d.
FBA-C		94.00 ± 4.51	n.d.	n.d.	n.d.	n.d.	n.d.	13.36 ± 2.00
FBA-D		n.d.	n.d.	n.d.	n.d.	3.44 ± 0.82	9.26 ± 1.56	n.d.
FBA-E		50.06 ± 4.45	n.d.	n.d.	n.d.	n.d.	n.d.	276.34 ± 7.62
FBA-F		59.96 ± 4.32	n.d.	n.d.	n.d.	n.d.	n.d.	20.90 ± 1.62
FBA-G		29.00 ± 3.59	6.04 ± 0.87	3.13 ± 0.29	n.d.	n.d.	n.d.	n.d.
FBA-H		23.29 ± 3.72	n.d.	3.56 ± 0.52	n.d.	n.d.	n.d.	n.d.
CBS 145949	Maize kernels	64.18 ± 5.34	n.d.	n.d.	n.d.	n.d.	n.d.	40.88 ± 4,04
FBA-B		80.40 ± 3.89	n.d.	n.d.	3.63 ± 1.02	n.d.	n.d.	79.33 ± 5,24
FBA-C		72.49 ± 3.27	n.d.	n.d.	n.d.	n.d.	n.d.	164.91 ± 8,16
FBA-D		41.45 ± 4.36	n.d.	n.d.	n.d.	n.d.	n.d.	77.29 ± 4,69
FBA-E		44.11 ± 3.63	n.d.	n.d.	n.d.	n.d.	n.d.	172.01 ± 9.96
FBA-F		n.d.	n.d.	n.d.	3.84 ± 1.56	n.d.	n.d.	90.02 ± 7.29
FBA-G		48.93 ± 2.34	n.d.	n.d.	n.d.	n.d.	n.d.	132.64 ± 6.88
FBA-H		54.54 ± 4.09	n.d.	n.d.	n.d.	n.d.	n.d.	107.62 ± 5.99

**n.d.* not detected

Xie et al. (2023) explored the link between FB1 biosynthesis and oxidative stress during *F. proliferatum* infection. It was observed that while H_2O_2 treatment inhibits fungal growth, it stimulates FB1 production and increases endogenous ROS levels. This suggests that FB1 helps the fungus tolerate oxidative stress, enhancing its virulence. The construction of a *Fusarium proliferatum* mutant (ΔFpfum21) with attenuated FB1 biosynthesis confirmed that the production of this mycotoxin is crucial for the fungus's virulence. This highlights that fumonisins not only contribute to phytotoxicity but are also crucial determinants of the pathogenicity of the Fusarium/banana system, giving *F. proliferatum* a significant competitive advantage in colonizing and damaging banana fruits.

The literature also evidences the production of several fumonisin analogs, such as FA1 and FA2 detected in this study, and other analogs like the dimethyl and N-3-hydroxypyridinium derivatives which belong to the fumonisin C series and fumonisin P series, respectively, by *F. proliferatum*. The production of these mycotoxins by this fungal species has been reported in vitro and detected in maize samples (Lazzaro et al. 2013; Tamura et al. 2015; Musser et al. 1995). Notably, this study is the first to report the production of FA1 and FA2 by *F. proliferatum* in two highly consumed food matrices: barley and banana.

Enniatins belong to the group of cyclodepsipeptides, which comprises also Beauvericins, Beauvenniatins and their analogues (Urbaniak et al. 2020). Originally isolated from cultures of *F. orthoceras* var. *enniatum* (later renamed *F. oxysporum*) from which they were named, these mycotoxins are produced by several *Fusarium* species, including *F. avenaceum*, *F. sambucinum*, *F. poae*, *F. sporotrichioides* and *F. tricinctum* which infect cereals and other commodities (Altomare et al. 2021). *Fusarium sacchari* was also reported among the *Fusarium* species that produce both Enniatins and Beauvericin (Mohamed and Al-Ani 2021; Tavakol Noorabadi et al. 2021). The primary toxic action of Enniatins is related to their ionophoric properties. The most important contributors to the dietary exposure of humans and animals to these mycotoxins are cereal grains and cereal grain-based products (EFSA 2014). On the other hand, due to their antimicrobial and cytotoxic properties, Enniatins are candidate as anti-cancer drugs (Urbaniak et al. 2020). Consistently with the data of EFSA (2014), in this study, the

