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Characterization of entomopathogenic fungi from vineyards in Argentina with potential as biological control agents against the European grapevine moth *Lobesia botrana*

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Abstract Biological control by entomopathogenic fungi is a possible alternative to chemical insecticides. As the grapevine moth *Lobesia botrana* (Lepidoptera: Tortricidae), recently introduced in Argentina, is now the major pest in most of the vineyards in the country despite quarantine regulations, native entomopathogenic fungi could be a preferable alternative to current synthetic insecticides. Thus, the aim of this study was to characterize native fungus strains isolated from 45 soil samples, using larvae of *L. botrana* as bait insect, and infected arthropods from the wine-growing region in the west of Argentina. Sixteen strains were identified, belonging to two species: four strains to *Beauveria bassiana* (Hypocreales: Cordycipitaceae) and 12 strains to *Metarhizium robertsii* (Hypocreales: Clavicipitaceae). Based on their physiological features, *M. robertsii* strains collected from the west region of Argentina seem to be the most tolerant to the high temperatures specific to this region. Therefore, being well adapted to Argentinean climate conditions, some of the native *M. robertsii* could be proposed as biological control agents against *L. botrana*.

Keywords *Beauveria bassiana* · *Metarhizium robertsii* · *Lobesia botrana* · Biocontrol · Viticulture · Argentina · *Hypocreales*

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Introduction

The European grapevine moth *Lobesia botrana* Denis & Schiffermüller (Lepidoptera: Tortricidae), the main pest in European vineyards (Delbac and Thiéry 2016), was recently introduced in the American continent. It reached Argentina in 2010, when it was declared a quarantine pest (National Service of Sanity and Quality of the Agri-food sector, SENASA, administrative resolution No. 122/2010). Despite regulations designed to protect grapevines against this introduced pest, based on compulsory large-scale insecticide treatments, *L. botrana* continued to spread and is now recorded in most Argentinean vineyards (López Plantey unpublished data).

Therefore, the use of entomopathogenic fungi as a control strategy for lepidoptera, known from ancient

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times, could be a preferable alternative to chemical treatment, since their natural metabolite activity is generally selective and prevents the emergence of resistance in pest populations (Butt et al. 2016). Commercial entomopathogenic fungi products used for biological pest control are mainly produced from *Beauveria* and *Metarhizium* strains. Several species within the *Beauveria* genus have been found to be highly effective for the control of a wide variety of phytophagous pests, including lepidopteran (Bidochka and Khachatourians 1991; de Faria and Wraight 2007; Safavi et al. 2010; Wraight et al. 2010 and Baskar et al. 2012). Concerning *L. botrana*, it has been shown that the *Beauveria bassiana* ITEM-1559 strain has a high capacity to control *L. botrana* larvae, causing 55% of deaths in Italian populations (Cozzi et al. 2013). Some studies have shown that *Metarhizium anisopliae* sensu lato can be highly effective to control Lepidopteran pests such as *Spodoptera litura,* Noctuidae (Sahayaraj and Borgio 2010). Those fungi are commonly found in both cultivated and undisturbed soils, although their natural distribution appears to be linked to habitat and agricultural practices (Meyling and Eilenberg 2007; Jaronski 2007; Meyling et al. 2009). Furthermore, the efficacy and persistence of these fungal treatments against pests are conditioned by biotic factors (Quesada-Moraga et al. 2007; Jaronski 2007; Thompson et al. 2006) and abiotic factors (Jaronski 2009). Therefore, a greater understanding of entomopathogenic fungus ecology is needed to find the optimal conditions and to define the limits in their use for biological control (Lacey et al. 2015).

If entomopathogenic fungi were to be implemented in Argentina to manage the *L. botrana* population, it should be essential to select the fungal strains whose survival and fitness are best adapted to such environmental conditions. As the performance of the fungi virulence may vary even among strains depending on biotic and abiotic factors, the main step in the implementation of the control strategy should be to isolate, identify and characterize native entomopathogenic fungal communities that are able to survive and preserve their pathogenicity under different climatic conditions. The western Argentinian wine-growing region is characterized by semi-arid weather with low precipitation (under 200 mm year^{-1}) mainly during winter, when the so-called *Zonda,* a warm, dry, downslope wind, can raise the temperature by over 15 °C in only a few hours, while the northern part is a semi-desertic mountainous area characterized by warm subtropical weather with rainfall concentrated during summer. The aim of this study was to characterize native fungus strains isolated from sample of soils, using larvae of *L. botrana* as bait insect, and infected arthropods from the wine-growing region in the west region of Argentina.

