J Pest Sci (2017) 90:925–933
DOI 10.1007/s10340-017-0841-4

ORIGINAL PAPER

Dactylopius opuntiae: control by the *Fusarium incarnatum–equiseti* species complex and confirmation of mortality by DNA fingerprinting

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Received: 13 August 2016 / Revised: 6 February 2017 / Accepted: 14 February 2017 / Published online: 24 February 2017

Abstract Cultivation of the prickly pear cactus, *Opuntia ficus-indica*, an important cattle food in the semiarid region of Brazil, has been increasingly compromised by predation by the cochineal scale *Dactylopius opuntiae*. Entomopathogenic fungi represent a low environmental impact alternative to controlling this insect pest, and the genus *Fusarium* has been described as a promising agent for that task. As such, we selected isolates of the *Fusarium incarnatum–equiseti* species complex (FIESC) to evaluate their potential biological control of *D. opuntiae* and confirmed their efficiency/presence in the field through morphological and molecular characteristics. The pathogenicities of 25 isolates of FIESC obtained from *D. opuntiae* were evaluated. The isolates URM6782, URM6778, and URM6811 demonstrated kill rates above 45%, and good sporulation characteristics, and were thus selected for field testing. The isolate URM6782 showed the highest kill rate in the field, and amplification profiles obtained using the ISSR UBC834 marker confirmed that the isolates released in the field were in fact the causal agents of the high mortality of *D. opuntiae*. We also observed natural biological control by native *Fusarium* species present in the field. Our results suggest that the isolates URM6782 and URM6778 show significant promise for controlling *D. opuntiae* and that the ISSR primer UBC834 can be used to monitor those isolates when released into the field. This work represents an initial study directed toward the biological control of *D. opuntiae* using novel isolates from the FIESC.

Keywords Biological control · FIESC · Cochineal · *Opuntia ficus-indica* · Molecular marker

Key message

- *Dactylopius opuntiae* has reduced the productivity of prickly pear, an important cattle food in the semiarid region of Brazil.
- Specific strains of the *Fusarium incarnatum–equiseti* species complex were found to be efficient in controlling this insect under both laboratory and field conditions.
- DNA fingerprints generated by the primer UBC834 confirmed that specific fungal strains caused the mortality of *D. opuntiae*. These molecular markers can be used to monitor fungal isolates in the field.
- The novel isolates from the *Fusarium incarnatum–equiseti* species complex can be used as an environmentally friendly alternative to control *D. opuntiae*.

Introduction

The prickly pear (*Opuntia ficus-indica* Miller) cactus is native to Mexico, but is now cultivated on most continents. Its wide dissemination is due to its usefulness as both a human and animal food resource (especially in arid and semiarid environments), for energy production, as a

Communicated by S.T. Jaronski.

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medicinal plant, in manufacturing cosmetics, for soil protection and conservation, and for the confection of adhesives, glue, fibers for handicrafts, paper, coloring agents, mucilage, plant antitranspirants, and in landscaping (Griffith 2004; Almeida et al. 2011).

The cultivation of this plant in the semiarid region of Brazil is important for the sustainability of cattle farming there. The agro-industrialization of this plant has generated a large number of products and derivatives, aggregating production value and creating jobs and income for the inhabitants of arid and semiarid regions (Oliveira et al. 2010). The cultivation of this green fodder cactus has been compromised, however, by the cochineal scale species *D. opuntiae* Cockerell, which is a major pest in many countries throughout the world (Lopes et al. 2009). Under favorable climatic conditions, *D. opuntiae* will limit plant growth, and serious infestations can result in the death of the cactus (Lima et al. 2011).

Dactylopius opuntiae is native to Mexico and neighboring countries in Central America. The scale was introduced into several areas, mainly to Australia, India, and South Africa, in order to control the prickly pear cacti (Cactaceae: Opuntia spp.), where they are considered noxious weeds (Foxcroft and Hoffmann 2000; Hosking et al. 1994). *Dactylopius opuntiae* is also encountered in countries such as France, Kenya, Madagascar, Pakistan, Sri Lanka, the USA (California and Texas), and Israel (Ben-Dov et al. 2013; Spodek et al. 2014).

These cochineal scales were introduced into northeastern Brazil with the objective of producing carmine food coloring, but soon became a regional plague, resulting in considerable economic losses to farmers and ranchers in the semiarid region, with reductions of up to 100% in cactus production (Chiacchio 2008).

