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SHORT COMMUNICATION

New records of hypocrealean fungi infecting aphids and whiteflies: pathogenicity against *Myzus persicae* and interaction with its predator *Eriopsis connexa*

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Occurrence of hypocrealean entomopathogenic fungi in Argentina is reported. Bioassays were performed to evaluate their pathogenicity against *Myzus persicae* and *Eriopsis connexa*. The findings underscore the importance of preserving these fungi and of investigating their potential for vector control.

Keywords: bioassays; entomopathogenic fungi; *Eriopsis connexa*; Hypocreales; *Myzus persicae*

The green-peach aphid *Myzus persicae* Sulzer (Hemiptera: Aphididae) is one of the major horticultural pests worldwide. It transmits more than 100 plant viruses (Blackman and Eastop 2000). Fungi are among the most common and significant microbial pathogens of both insects and arachnids, such as mites and spiders. At least 16 species of fungi are known to infect aphids in nature, and several species frequently cause epizootics among aphid populations (Lacey, Frutos, Kaya, and Vail 2001; Pell, Eilenberg, Hajek, and Steinkraus 2001; Chen, Li, and Feng 2008). In Argentina, few entomopathogenic fungi for Aphididae have been documented, mainly the Entomophthorales fungi (López Lastra and Scorsetti 2006, 2007; Scorsetti, Humber, Garcia, and López Lastra 2007; Cedola and Greco 2010; Scorsetti, Macia, Steinkraus, and Lopez Lastra 2010).

Aphids are also attacked by other natural enemies including various arthropod predators and hymenopterous parasitoids. Coccinellids are a major group of natural enemies that could be considered generalist predators, but nevertheless show a certain preference for soft-body prey such as the whiteflies, aphids, mealybugs, and mites (Michaud and Grant 2003). The coccinellid, *Eriopsis connexa* (Germar) (Coleoptera: Coccinellidae) is common in agroecosystems of the Neotropical Region in horticultural crops and is considered as a potential control agent (Sarmiento et al. 2007).

Entomopathogenic fungi can have detrimental effects on intraguild predators. Sixteen genera of coccinellids have been recorded as having been infected by the entomopathogenic fungus *Beauveria bassiana* Vuill. (Ascomycota: Hypocreales) (Yeo 2000). Other species of entomopathogenic fungi have also been found to be pathogenic for coccinellids (James and Lighthart 1994). On the basis of these

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considerations, a greater understanding of the interactions among aphids, entomopathogenic fungi, and natural enemies of the aphids is clearly essential for a more effective aphid control.

The objectives of this study were to provide new records of natural infections of entomopathogenic fungi on insects (aphids and whiteflies) in Argentina, to select native entomopathogenic fungi that are virulent against *M. persicae*, and to evaluate the direct virulence of a selected isolate against *E. connexa*.

Entomopathogenic fungi were isolated from pests among the Hemiptera (Aphididae, Aleyrodida) as well as from forest soil samples (Table 1). Isolates were collected from the Argentine provinces of Buenos Aires, Corrientes and Tierra de Fuego. Collections of insects were made following the methodology described by Scorsetti et al. (2007). Fungal species were identified according to the taxonomic keys and monographs in Gams (1971), Samson (1974), and Zare and Gams (2001). Monosporic isolates were obtained from infected insects as described by Lecuona (1996). Isolates were deposited at the Culture Collection of the Instituto de Botánica Carlos Spegazzini (LPSC), La Plata, Argentina and at the USDA-ARS Collection of Entomopathogenic Fungal Cultures (ARSEF), Ithaca, New York.

The aphid *M. persicae* was cultured and assayed on cabbage (*Brassica oleracea* var. *capitata* L.) after Forbes, Frazer, and Chan (1985). Aphids were kept in ventilated cages at an insectary at $25 \pm 1^\circ\text{C}$ and $70 \pm 5\%$ relative humidity with a 16:8-h light:dark regime. *E. connexa* was reared according to Matsuka and Niiijima (1985)

Table 1. List of entomopathogenic fungi isolated from insect pests, host insect and locality.