Materials and methods

Biological material

A total of 45 samples of soil and three infected arthropods (insects and mites found during the soil collection) were collected in 2014 along a 1945 km transect across the vine growing area in western Argentina. The samples were collected in seven provinces: Mendoza, San Juan, Catamarca, La Rioja, Salta, Neuquén and Río Negro. Four soil sub-samples were collected from each of the 45 sites. These subsamples were taken at a depth of 20 cm, close to the vine trunk. Each subsample was sieved up to a mesh size of 45 and unified to form a single sample which was preserved at 4 °C until its use (four months) (Inglis et al. 2012; Quesada-Moraga et al. 2007).

The entomopathogenic fungi were isolated from the soil adapting the technique of insect bait proposed by Meyling (2007). The adjustment consisted in the use of L4 and L5 instar larvae of *L. botrana* that were reared in an artificial medium (Ferreira et al. 2003) at the Entomology Laboratory of the Universidad Nacional de Cuyo (Mendoza, Argentina). The isolation technique consisted in distributing each sample into three glass Petri dishes (9 cm in diameter) with 20 g of humidified soil and five *L. botrana* larvae in each (n = 15 larvae per sample). The Petri dishes were conserved for seven days at controlled temperature and photoperiod (24 °C and L:D 12:12, respectively). After seven days of exposure to soil, dead larvae were treated with a solution of sodium hypochlorite (1% in volume) for 30 s for external disinfection and washed with sterilised distillate water and conserved in a humidity chamber (> 70%) to facilitate sporulation. After four days, larvae were placed in Petri dishes with a nutritive medium of Potato Dextrose Agar (PDA) 2% Biokar diagnostics® and added streptomycin SIGMA® (50 mg l^{-1}). For the infected arthropods occasionally found in the field during the soil sample

Table 1 Entomopathogenic strains collected from the vine-growing area of the west coast of Argentina, listed in alphabetic order

Strain	Genus	Substrate	GenBank	GPS localization
ACA	*Beauveria*	Acari	MG188796	S 32° 50.272 W 68° 45.934
BsoilAR-4.1(2)	*Beauveria*	*L. botrana* (in soil)	MH070615	S 33° 05.111 W 68° 54.540
BinsAR-1	*Beauveria*	Heteroptera	MK492665	S 23° 49.130 W 64° 47.250
BinsAR-2	*Beauveria*	Coleoptera	MH070614	S 33° 00.261 W 68° 52.398
MsoilAR-2.5(2)	*Metarhizium*	*L. botrana* (in soil)	MH070611	S 33° 01.774 W 68° 57.847
MsoilAR-4.3	*Metarhizium*	*L. botrana* (in soil)	MH070613	S 33° 05.119 W 68° 54.521
MsoilAR-18.5(3)	*Metarhizium*	*L. botrana* (in soil)	MH070612	S 29° 15.392 W 67° 25.868
MsoilAR-14.3(3)	*Metarhizium*	*L. botrana* (in soil)	MH070610	S 26° 12.151 W 65° 56.334
MsoilAR-2.3(2)	*Metarhizium*	*L. botrana* (in soil)	MK492657	S 33° 01.879 W 68° 57.731
MsoilAR-2.3(4)	*Metarhizium*	*L. botrana* (in soil)	MK492658	S 33° 01.874 W 68° 57.897
MsoilAR-10	*Metarhizium*	*L. botrana* (in soil)	MK492659	S 26° 05.952 W 65° 57.957
MsoilAR-15	*Metarhizium*	*L. botrana* (in soil)	MK462660	S 26° 12.029 W 65° 56.509
MsoilAR-16.9	*Metarhizium*	*L. botrana* (in soil)	MK462661	S 29° 09.235 W 67° 23.349
MsoilAR-16.7	*Metarhizium*	*L. botrana* (in soil)	MK492662	S 29° 09.275 W 67° 23.185
MsoilAR-17.2	*Metarhizium*	*L. botrana* (in soil)	MK492663	S 29° 11.992 W 67° 25.108
MsoilAR-17.5	*Metarhizium*	*L. botrana* (in soil)	MK492664	S 29° 12.011 W 67° 25.144

collection, the isolation procedure was similar to that of soil-isolated strains. Infected arthropods were submitted to an external disinfection and placed directly in Petri dishes with the same nutritive medium as soil strains.

Monosporic entomopathogenic fungi strains were obtained from the original cultures, using the methodology proposed by Inglis et al. (2012). Fungi growth and multiplication were observed on PDA, in 50-mm Petri dishes. The fungi collection was stored in the Phytopathology laboratory of the IBAM-FCA (Instituto de Biología Agrícola de Mendoza) by three methods: on glycerol 10% at − 80 °C, on PDA at 4 °C and lyophilised (Table 1).