Researchers are continually investigating techniques for controlling insect pests that result in only low-level environmental impacts. The use of entomopathogenic fungi has demonstrated significant potential as an efficient and secure method of insect control in Brazil and other countries (Moino Jr. 2000; Lacey et al. 2015).

The genus *Fusarium* has been investigated since the mid-1980s as a promising biological agent for insect control as it demonstrates a broad appetite for host insects of the orders Coleoptera, Diptera, Hemiptera, Hymenoptera, and Lepidoptera (Teetor-Barsch and Roberts 1983). These authors cite as advantages the fact that some of those fungal species demonstrate high host specificity, do not damage the plants, and are easily grown in the laboratory. The *Fusarium incarnatum–equiseti* species complex (FIESC) is considered especially rich in terms of *Fusarium* species isolated from insects. These species have not yet been assigned Latin binomials and are only identified by Arabic numerals; their different haplotypes are identified by lowercase Roman letters, following O'Donnell et al. (2009). Of the 30 phylogenetic species known to the FIESC, 15 were isolated from insects and have been reported as pathogens of various species of insects demonstrating their potential for insect control (Addario and Turchetti 2011; O'Donnell et al. 2012; Liu et al. 2014).

In order to successfully employ entomopathogenic *Fusarium* species to control carmine cochineal scale, research efforts must be focused on the host specificities of the isolates and on monitoring techniques that can gauge the presence and establishment of the fungi when liberated into the field. The application of molecular techniques to bioassays allows greater agility in detecting and identifying the infecting fungi, as it is now possible to identify the individual products (fungi) and distinguish them from others coexisting in the field, as well as track and monitor their stabilities and impacts on the environment (Destéfano et al. 2004; Tiago et al. 2016).

As such, we selected isolates of the FIESC with high pathogenicity toward *D. opuntiae* and developed morphological and molecular techniques to detect their presence and confirm their efficiency in the field.

Materials and methods

Fungal isolates

Twenty-five isolates of *Fusarium* obtained from *D. opuntiae* specimens collected in the field had previously been molecularly identified as belonging to the FIESC-based portions of the translation elongation factor 1-alpha gene (TEF1). These isolates were deposited in the fungal collections at the Federal University of Pernambuco (URM) (Table 1).

Source and maintenance of *Dactylopius opuntiae* under greenhouse conditions

Specimens of the cochineal scale *D. opuntiae* derived from cactus cladodes from different municipalities in the semiarid region of Pernambuco State were maintained under greenhouse conditions on infected (but otherwise healthy) cacti. The colonies were held at 34 °C (±3 °C) for 30 days, and the adult insects were then used in infection bioassays.

Pathogenicity of isolates of the *Fusarium incarnatum–equiseti* species complex under laboratory conditions

Pathogenicity tests utilized 25 isolates of *Fusarium* belonging to the FIESC; these fungi were cultivated in

Table 1 Isolates of the *Fusarium incarnatum–equiseti* species complex obtained of *Dactylopius opuntiae* in semiarid region of Brazil and their accession numbers in the URM Culture Collection (University Recife Mycologia)

Species complex	Fusarium-ID database[a]	Accession number isolates
Fusarium incarnatum–equiseti species complex (FIESC)	FIESC 16	URM6808
	FIESC 17	URM6801
	FIESC 20	URM6792, URM6779, URM6776, URM6777, URM6803, URM6804, URM6782, URM6793, URM6783, URM6809, URM6805, URM6778, URM6784, URM6802, URM6781, URM6807, URM6806, URM6811, URM6785, URM6788, URM6780, URM6787
	FIESC 28	URM6789

[a] Web site dedicated to identifying fusaria

potato dextrose agar (PDA) medium and incubated in an adapted biochemical oxygen demand (BOD) growth chamber (26 ± 1 °C) to promote growth and sporulation. After 8 days of growth, a suspension of 10^7 conidia/mL was prepared in Tween 80® (0.01%), following the methodology described by Alves and Moraes (1998).