Fungus species	Access No.	Host insect/substrate	Locality
<i>Lecanicillium lecanii</i>	LPSC 1089/	<i>Trialeurodes vaporariorum</i> /	El Peligro, La Plata,
	ARSEF 7207	<i>Apium graveolens</i>	Buenos Aires
	LPSC 1035/	<i>M. persicae</i> / <i>Brassica oleraceae</i>	La Plata, Buenos
	ARSEF 7462		Aires
<i>Lecanicillium longisporum</i>	LPSC 1090/	<i>T. vaporariorum</i> / <i>A. graveolens</i>	El Peligro, La Plata,
	ARSEF 7461		Buenos Aires
	LPSC 1092/	<i>Capitophorus elaeagnil</i> / <i>Cynara scolymus</i>	General Mansilla La
	ARSEF 7459		Plata, Buenos Aires
* <i>Lecanicillium muscarium</i>	LPSC 1091	<i>M. persicae</i> / <i>Capsicum annum</i>	Olmos, La Plata,
			Buenos Aires
	LPSC 1034/	<i>M. persicae</i> / <i>B. oleraceae</i>	La Plata, Buenos
	ARSEF 7782		Aires
<i>Isaria fumosorosea</i>	LPSC 1093/	<i>T. vaporariorum</i> / <i>A. graveolens</i>	El Peligro, La Plata,
	ARSEF 7460		Buenos Aires
	LPSC 1094/	<i>Aphis fabae</i> / <i>Solanum melongena</i>	General Mansilla, La
	ARSEF 7785		Plata, Buenos Aires
<i>Isaria javanica</i>	LPSC 1095/	<i>T. vaporariorum</i> / <i>A. graveolens</i>	El Peligro, La Plata,
	ARSEF 7205		Buenos Aires
	LPSC 1036	<i>Bemisia tabaci</i> / <i>Cucumis sativus</i>	Monte Caseros,
<i>Tolypocladium cylindrosporum</i>			Corrientes
	LPSC 1096/	<i>T. vaporariorum</i> / <i>Lycopersicon esculentum</i>	Colonia Urquiza, La
	ARSEF 7477		Plata, Buenos Aires
	LPSC 1065	Soil/ <i>Nothofagus pumilio</i>	Ushuaia, Tierra del
			Fuego

and under the same conditions as for *M. persicae* and fed *ad libitum* with nymphs and adults of the bird cherry-oat aphid, *Rhopalosiphum padi*, raised on wheat seedlings.

Twelve strains of native entomopathogenic fungi were used in this study and fungal pathogenicity was tested on *M. persicae* adults. Fungal isolates were cultured on 2% (v/v) malt-extract agar in Petri dishes and incubated for 7 days at 25°C in the dark. Conidia were then harvested with a disposable cell scraper (Fisherbrand®) and placed into test tubes containing 0.01% (v/v) Tween 80® (Merck). The suspension was adjusted to 1×10^7 conidia ml⁻¹ after counting in a Neubauer hemacytometer. The conidial viability of each isolate was assessed after 24 h through the use of the techniques described by Lane, Humphreys, Thompson, and Trinci (1988). Viability ranged between 93 and 100% on all occasions. The test insects were sprayed with 300 µl of a conidial suspension from a 35-ml glass atomizer, while the control insects were sprayed with 300 µl of 0.01% (v/v) Tween 80® alone. The experiment consisted of four replicate test groups and a control group, with all groups containing 20 insects each. Thereafter, the insects were arranged on a cabbage leaf and placed in a 35-mm Petri dish containing a sterilized moistened filter paper to maintain a 95% relative humidity. Both the treated and the control insects were incubated at $24 \pm 1^\circ\text{C}$ with a 16:8-h light:dark photoperiod. The cumulative mortality was recorded daily for 10 days. Dead insects were removed daily and their surfaces sterilized according to Lacey and Brooks (1997). The experiments were repeated three times under comparable laboratory conditions. The median lethal time (MLT) was estimated according to the methodology cited by Lecuona and Díaz (2001). The cumulative-mortality data were evaluated by the analysis of variance and their means by Fisher's least-significant-difference multiple-range test option ($P < 0.05$) through the use of a version of the InfoStat 2007 software (InfoStat 2001). Ten days post inoculation of the aphids in the pathogenicity test, we observed no significant differences between the treatments ($F = 4.05$, $df = 11$, $P = 0.4362$), and the mortality of the controls did not exceed 20%.