Morpho-physiological characterization

The fungal strains were morphologically characterized based on their colony color, shape, and type of edge, as described by Humber (2012). Furthermore, each of the strains was colored using Gueguen's technique (Langeron and Vanbreuseghem 1952), observed in a Carl Zeiss© Axioplan® microscope at 1000 magnifications, and characterized according to the shape and size of conidiophores and conidia (n = 70 per sample) (Humber 2012). The length, width and the length/width ratio were measured for each strain, and the results were analyzed performing a one-way ANOVA with the InfoStat 2017p® software to test each variable separately and compare strains. Means were separated using the LSD Fisher test (alpha = 0.05).

In order to provide a physiological characterization of these fungi, the growth area of the colonies was assessed on PDA at five different temperatures (4 °C, 17 °C, 21 °C, 24 °C and 35 °C) using the UAT-CONICET® incubators (variation ∼ 1 °C). For this purpose, the colony area of each fungus strain was recorded daily, until it reached the edge of 55 mm-diameter Petri dishes (Boiteux et al. 2014). The average data of area growth on the tenth day for five biological repetitions (for each strain at different temperature conditions) was statistically analyzed performing a one-way ANOVA with the InfoStat 2017p® software to test the effect of strain for each temperature. Means were separated using the LSD Fisher test (alpha = 0.05).

Molecular identification

DNA extraction

The mycelium obtained from each of the fungi strains and cultivated as monosporic colonies was chopped

separately in 1.5 ml microtubes containing 500 µl of potato dextrose broth and then incubated at 24 °C for 72–96 h. The microtubes were centrifuged for 10 min at 13,000 rpm and placed in an ultra-freezer (− 80 °C) for a minimum of 3 h before lyophilisation in a Christ© alpha 1-4 LD plus® lyophilizer for a minimum of 16 h.

The DNA extraction was performed using the technique described by Zola and Pukkila (1986), with some adaptations. The lyophilized mycelium was lacerated mechanically with glass beads and resuspended in CTAB buffer extraction 1× (2 M Tris HCl at pH 8, 0.7 M ClNa, 0.5 M EDTA, 13.7 mM CTAB and 1% w/v PVP-40). The microtubes were placed in a water bath at 65 °C for 60 min. Isoamyl–chloroform (24:1) was added later to the microtubes and centrifuged for 10 min at 13,000 rpm. The supernatant was transferred to a new microtube to which isopropyl alcohol was added and kept cold (− 24 °C) for 2 h. Afterwards, the microtubes were centrifuged for 10 min at 13,000 rpm. The pellet was washed with 70% ethyl alcohol, centrifuging for 10 min at 13,000 rpm. The formed pellets were dried in a Savant© Speed Vac Concentrator® for 7 min. Finally, the obtained DNA was resuspended in 50 µl of miliq® water. The quality of DNA samples was verified by migration on electrophoresis 2% agarose gel at 100 V for 35 min. The DNA concentration was measured for each of the samples by means of the Nanodrop® spectrophotometer.

PCR and sequencing

The molecular identification and the phylogenetic characterisation of the Argentinean entomopathogenic fungi strains was carried out by using a partial sequence of the nuclear protein-encoding gene, the translation elongation factor 1-alpha (EF-1α). The fungi strains identified as *Beauveria* spp., according to the morphological identification, were also characterized by molecular methods using the primers 983F (5′-GCYCCYGGHCAYCGTGAYTTYAT) and 2218R (5′-ATGACACCRACRGCRACRGTYTG) and a touchdown PCR procedure, following the instructions by Rehner and Buckley (2005).

The fungi samples morphologically identified as *Metarhizium* were characterized by using the primers EF1T (5′-ATGGGTAAGGAGGACAAGAC) and EF2T (5′-GGAAGTACCAGTGATCATGTT) that amplified another fragment of the same nuclear protein-encoding gene, following the PCR conditions described in Bischoff et al. (2009). Finally, all samples were run in 2% Agarose gel at 100 V for 35 min to determine the presence and the quality of the specific band amplification (∼ 1200 pb for both primers).

All the samples were sequenced by the Beckam Coulter Genomics© company. The lower quality parts of each sequence were removed before making the multiple-sequence alignments of translation EF1-α (1200 bp long) by using the CLUSTAL W software (Thompson et al. 1994). Phylogenetic analyses included all the sequences produced for the present study and some supplementary sequences available in the NCBI GenBank® (Rehner and Buckley 2005; Bischoff et al. 2009; Meyling et al. 2009; Nishi et al. 2011, 2013; Zhang et al. 2013; Rehner et al. 2006, 2011; Denier and Bulmer 2015; Steinwender et al. 2014; Kepler et al. 2015; Rezende et al. 2015).