Cladodes of *O. ficus-indica* infected with adult females of *D. opuntiae* were first sprayed with a neutral detergent (2%) to diminish the waxy surface covering the cochineal scale. After 3 h, the cladodes were then uniformly sprayed (manually) with 4 mL/cladodes of the fungal suspension (10^7 conidia/mL) in Tween 80® (0.01%). In the control treatment, the insects were sprayed only with Tween 80® (0.01%). After treatment, the cladodes were maintained at 28 ± 1 °C. Evaluations of insect mortality were performed 12 days after inoculation with the fungi (spraying) by delimiting three 8 cm × 8 cm areas on each cladode and evaluating 50 insects in each area, totaling 150 insects evaluated for each *Fusarium* isolate studied. The 150 insects were chosen at random, but it was not possible to ascertain whether they were living or dead due the sessile nature of adult cochineal females and their waxy coverings. Mortality due to fungal infection was confirmed by disinfecting the insects with 70% alcohol for 30 s, immersion in a solution of 4% sodium hypochlorite for 2 min, with subsequent rinsing in sterile distilled water (3×). The insect bodies were then transferred to Petri dishes containing Sabouraud medium + chloramphenicol (0.05%) and maintained at 28 ± 2 °C to cultivate the causal agents.

Six separate tests were carried out, each involving a different subset of isolates. The experimental regime was totally randomized, with three repetitions. The data were submitted to ANOVA, and the means were compared using the Tukey test at a 5% level of probability, using Assistat 7.7 Beta software (Silva and Azevedo 2009).

Bioassay 1 involved the isolates URM6782, URM6802, URM6787, URM6807; bioassay 2 involved the isolates URM6782, URM6789, URM6801, URM6778, URM6784; bioassay 3 involved the isolates URM6782, URM6776, URM6804, URM6808, URM6803, URM6777; bioassay 4 involved the isolates URM6782, URM6779, URM6792, URM6785, URM6780. URM6783; bioassay 5 involved the isolates URM6782, URM6793, URM6811, URM6806, URM6809; bioassay 6 involved the isolates URM6782, URM6781, URM6788, URM6805.

Isolates that demonstrated death rates above 45% and good sporulation were utilized in the field experiments.

Field experiments

The isolates selected for field trials were cultivated in liquid potato dextrose medium (PD) with incubation for 8 days in a BOD chamber (26 ± 1 °C). After that period, suspensions of 10^7 conidia/mL were prepared in Tween 80® (0.01%) (Alves and Moraes 1998).

Field experiments were undertaken on private property in a *O. ficus-indica* cultivation field that was naturally infested by *D. opuntiae*. The fungi were applied by manual spraying them on plants demonstrating high densities of cochineal scale; the fungal isolates were applied separately, at the same times of the day, to individual plant (10 mL/cladodes of the fungal suspension [10^7 conidia/mL]). The control treatment consisted of spraying only with Tween 80® (0.01%). During the field trials, the mean temperature varied between 18 and 25.6 °C and the relative humidity (RH) between 72% (09:00 a.m.) and 57% (03:00 p.m.).

Fifteen days after spraying, three cladodes from each plant were removed to the laboratory for analysis to confirm adult insect mortality. Fifty insects per cladode were selected within a 10 cm × 10 cm area, totaling 150 cochineal per applied fungal isolate. The insects were selected at random and superficially disinfected by sequential immersion in 70% alcohol for 30 s and 4% sodium hypochlorite for 2 min, and subsequently rinsed three times in sterile distilled water; the insect bodies were

then transferred to a humid BOD chamber and maintained at 26 ± 1 °C until fungal sporulation. After 5 days, the fungal structures were spread on Petri dishes containing PDA medium with added chloramphenicol (0.05%) and maintained at 26 ± 1 °C for 7 days to confirm the causal agent of insect mortality.

The *Fusarium* isolates responsible for insect mortality were identified by microscopic morphological analyses, based on colony and conidia characteristics, using the slide culture technique (Riddell 1950) allied with the ISSR molecular marker technique inter-simple sequence repeats (ISSR). The UBC834 primer (5′-AGAGAGAGAGAGAGAGYT-3′) was selected for that process (Tiago et al. 2016).

To analyze the DNA fingerprints of the fungi using the UBC834 primer, the isolates were cultivated in liquid Czapek medium (Lacaz et al. 2002) and maintained under agitation at 28 °C for 120 h. The mycelia were then collected by vacuum filtration and washed with sterilized distilled water. DNA extraction was performed using the *Wizard Genomic DNA Purification Kit* (Promega). DNA concentrations were determined using a Qubit® fluorometer (Invitrogen). The amplification reactions were performed in a final volume of 25 µL, with the following component concentrations: 1× reaction buffer (20 mM Tris–HCl, pH 8.4; 50 mM KCl), 1.8 mM MgCl2, 0.4 mM dNTP, 0.20 µM primer, 0.04 U-µL Taq DNA polymerase (Invitrogen, Life Technologies), and 25 ng of DNA. Amplification was performed in a thermal cycler (Viriti® 96 well Thermal Cycler, Applied Biosystems®) using the following program: initial denaturing at 94 °C for 5 min, followed by 40 cycles at 94 °C for 30 s, 52 °C for 45 s, 72 °C for 2 min, with a final extension at 72 °C for 6 min. The amplification products were separated by electrophoresis in 1.5% agarose gel with a 3 V/cm distance between the electrodes in 1× TBE running buffer (pH 8.0), incorporating a 1 Kb molecular weight marker (Invitrogen Life Technologies). The gel was stained with *Syber Green*® and photographed and analyzed using a photodocumentation system (L.PIX).