The 12 isolates were highly infective to adults of *M. persicae* and mortality ranged between 83 and 100% (Table 2). We observed significant differences between the average times of death (MLT) for each isolate ($F = 12.84$, $df = 11$, $P \leq 0.0001$; Table 2). Two strains of *Lecanicillium muscarium* (LPSC 1034 and 1093) were the most effective isolates (at 100% mortality); but since *L. muscarium* (LPSC 1034) had a shorter MLT (2.81 ± 0.25 days), it was selected for dose-response virulence tests and the *E. connexa* bioassays.

After evaluating the biological activity of the different fungal isolates, multiple-concentration assays were done with the most effective isolate as judged by its higher virulence and the lower MLT. Five serial concentrations of conidia, ranging from 1×10^2 to 1×10^6 conidia ml⁻¹, were assayed. The inocula were obtained by the same methodology as used for the virulence tests. Four replicates groups and a control group, all containing 20 insects, were treated at each dose. The cumulative mortality was recorded daily for 10 days. Dead insects were removed daily and their surfaces sterilized according to Lacey and Brooks (1997). The results of the dose-response test (LC 50) were analyzed by Probit analysis (Chi 1997). After 10 days a LC₅₀ of 1.6×10^3 conidia ml⁻¹ ($\alpha = 0.05$) ($\chi^2 = 0.74$, $df = 2$, $LC = 696.58/3255.61$) was obtained. This isolate showed higher virulence than Fournier and Brodeur

Table 2. Results of conidial germination, median lethal time (MLT) and cumulative mortality of fungal isolates evaluated against *M. persicae*.

Fungus species	Conidial viability (SD)	% Mortality (SD)	MLT (SD)*
<i>L. lecanii</i> LPSC 1089/ARSEF 7207	100%	96.07 (6.79)	4.32 (0.75) e
<i>L. lecanii</i> LPSC 1035/ARSEF 7462	93.1% (± 1.3)	92.15 (8.98)	4.19 (0.68) cd
<i>L. longisporum</i> LPSC 1090/ARSEF 7461	100%	90.66 (3.23)	4.78 (0.19) de
<i>L. longisporum</i> LPSC 1092/ARSEF 7459	100%	95.83 (7.21)	3.35 (0.26) ab
<i>L. longisporum</i> LPSC 1091	100%	95.83 (7.21)	4.95 (0.4) e
<i>L. muscarium</i> LPSC 1034/ARSEF 7782	98% (± 3.5)	100	2.81 (0.25) a
<i>L. muscarium</i> LPSC 1093/ARSEF 7460	95.3% (± 1.8)	100	3.9 (0.7) bc
<i>L. muscarium</i> LPSC 1094/ARSEF 7785	100%	88.23 (11.76)	4.32 (0.3) cde
<i>I. fumosorosea</i> LPSC 1095/ARSEF 7205	96.5% (± 3.15)	95.83 (3.60)	3.25 (0.1) ab
<i>I. fumosorosea</i> LPSC 1036	100%	89.58 (13.01)	3.82 (0.54) bc
<i>I. javanica</i> LPSC 1096/ARSEF 7477	98.8% (± 0.2)	92.56 (12.87)	3.1 (0.2) ab
<i>T. cylindrosporum</i> LPSC 1065	100%	83.75% (± 8.53)	5.92 (0.25) f

*Data are given in mean \pm SD; values followed by the same letters do not differ significantly according to least-significant-difference test ($P=0.05$).