Two analyses were conducted with MEGA 7 (Tamura et al. 2013): maximum likelihood parsimony and maximum parsimony. Branch support was estimated by bootstrapping with 500 replicates.

Results

Characterization by morphological and molecular criteria

Out of the total collected samples (n = 45), 29% had a positive result, with at least one strain that could be isolated. Sixteen entomopathogenic fungi strains could be isolated and cultivated directly from soil samples or infected arthropods (Table 1) and they were differentiated into two well-defined groups according to the morphological criteria. For the first group, four strains were identified as *Beauveria* sp.: ACA, BinsAR-1 and BinsAR-2 from an infected hemipteran, coleopteran and mite and BsoilAR-4.1(2), which was isolated from the soil. These strains were collected in Mendoza province, the main wine-growing area of central Argentina. The fungal colonies on PDA displayed a regular round shape with well-defined edges and a compact cottony aspect, with a clear white coloring and light beige on the reverse, which specifically characterized these strains. Under the microscope, the fungus presented zigzagging conidiophores (sympodulospores) and conidia

Table 2 Conidial sizes of the studied strains of *Beauveria bassiana* and *Metarhizium robertsii*

Strain	Genus	Length (μm) F = 1465.26; df = 6, 406 ($p < 0.0001$)		Width (μm) F = 194.00; df = 6, 406 ($p < 0.0001$)		Length/width ratio F = 345.99; df = 6, 406 ($p < 0.0001$)	
		Mean (SE)		Mean (SE)		Mean (SE)	
ACA	*Beauveria*	1.92 (0.32)	a	1.53 (0.25)	a	1.27 (0.19)	a
BsoilAR-4.1(2)	*Beauveria*	1.88 (0.28)	a	1.56 (0.26)	a	1.22 (0.19)	a
BinsAR-2	*Beauveria*	2.66 (0.31)	b	2.24 (0.28)	b	1.20 (0.14)	a
MsoilAR-2.5(2)	*Metarhizium*	5.85 (0.44)	c	2.38 (0.25)	c	2.49 (0.30)	b
MsoilAR-4.3	*Metarhizium*	6.47 (0.50)	d	2.59 (0.33)	d	2.53 (0.33)	b
MsoilAR-18.5(3)	*Metarhizium*	7.24 (0.63)	e	2.89 (0.28)	e	2.53 (0.34)	b
MsoilAR-14.3(3)	*Metarhizium*	6.55 (0.52)	d	2.67 (0.27)	d	2.47 (0.28)	b

Values with the same letter indicate not significant differences ($p < 0.05$) for each measurement. Strains are listed in alphabetic order

showed, on average, a globular shape with a length of 2.23 μm (1.17–3.45 μm), a width of 1.84 μm (1.08–2.81 μm) and a length/width ratio of 1.23 (0.86–1.75) (Table 2). Genetic analysis, based on the EF-1α amplification, confirmed that the four sequences, morphologically identified as *Beauveria* sp., matched at 100% with *B. bassiana* sequences published on the NCBI GenBank (Fig. 1).

Twelve isolates were identified as *Metarhizium* sp. according to morphological criteria. All these strains were recuperated from the vineyard soils along the 1945 km transect across the western Argentinean vineyards (over 95% of the total Argentinean grape growing area). Microscopic observations revealed that the *Metarhizium* conidia were arranged in long chains and had a cylindrical shape with an average length of 6.58 μm (5.12–8.58 μm), a width of 2.65 μm (1.68–3.53 μm) and a length/width ratio of 2.51 (1.82–3.78) (Table 2).

Genetic analysis, based on the gene EF-1α amplification, confirmed that the 12 sequences morphologically identified as *Metarhizium* sp. matched at 99% the *Metarhizium robertsii* sequences published on the NCBI GenBank (Fig. 2). Among the 12 Argentinean *Metarhizium* strains, two distinct genotypes were identified: a first genotype that included the two isolates from Mendoza (MsoilAR-2.5(2), MsoilAR-4.3) and one from the northern region (MsoilAR-18.5(3)), and a second genotype including the strain from Salta province (MsoilAR-14.3). A high degree of similarity, greater than 90%, was observed within the *Metarhizium* group as well as in the *Beauveria* group.