The data were submitted to analysis of variance (ANOVA) and the means compared using the Tukey test and a 5% level of probability, using Assistat 7.7 Beta software (Silva and Azevedo 2009). These analyses incorporated data obtained from the morphological identifications.

Results

Laboratory testing of pathogenicity

After performing a pilot bioassay, it was observed that infection by *Fusarium* isolate URM6782 resulted in a high death rate of *D. opuntiae* and that variety was thereafter used as a standard. Pathogenicity tests revealed that the confirmed mortality rates of *D. opuntiae* caused by *Fusarium* isolates varied from 8 to 66.66% in the different bioassays (Fig. 1). In bioassay (a), we observed differences in mortality rates only between the URM6782 isolate and the control group. In bioassay (b), only the URM6784 isolate did not differ significantly from the control group, but the isolates URM6782 and URM6778 demonstrated high and equivalent mortality rates. In bioassay (c), the URM6776 isolate differed from the control group and from isolate URM6804. In bioassay (d), only the isolate URM6792 did not differ from the control group, but did differ from URM6782. In bioassay (e), significant differences were observed between the mortality rates of isolates URM6782, URM6806, and URM6809. In bioassay (f), significant differences were observed only between isolate URM6782 and the control group. Among the 25 isolates examined, five resulted in cochineal scale death rates greater than 45% [URM6782 = 66.66% (Fig. 1a), URM 6778 = 58% (Fig. 1b), URM6776 = 57.33% (Fig. 1c), URM6777 = 47.33% (Fig. 1c), and URM6811 = 46% (Fig. 1e)]. Only the isolates URM6782, URM6778 and URM6811 (all of the species FIESC 20), were selected for field trials because they demonstrated adequate sporulation.

Field tests of pathogenicity

The isolates URM6782 and URM6778 (based on their morphological characteristics) caused 42.66 and 40.66% of the confirmed mortality of *D. opuntiae* in field tests, respectively, with no statistical differences between them. The isolate URM6811 demonstrated a confirmed death rate of 14%, significantly differing from the other isolates, but not from the control (5.33%) (Table 2).

Among the isolates recuperated (42.66, 40.66, 14% respectively) from the field, 84.52% demonstrated the same molecular profile as isolate URM 6782 (Fig. 2a), 54.17% demonstrated the molecular profile of URM 6778 (Fig. 2b), and 19.76% demonstrated the profile of URM 6811 (Fig. 2c). Significant differences were observed between the killing efficiencies of these three isolates when applied in the field, with URM 6782 demonstrating the greatest efficiency (Table 2). In the control group, among the eight isolates recovered, none of them demonstrated a molecular profile corresponding to either of the three isolates that were introduced, indicating biological control by native *Fusarium* isolates in the area (Fig. 2c). The UBC834 primer was found to efficiently differentiate between the introduced and native isolates. The three isolates tested demonstrated lower mortality rates in the field than in the laboratory, although under both conditions the URM 6782 isolate was efficient at controlling insect pests.