(2000) had reported using *Lecanicillium* sp. against *M. persicae* with a LC_{50} of 2.3×10^6 conidia ml^{-1} .

Virulence tests against *E. connexa* were carried out by a modified dip method according to Cottrell and Shapiro-Ilan (2003). The inocula were obtained by the same methodology as used for the tests against aphids. The bioassays were performed on eggs, larvae, pupae, and adults at the same time. Two doses were used, 1×10^7 conidia ml^{-1} and the dose producing a 50% killing level ($LC_{50} = 1.6 \times 10^3$ conidia ml^{-1}) obtained from previous assays. The assay consisted of four replicate test groups and a control group, with all groups containing 20 insects each. Each beetle was transferred to a sterile 9-cm Petri dish and was maintained in a plastic container ($30 \times 16 \times 9$ cm) placed at 25°C, 14:10 h light:dark photoperiod and 88% relative humidity. The experiments were repeated three times under comparable laboratory conditions. The insects were fed in the same way as during rearing. Dead insects were removed daily and their surfaces sterilized according to Lacey and Brooks (1997).

The results of the virulence test against *E. connexa*, showed low cumulative mortality, with the mortality of the controls of only $2.2 \pm 0.57\%$, and no infection by entomopathogenic fungi registered. At the higher dose (1×10^7 conidia ml^{-1}) first and second instar larvae proved the most susceptible with $45 \pm 5\%$ and $30 \pm 5\%$ mortalities, respectively. The LC_{50} (1.6×10^3 conidia ml^{-1}) doses only caused a cumulative mortality of $5 \pm 0\%$ on first instar larvae. No eggs, pupae, or adults were found infected at both doses. All dead insects were infected by *L. muscarium*. Consistent with Steenberg and Harding (2009), these preliminary data indicate that larvae are more susceptible to infection than other developmental stages.

We found a considerable diversity of Hypocreales fungi on pests of Argentine horticultural crop systems. Among the six species recorded here, the two species isolated from aphids, *Lecanicillium longisporum* and *L. muscarium* (Table 1), are new records for South America; while the fungus *Lecanicillium lecanii*, isolated from *M. persicae*, represents a new record for Argentina. Although in South America there are reports of entomopathogenic fungi that infect other agricultural insect pests (Aruta, Carrillo, and González 1974; Lange 1996; Méndez Sánchez, Freitas, and Roberts 2001; Edelstein and Lecuona 2003; Pelizza, Cabello, and Lange 2010), there is little few documentation of such fungi infecting aphids (López Lastra and Scorsetti 2006; Scorsetti et al. 2007, 2010). These new records thus represent an extension of the aphid-host range for the Hypocreales fungi in South America.

Because of their host specificities, most fungal entomopathogens pose little risk to nontarget hosts (Rashki, Kharazi-pakdel, Allahyari, and van Alphen 2009), but possible fungal activities against beneficial arthropods in the target environment must also be considered. A consideration must be made of adult aphid predators' susceptibilities to the fungus, mainly with respect to damage attributable to direct and/or indirect infection as sublethal effects. Thus, the survey of native fungal species within the same natural environments of the target species is of high priority.

The strain of *L. muscarium* (LPSC 1034) was isolated from *M. persicae* on *Brassica oleraceae* crops in La Plata, Buenos Aires province. This fungus seems to be a good candidate for further development as a potential biological-control agent against aphid pests. Furthermore, our results support the hypothesis that the more virulent fungal strains are isolated from the target organism itself (Shah and Pell 2003), thus increasing the chances of having success in the realization of effective formulations for biological control of insect pests.

Further studies of the interactions with natural enemies need to be carried out under field conditions in order to confirm whether the laboratory results with this isolate do, in fact, accurately predict its performance in the field.

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