Table 5 Performance of the mycotoxin determination and quantification evaluated by the parameters: the linearity and recovery. Studied mycotoxins were: FUS = fusaproliferin, FA_1 = fumonisin A_1, FA_2 = fumonisin A_2, FB_1 = fumonisin B_1, FB_2 = fumonisin B_2, HFB_1 = hydrolysed fumonisin B_1, FC = Fusarin C, MON = moniliformin, ENB_2 = enniatin B_2, ENB_3 = enniatin B_3, ENB_4 = enniatin B_4

Validation item	Substrate	FUS	FA_1	FA_2	FB_1	FB_2	HFB_1	FC	MON	ENB_2	ENB_3	ENB_4
Linearity (r)	Banana	0.9987	0.9962	0.999	0.9974	0.996	0.9997	0.9961	0.9999	0.9972	0.998	0.9974
Recovery (%)		98.7	97.5	101.3	100.1	101.7	99.2	97.8	102.6	100.9	98.8	101.7
LOD		1.98	1.67	1.28	1.72	1.46	1.06	1.37	1.59	1.02	1.50	1.24
Linearity (r)	Barley kernels	0.9985	0.9997	0.9983	0.9982	0.9995	0.9986	0.9963	0.9961	0.9978	0.9972	0.9976
Recovery (%)		99.6	97.3	100.4	98.3	100.2	101.9	99.7	97.9	102	100.3	98.1
LOD		1.81	1.33	0.98	1.89	1.20	0.93	2.11	0.80	1.15	0.71	2.02
Linearity (r)	Maize kernels	0.9963	0.9986	0.9965	0.9981	0.9987	0.9971	0.9977	0.9993	0.9989	0.9966	0.9985
Recovery (%)		101.1	99.4	97.7	102.3	100.8	100.7	98.4	101.5	99.9	97.6	102.8
LOD		1.85	0.85	2.07	1.11	1.54	2.15	1.76	1.41	0.76	1.98	1.63

greatest amounts of Enniatins were produced by *F. sacchari* isolates on barley and maize kernels and only two isolates of this *Fusarium* species produced mycotoxins of this group in banana peel.

Fusaproliferinis are produced by *F. proliferatum*, after which it was named, and other related *Fusarium* species (Ćeranić et al. 2021). Several fungi from other distant taxonomic groups were also reported to produce fusaproliferin or the deacetylated derivative, known as siccanol or terpestacin.

Fusarin C, a tetramethylated heptaketide fused to homoserine, is a metabolite with mutagenic properties. Although it is regarded as an emerging mycotoxin it is not legally regulated (Gasser et al. 2023). Originally, Fusarin C was reported as a mycotoxin typically produced by the plant pathogen *F. verticillioides* in maize (Wentzel et al. 1984). Subsequently, it was reported as a secondary metabolite of a number of other *Fusarium* species, such as *F. avenaceum*, *F. crookwellense*, *F. culmorum*, *F. graminearum*, *F. poae*, *F. proliferatum*, *F. sporotrichioides*, *F. tricinctum*, *F. venenatum* and *F. verticillioides* (Desjardins and Proctor 2007; Gaffoor et al. 2005; Gasser et al. 2023; Song et al. 2004; Studt et al. 2012; Thrane 1988). In this study, both *F. proliferatum* and *F. sacchari* isolates from banana produced Fusarin C, but their ability to produce this metabolite differed substantially. All isolates of both fungal species produced this mycotoxin in maize kernels and all isolates of *F. proliferatum* produced it in barley kernels as well as in banana peel. By contrast, only three isolates of *F. sacchari* out of eight produced Fusarin C in barley kernels and only one isolate out of eight produced it in banana peel. In general, based on the findings of this study the risk of contamination of banana fruit with Fusarin C can be considered low.