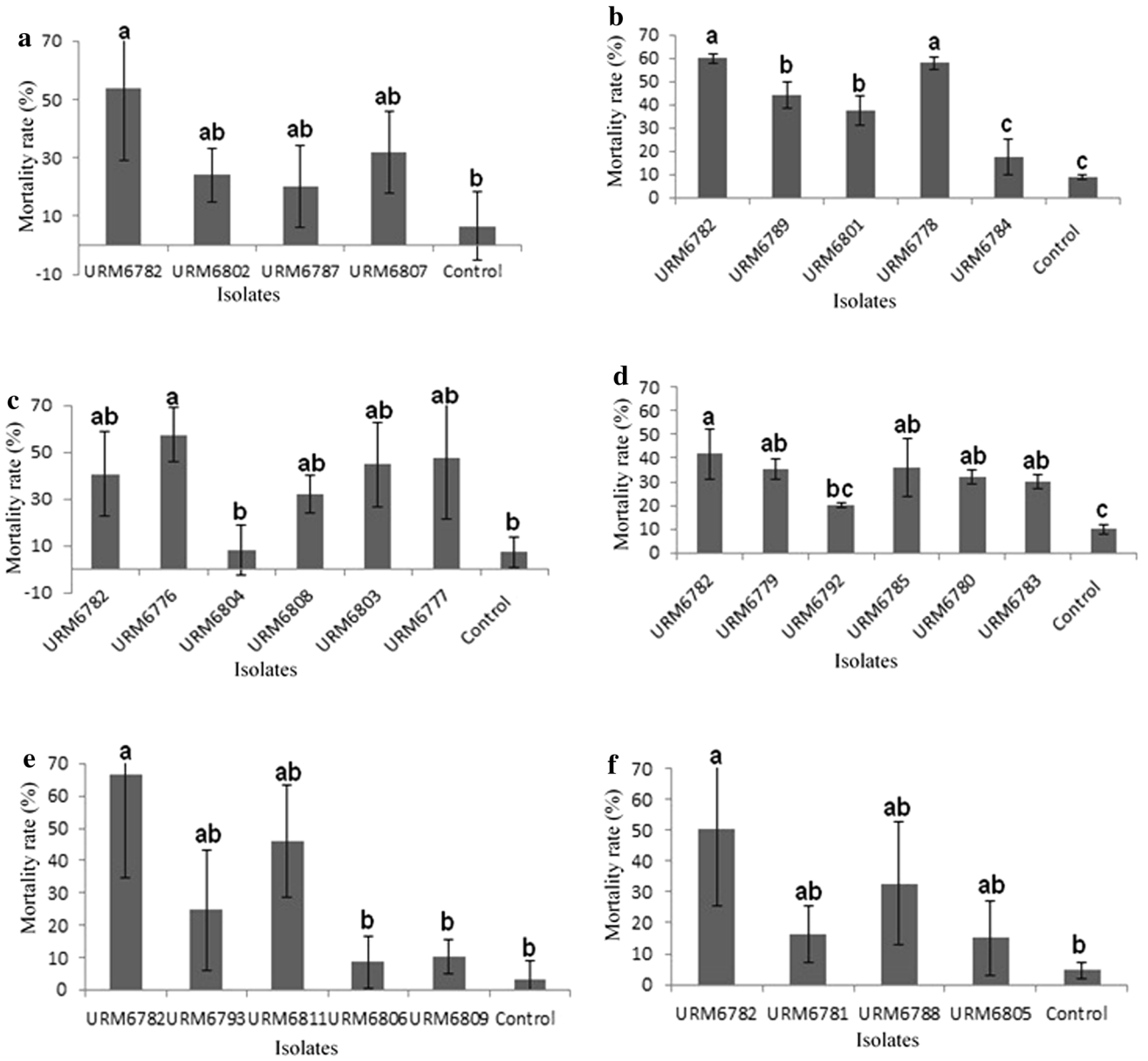

Fig. 1 Confirmed mortalities of adult *Dactylopius opuntiae* scale-insects (females) 12 days after spraying with isolates of the *Fusarium incarnatum–equiseti* species complex, in six bioassays. The isolates were identified using morphological characteristics. **a** Bioassay I—URM6782, URM6802, URM6787, URM6807; **b** Bioassay II—URM6782, URM6789, URM6801, URM6778, URM6784; **c** Bioassay III—URM6782, URM6776, URM6804, URM6808, URM6803, URM6777; **d** Bioassay IV—URM6782, URM6779, URM6792, URM6785, URM6780. URM6783; **e** Bioassay V—URM6782, URM6793, URM6811, URM6806, URM6809; **f** Bioassay VI—URM6782, URM6781, URM6788, URM6805

Discussion

Researchers have demonstrated that many insect species can be efficiently controlled by different species of *Fusarium*: *Fusarium solani* (Mart.) Sacc. was described occurring on *Tetanops myopaeformis* (Röder) (Diptera: Ulidiidae) (Majumbar et al. 2008) and on *Brahmina coriacea* (Hope) (Coleoptera: Scarabaeidae) (Anupam et al. 2012); *Fusarium pallidoroseum* (Cooke) Sacc. was able to cause, in only 9 days after inoculation, the death of 100% of *Lymantria obfuscate* (Walker) (Lymantriidae: Lepidoptera) (Munshi et al. 2008); *Fusarium lateritium* (Nees) was found to be efficient in controlling *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) (Anand and Tiwary 2009); the first isolation of *Fusarium verticillioides* (Sacc.) Nirenberg was described from the grasshopper *Tropidacris collaris* (Stoll) (Orthoptera: Acridoidea: Romaleidae) in 2011, and its pathogenicity was likewise confirmed against