Temperature-dependent growth of *B. bassiana* and *M. robertsii*

The physiological characterisation of *B. bassiana* and *M. robertsii* fungi, by the assessment of colony growth area across a wide temperature range, was done in order to test their capacity to adapt to the local conditions. From the two species, at least two strains we selected came from the north and at least two from the central region of Argentina. So we were able to analyse three strains of *B. bassiana* (ACA, BinsAR-2 and BsoilAR-4.1(2)) collected from central Argentina, and four *M. robertsii* strains: two collected from the same region in central Argentina (MsoilAR-2.5(2) and MsoilAR-4.3) and two other *M. robertsii* strains (MsoilAR-18.5(3) and MsoilAR-14.3(3)) collected from northern Argentina. As only one strain of *B. bassiana* was collected from northern Argentina (BinsAR-1), it was excluded from the analysis.

Two *B. bassiana* (ACA and BinsAR2) and the four *M. robertsii* strains showed low growth areas at 17 °C. Only BsoilAR4.1(2) showed statistically higher growth than the other strains. At 4 °C growth was very low for all strains. However, BsoilAR4.1(2) was significantly higher than the others. Different responses were obtained at the optimal temperature growth (21 °C and 24 °C). This behavior could not be attributed to the geographical origin of the strains or species (Table 3). However, at the highest temperature tested (35 °C) all four *M. robertsii* strains and one *B.* bassiana (BsoilAR-4.1(2)) still showed a larger growth area than the other two *B. bassiana* strains