Moniliformin is a mycotoxin with low molecular weight primarily produced in cereals by a number of *Fusarium* species, including *F. avenaceum*, *F. subglutinans* and *F. proliferatum*. Like Fusarin C, it is an unregulated mycotoxin (Gasser et al. 2023). In this study, Moniliformin was detected only in the mycotoxin profile of two *F. proliferatum* isolates grown on barley kernels.

Overall, of the 11 detected mycotoxins, four (Fumonisins A2 and B1, Fusaproliferin and Moniliformin) were produced exclusively by *F. proliferatum*, four (Enniatins B_2, B_3 and B_4 and Fumonisin B2) were produced exclusively by *F. sacchari* and only three (Fusarin C, Fumonisin A1 and Hydrolysed Fumonisin B_1) were in common between the two *Fusarium* species. Substantial quantitative differences were also observed between the mycotoxin profiles of the two species. Moreover, there were noticeable differences in the mycotoxigenic potential among isolates of the same species. Fumonisins A1 and A2 were the prevalent mycotoxins produced by *F. proliferatum* isolates, with the highest

concentrations in maize kernels, varying from 103.68 to 1757.35 mg/kg and from 244.78 to 677.85 mg/kg, respectively. Fusarin C was another major mycotoxin in the metabolite profile of *F. proliferatum* isolates, with concentrations varying from 89.35 to 258.62, 58.05 to 138.02 and 23.81 to 97.88 mg/kg in maize, barley and banana, respectively. Fusarin C and Enniatin B2 were the prevalent mycotoxins in the metabolite profile of *F. sacchari* isolates. However, only a single isolate of this *Fusarium* species produced Fusarin C in banana peel, indicating the ability of producing mycotoxins varies among diverse isolates of the same species and confirming the crucial role of culture medium for the expression of mycotoxigenic potential of *Fusarium* isolates, as demonstrated in numerous previuos studies (e.g. Shi et al. 2017). The most relevant difference between the mycotoxin profiles of the two *Fusarium* species was the high amount of Fumonisins A1 and A2 produced by *F. proliferatum* in the peel of artificially inoculated banana fruit. In general, among the three diverse matrices tested in this study, banana peel was the least conducive for the production of mycotoxins. Other mycotoxins, such as Fusaproliferin, Fumonisin B1, Hydrolysed Fumonisin B1 and Moniliformin were detected only in traces.

Conclusions

In this study, it was demonstrated that *F. proliferatum* and *F. sacchari*, singularly or in association, were responsible for the post-harvest rot observed in southern Italy on 'Lady Finger' banana fruit imported from Costa Rica. The comparison of the metabolite profile of these two *Fusarium* species provided enough evidence to hypothesize some mycotoxins they produce, such as Fumonisins, could act as virulence factors in this pathosystem. Understanding the mechanisms by which these mycotoxins affect pathogen-host interactions could pave the way for new approaches to mitigating the impact of Fusarium diseases in banana crops. Moreover, the analysis of the mycotoxigenic potential of *F. proliferatum* and *F. sacchari* on different matrices provided information that can contribute to evaluate the risk of contamination by mycotoxins and to improve the legal regulation of mycotoxin concentrations in agricultural products, foods and feeds.

Author contribution Conti Taguali S.: Methodology, Data curation, Formal analysis, Writing—original draft; Riolo M.: Investigation, Methodology, Data curation, Formal analysis, Visualization, Supervision, Writing—original draft, Writing—review and editing; Dopazo V.: Investigation, Methodology, Formal analysis; Meca G.: Visualization, Investigation, Methodology; Cacciola SO.: Investigation, Methodology, Visualization, Writing—review and editing, Supervision.

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Data availability The data generated and analyzed during this study are included in the manuscript and all the raw data generated and/or analyzed during the current study are available from the corresponding author on request.

Declarations

Ethical approval This article does not contain any studies with human participants or animalsperformed by any of the authors.

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