Table 2 Mortality rates of *Dactylopius opuntiae* caused by isolates of the *Fusarium incarnatum–equiseti* species complex (FIESC 20) applied in the field; isolate identities were based on morphological characteristics and confirmed by DNA fingerprinting

Treatment	Mortality rate (%)/from isolated used	
	Based on morphological identification	Verified by molecular confirmation
URM6782	42.66 ± 9.01a	84.52% ± 5.2a
URM6778	40.66 ± 6.11a	54.17% ± 9.8b
URM6811	14.00 ± 6.00b	19.76% ± 5.3c
Control	5.33 b ± 1.15b	0.0% ± 0.0d

Means followed by the same letter on same column are not significantly different by Tukey test ($P \leq 0.05$)

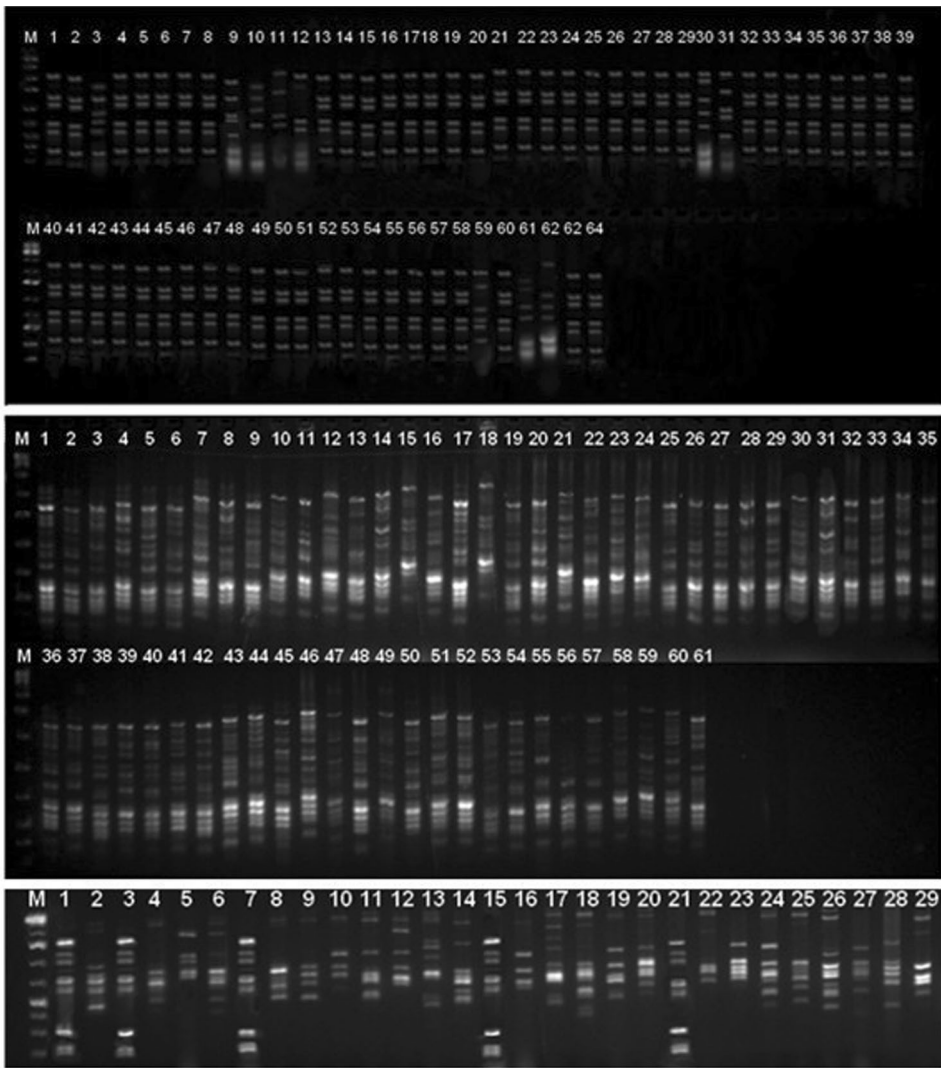

Fig. 2 Amplification profiles of the three isolates of the *Fusarium incarnatum–equiseti* species complex (FIESC-20) utilizing the UBC834 primer ISSR marker. **a** Profiles of the URM6782 isolate (*lane 1*, profile of the isolate before being released in the field; lanes 2–64 profiles of the fungal isolates after release [*lanes* 2, *4–8, 13–29, 32–58, 60, 62–64*] have the same profile of the *lane 1*). **b** Profiles of the URM6778 isolate (*lane 1*, profile of the isolate before being released in the field; *lanes 2–61*, profiles of the fungal isolates after release [*lanes 2, 3, 5, 6, 9, 11, 13, 17, 19, 20, 22, 25–29, 32, 33, 35–42, 45, 48, 50, 53, 54–61*] have the same profile of the *lane 1*). **c** Profiles of the isolate URM6811 and of the control group (*lane 1*, profile of the isolate before being released in the field; *lanes 2–21*, profiles of the fungal isolates after release [3, 7, 15–21] have the same profile of the *lane 1*). *Lanes 22–29*, profiles of the isolates of the control group (no profile was the same isolates released in field). Lane M, 1 Kb molecular weight marker

the grasshopper *Ronderosia bergi* (Stål) (Acridoidea: Acrididae: Melanoplinae) (Pelizza et al. 2011); *Fusarium avenaceum* (Fries) Sacc. was reported to be a pathogen for *Sitophilus oryzae* (L.) adults (Coleoptera: Curculionidae) (Batta 2012); *Fusarium proliferatum* Nirenberg was observed to cause mortality in *Thaumastocoris peregrinus* (Carpintero and Dellapé) (Hemiptera: Thaumastocoridae) (Lazo 2012) and to grow on the cadavers of larva, pupa, and adults of *Dryocosmus kuriphilus* (Yasumatsu) (Hymenoptera: Cynipidae) in the field (Tosi et al. 2015); *Fusarium oxysporum* Schlecht was observed to cause mortality in *Chloropulvinaria* (*Pulvinaria*) *psidii* (Maskel)

(Hemiptera: Coccidae) (Golpalakrishnan and Naraynan 1989).

Isolates of the FIESC have also been reported as causing mortality in adult females of *Matsucoccus matsumurae* (Kuwana) (Hemiptera: Coccoidea: Matsucoccidae) (Liu et al. 2014) and was observed to cause mortality in *Coccus hesperidum* L. (Hemiptera: Coccidae) (Fan et al. 2014). Isolates of FIESC 25-b and FIESC 3-b were likewise observed to cause high death rates (60–70%) in the chestnut gall wasp *Dryocosmus kuriphilus* (Yasumatsu) (Hymenoptera: Cynipidae) (Addario and Turchetti 2011).