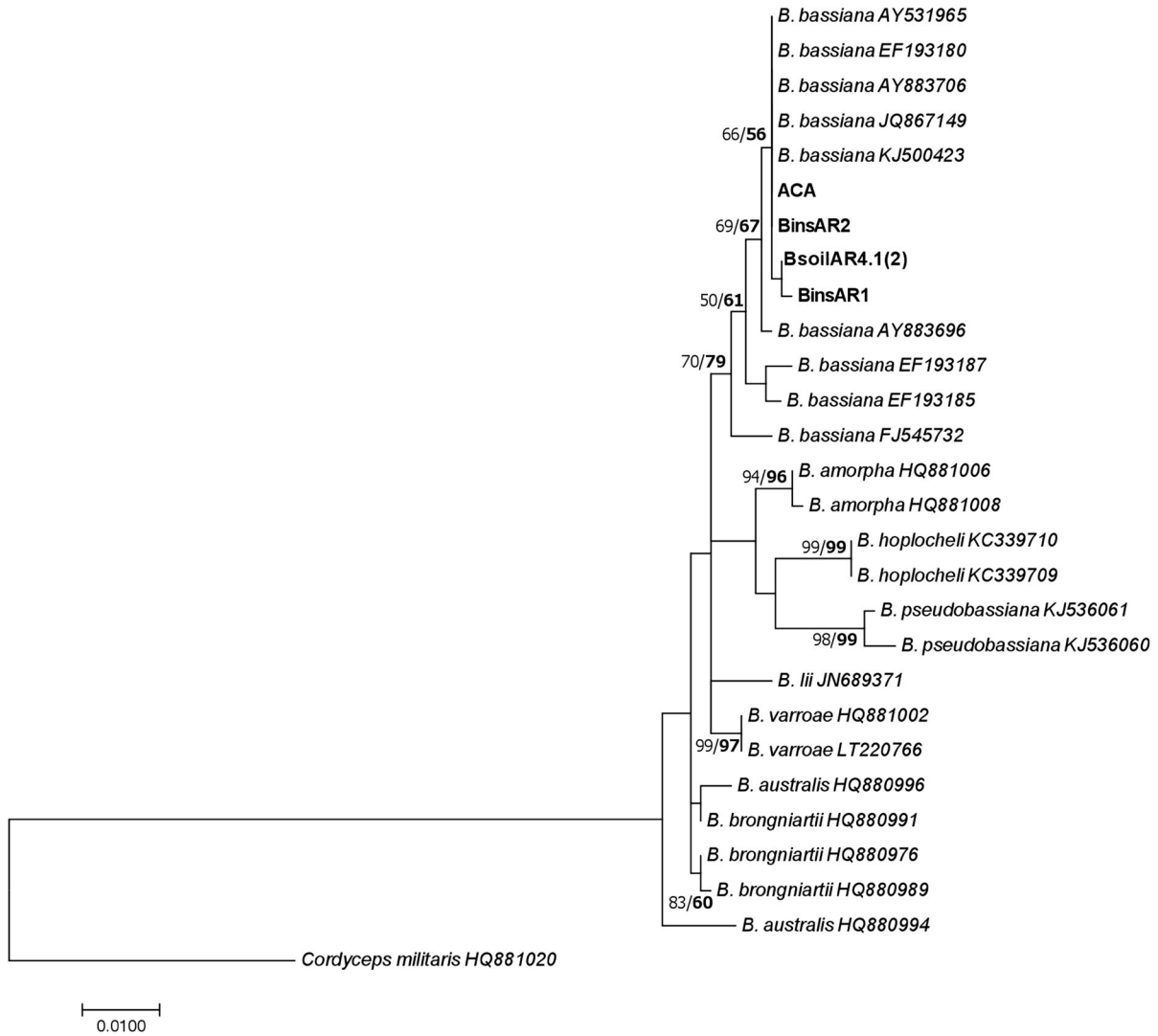

Fig. 1 Phylogeny of *Beauveria* as shown by joint maximum likelihood analysis of the elongation factor 1-α (TEF). Values indicated correspond to bootstrap ≥ 60% for maximum likelihood and maximum parsimony methods. Strains in bold indicate the origin from Argentinean vineyards. The scale bar represents 0.0100 substitutions per nucleotide position

(ACA and BinsAR2), which did not grow at 35 °C (Table 3).

Discussion

All the entomopathogenic fungi isolated along the 1945 km of the wine-growing region of the west of Argentina were characterized using morphological and genetic criteria. They were also characterized based on the temperature range of growth. The study of this physiological characteristic was significant, taking in account the great distance existing between the first sample taken in the north and the last one in the south of the country. Out of the entomopathogenic strains specifically baited from the soil with the *Lobesia botrana* larvae, the majority (12 out of a total of 16 isolated strains) were identified as *Metarhizium* spp. according to morphological criteria and only one strain was attributed to the *Beauveria* spp. Goble et al. (2010) also use insect pest larvae (*Ceratitis capitata* (Diptera: Tephritidae) and *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae)) as bait and compare the number of entomopathogenic strains isolated through

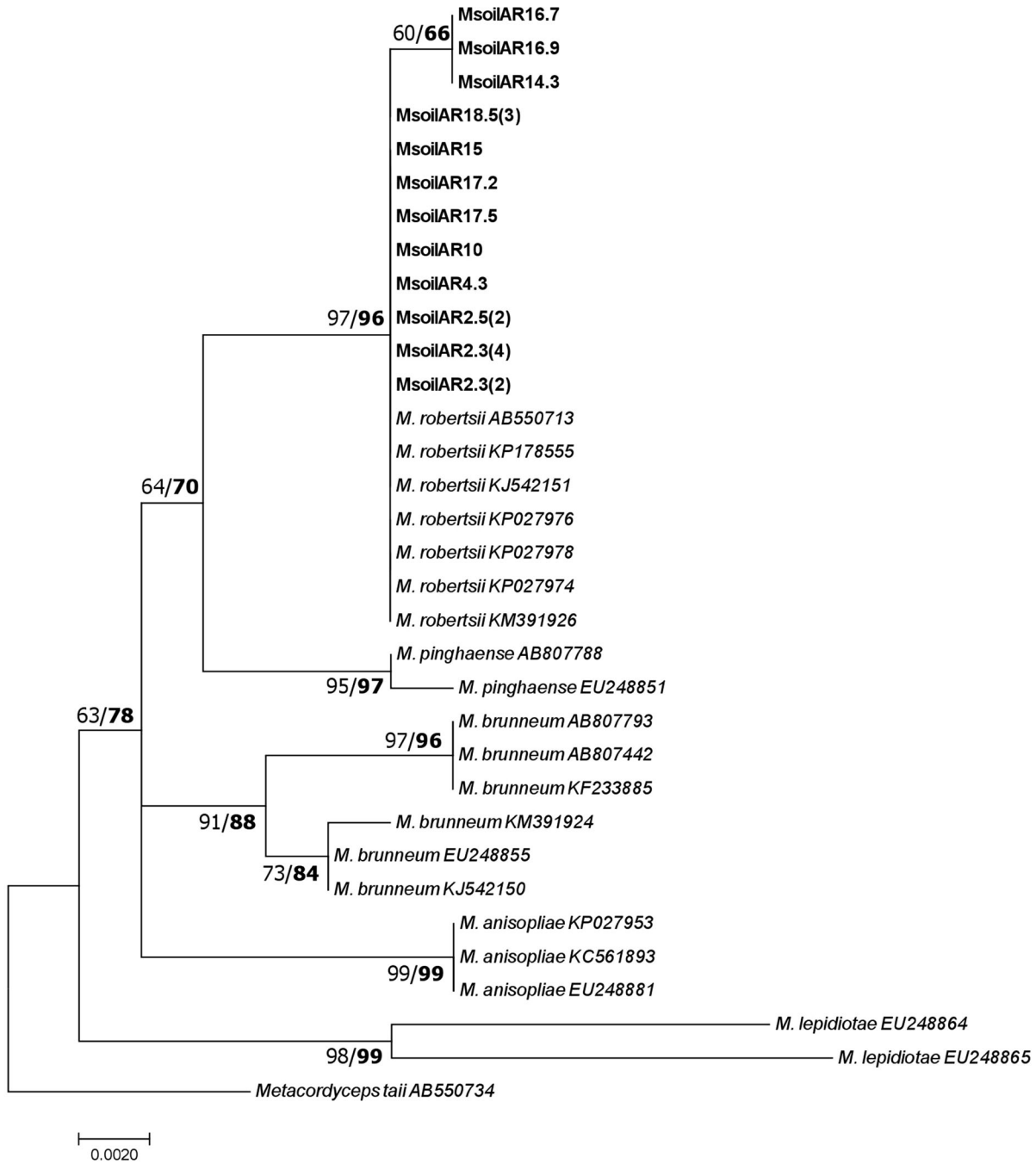

Fig. 2 Phylogeny of *Metarhizium* as shown by joint maximum likelihood analysis of the elongation factor 1-α (TEF). Values indicated correspond to bootstrap $\geq 60\%$ for maximum likelihood and maximum parsimony methods. Strains in bold indicate the origin from Argentinean vineyards. The scale bar represents 0.0020 substitutions per nucleotide position

the use of these pests with the standard technique using *Galleria mellonella* (Lepidoptera: Pyralidae). They found that there is a greater susceptibility of *G. mellonella* to entomopathogenic fungi, resulting in an excellent bait insect. Nevertheless, from a pest management point of view, the importance of the pathogenicity of the organisms found on target insect is not discussed. The use of the target insect as bait

Table 3 Comparison of growth area (cm^2) of *Beauveria* and *Metarhizium* strains at five different temperatures (°C) on the tenth day of culture

Strain	4 °C F = 11.02; df = 6, 14 (p = 0.0001)		17 °C F = 17.25; df = 6, 14 ($p < 0.0001$)		21 °C F = 3.22; df = 6, 14 (p = 0.0335)		24 °C F = 6.77; df = 6, 14 (p = 0.0016)		35 °C F = 22.55; df = 6, 14 ($p < 0.0001$)	
	Mean (SE)		Mean (SE)		Mean (SE)		Mean (SE)		Mean (SE)	
ACA	0.24 (0.03)	b	1.44 (0.16)	a	7.62 (0.31)	a b c	4.54 (0.49)	a	0.31 (0.09)	a
BsoilAR4.1(2)	0.33 (0.04)	c	6.61 (2.15)	b	8.89 (2.26)	b c	9.97 (0.97)	d	3.59 (0.38)	c
BinsAR2	0.21 (0.01)	a b	1.21 (0.14)	a	8.83 (0.72)	b c	6.32 (0.76)	a b	0.54 (0.09)	a
MsoilAR2.5(2)	0.24 (0.03)	b	2.15 (0.12)	a	11.95 (5.79)	c	9.00 (1.23)	c d	3.18 (1.13)	b c
MsoilAR4.3	0.20 (0.02)	a	1.70 (0.14)	a	5.80 (0.49)	a b	4.91 (0.68)	a	2.48 (0.48)	b
MsoilAR14.3	0.18 (0.01)	a	1.57 (0.13)	a	8.81 (1.51)	b c	7.33 (2.81)	b c	3.61 (0.30)	c
MsoilAR18.5(3)	0.24 (0.04)	b	0.97 (0.08)	a	3.77 (1.54)	a	5.45 (1.32)	a b	2.92 (0.18)	b c

Strain name in bold type indicates origin in the north of Argentina; strain name in regular type indicates origin in the central region. Different letters indicate significant differences (α = 0.05) in mean relative mycelial growth between strains at the same incubation temperature. Strains are listed in alphabetic order

insect would allow to advance on the pathogenicity studies, reducing the use of time resources and ensuring a possible inclusion in a management program. Three isolated *Beauveria* spp. were obtained directly from arthropods (hemipteran and mite). The finding of only two genera of entomopathogenic fungi in the samples coincides with what was reported by other authors, who report that although entomopathogenic fungi are common inhabitants of the soil, their diversity is low, with only one or two species coexisting (Goble et al. 2010; Quesada-Moraga et al. 2007; Keller et al. 2003).