Pathogenicity tests are necessary to select isolates of entomopathogenic fungi that can be used in biological control situations.

The death rates associated with the FIESC-20 isolates tested here allowed the selection of those most efficient in controlling *D. opuntiae* representing the first step in a biological control program directed against that cochineal scale pest. A number of these isolates were studied in relation to their compatibility with plant extracts toxic to insects, and the isolate URM6778, in combination with the water extract of *Ricinus communis* caused 100% mortality in *D. opuntiae* (Santos et al. 2016).

The observed variations in the death rates of cochineal scale exposed to the isolate URM6782 in the different bioassays may reflect variations in the quantities of wax covering the insects. In spite of the fact that the cladodes had been sprayed with neutral detergent to reduce those wax coatings, considerable amounts of that substance still remained on the exoskeletons of the insects. Additionally, Carvalho et al. (2005) observed that in spite of being able to standardize the number of days after cladode infestation by cochineal scale, their distributions on the cactus and the quantities of wax covering their bodies could not be controlled, making it difficult for the fungus to penetrate the wax layers and infect the individuals located inside colony interiors.

According to Alves (1998), the choice of entomopathogenic fungal isolates to be used in biological control programs must be based on their high pathogenicity to that insect target and the existence of adequate fungal production. As such, we selected those isolates that demonstrated the greatest cochineal scale mortality rates as well as good sporulation characteristics for the field tests.

The lower mortality rates observed in the field trials, as compared to laboratory tests, probably reflect environmental factors. Field conditions are difficult to control, and various factors can interfere in the efficiency of fungal control of *D. opuntiae*. After spraying, the fungi and their spores are exposed to factors such as solar UV radiation, temperature, relative humidity, and rainfall variations that can compromise their survival, propagation, and host infection (Goettel et al. 2000). Temperature and relative humidity may have affected the field trials, as during the spraying period temperatures varied between 18 and 25.6 °C and the relative humidity (RU) between 72 and 57%. Under laboratory conditions, by contrast, temperature and humidity could be controlled and the fungal spores were not exposed to any UV radiation.

The ability to monitor the dispersal and establishment of a fungus once it is liberated into the field is of critical importance for efficiency testing (Leal et al. 1997), as *Fusarium* is an abundant and cosmopolitan fungus and researchers need means to distinguish it from native isolates. The absence of clear morphological characteristics that could definitively distinguish *Fusarium* species has resulted in ample taxonomic circumscriptions, making field monitoring difficult. Additionally, their morphological characteristics are influenced by environmental factors, with requirements for specific conditions for manifesting optimal identification morphologies—which requires more time and effort but often results in imprecise or unreliable identifications (Leslie and Summerell 2006; O'Donnell et al. 2009, 2012). As such, molecular data combined with morphological characteristics allow more rapid and reliable identifications of isolates released into the field. DNA fingerprinting has been found to be satisfactorily efficient among the different techniques that can be used to accompany isolates tested in field trials and distinguish between them and native isolates (and thus monitor their entomopathogenicity) (Destéfano et al. 2004).

Our research group has been working to refine this technique. Preliminary tests using ISSR primers to identify the FIESC isolates identified in the present work showed that the UBC834 primer generated differences in the amplification profiles of all of the isolates tested (Tiago et al. 2016). The analysis of the amplification profiles by this primer, as seen in the present work, has allowed us to determine that most of the FIESC-20 phenotypes re-isolated from *D. opuntiae* insect cadavers were the same as the three utilized to infect them, therefore providing precise information concerning mortality and demonstrating the efficiency of that primer in DNA fingerprinting the entomopathogens tested and distinguishing them from native isolates.

A number of workers have suggested the use of ISSR primers to separate intraspecific pathogenic phenotypes of *Fusarium* to map their distributions in the field (Kiprop et al. 2002; Bayraktar et al. 2007; Sharma et al. 2009; Baysal et al. 2010), but there have been no reports of studies using DNA fingerprinting to actually monitor entomopathogenic fungi.

Some of the *Fusarium* phenotypes recovered from *D. opuntiae* in the present study were not the same as those introduced into the field, indicating that native isolates are naturally controlling that insect, even though at low rates.

This fact is of particular relevance in considering strategies for using those entomopathogens in biological insect control.

The strategies for introducing entomopathogenic fungi can be based on either saturation or on incremental approaches. The saturation strategy is designed to rapidly suppress a pest by applying large inoculates of a pathogen (even in regions where that pest might not be present). The pathogen, which is normally expected to act rapidly, should be effective independent of the population density of the pest and any natural isolates in the area, assuming, therefore, characteristics of a chemical insecticide application. The incremental strategy, on the other hand, assumes that a pathogen is already present at the site (although its occurrence on the pest is behind the infection curve and will probably not avoid damage to the plants in question). As such, the pathogen will require a boost in its density that can anticipate an epizootic event and reduce pest populations before they reach economic damage levels (Alves and Lecuona 1998). Thus, the choice of which strategy will best result in pest population reductions will depend on economic, ecological, toxicological, and social contexts.

Independent of which strategy will be used, however, the principal objective of biological control is to maintain insect pests at population levels that do not economically impact the crop but still maintain an ecological equilibrium (Alves 1998).

The data obtained in the present work reinforce the necessity of monitoring biological control agents at the genome level, both before and after their application in the field, to certify their effective and specific actions in controlling carmine cochineal scale. The use of morphological and molecular studies will therefore generate greater confidence in the use of entomopathogenic agents in biological control programs and increase their effectiveness.

The isolates URM6782 and URM6778 controlled cochineal scale insects. The present work represents the first phase of studies investigating the biological control of *D. opuntiae* through the use of new isolates from the FIESC, and additional studies will still be necessary to determine the methodologies that will be necessary for commercial production of the most efficient isolates as economically viable and efficient alternatives for large-scale control of insect pests attacking the edible cactus *O. ficus-indica*.

Author contributions

PVT, AFC and NTO conceived and designed the research. MPCL, PVT and LVM conducted experiments. MPCL, PVT and NTO analyzed the data. All authors wrote, read and approved the manuscript.

Acknowledgements Research supported by the Programa Nacional de Pós-Doutorado-Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (PNPD-CAPES; Pos-Doctorate grant given to the first author).

Funding This study was funded by Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES (Grant No. 2913-2011).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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

Informed consent Informed consent was obtained from all individual participants included in the study.

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