Nonetheless, as in many genera of the Hypocreales order, the *Metarhizium* species could not be easily distinguished based on morphological features (Rehner and Buckley 2005; Tsui et al. 2006). Based on the data for EF-1α, all the 12 *Metarhizium* strains isolated from the wine-growing area in Argentina were identified as *Metarhizium robertsii*. The high prevalence of *M. robertsii* in Argentinean grape growing areas is consistent with the results by Bidochka et al. (2001) and Bidochka and Small (2005) in Canada, where this species was most frequent in soils from managed lands. However, Steinwender et al. (2014) described a different situation in agricultural fields and surrounding hedgerow in Denmark, with the co-occurrence of the least four *Metarhizium* species.

Four out of the 16 Argentinean strains collected in the central part of the country showed typical *Beauveria* morphological features. The species within this genus are very difficult to differentiate (Rehner et al. 2011; Oda et al. 2014), probably because of the existence of a *Beauveria* species complex. The use of translation EF-1α allowed us to identify all four strains as *Beauveria bassiana* sensu stricto El Kichaoui et al. (2017) have reported in the Gaza Strip the existence of the same two genera of entomopathogenic fungi in an agricultural environment that is fairly similar to that in western Argentina.

In this study, we have shown that the three *B. bassiana* strains and the four *M. robertsii* strains had an optimum growth at 21 °C, with a minimum growth at 4 °C, as many of the mesophilic fungi (Fargues et al. 1992). But the most striking result of this study is the capacity of all four *M. robertsii* strains, assessed in terms of colony area growth, to keep developing well even at the high temperatures tested (35 °C). Furthermore, the four *Metarhizium* strains showed the same growth fitness (area) at this high temperature, despite their origin (wine-growing area of central and northern Argentina). Bischoff et al. (2009) mentioned that the genus is frequently isolated from tropical and temperate regions, as we saw in our study. Meyling and Eilenberg (2007) explain that this tolerance to temporal high temperature could be an adaptation of a specific genetic group to agroecosystem conditions, which appears to be more important than the geographical origin of the strains (Uma Devi et al. 2005). A genetically-distinct *M. anisopliae* strain group,

collected from the agricultural area in Canada (hay, alfalfa, corn, wheat, grapes, etc.), had also shown an ability for growth at high temperatures (i.e. 37 °C), while a second genetically distinct group, associated to forest areas, showed a cold-active growth ability (i.e. 8 °C). As those groups were morphologically indistinguishable, there were considered as different cryptic species (Bidochka and Small 2005). This ability to grow at high temperature should therefore act as selective force in determining population distribution patterns (Bidochka et al. 2001).

Considering the high summer temperatures, the thermal range—which can reach 20 °C—, scarce rainfalls and strong winds, it is obvious that the use of biocontrol agents as a way to control *L. botrana* in Argentina requires the selection of entomopathogenic fungi with the capacity to tolerate such conditions. Bearing this in mind, native *M. robertsii* strains, in particular, seem to show a specific growth-ability at high temperatures, being adapted to the climate conditions of northern and also central Argentina. *B. bassiana* strain BsoilAR-4.1(2) could also be considered as good candidates to be used as biocontrol agents against *L. botrana*, mainly in the wine-growing environment of central Argentina. The mentioned physiological adaptation characteristics of isolated strains, together with the essential fact that the fungi were specifically obtained from the use of *L. botrana* larvae as a bait insect (Klingen et al. 2002), in conjunction with a low negative impact agroecosystem management can provide evidence in favor of the use of these fungi as microbiological control agents.

It has been shown that the persistence of entomopathogenic fungi in agricultural areas could also be influenced by agricultural practices, such as organic or conventional production systems, and by fungicide applications (Lacey et al. 2015). However, in viticulture, as a perennial cropping system with a high potential to serve as a habitat to entomopathogenic fungi, the abundance of *Metarhizium* spp. does not seem to be impacted by these agricultural practices (Uzman et al. 2019). Experiences carried out in the Laboratorio de Fitopatología (UNCuyo) show that the main fungicides used in Argentine viticulture would not have a negative effect on the normal development of native isolates, producing fungistatic, rather than fungicidal effects (unpublished data). These findings have significant implications for their use as microbiological control agents in viticulture.

In an integrated pest management program, the correct combination of different tools to manage pest population is essential if one is to maintain the program under a certain economic threshold. Entomopathogenic fungi could be an important tool in the context of a control strategy, in combination with an integrated pest management program in which these organisms can act as a complementary tool to the use of pesticides. This could reduce the application of synthetic products and allow their rotation, with a view to reducing the risks of selecting resistant individuals, while having a low negative environmental impact and reducing the risks of killing non-target insects as the strains were selected for their action against the studied pest. Strain characterisation according to pathogenic potential against these grapevine pests in Argentina will be the topic of further study carried out by our work team.

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Compliance with ethical standards

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

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