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Natural occurrence of entomopathogenic fungi (*Zygomycetes*: *Entomophthorales*) of aphid (*Hemiptera*: *Aphididae*) pests of horticultural crops in Argentina

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Abstract. A three-year survey of entomophthoralean pathogenic fungi of aphids from horticultural crops in La Plata, Argentina, was conducted. Nine species of aphids, including *Aphis fabae* Scopoli, *Aphis gossypii* Glover, *Brevicoryne brassicae* (L.), *Lipaphis erysimi* (Kaltenbach), *Macrosiphum euphorbiae* (Thomas), *Myzus* sp., *Myzus persicae* (Sulzer), *Nasonovia ribisnigri* (Mosley) and *Capitophorus elaeagni* (del Guercio) were recorded as hosts of entomopathogenic fungi. Six species of Entomophthorales that infected and killed aphids were found in vegetable crops. The fungal species identified were *Conidiobolus obscurus* (Hall & Dunn) Remaudière & Keller, *Entomophthora planchoniana* Cornu, *Neozygites fresenii* (Nowakowski) Remaudière & Keller, *Pandora neoaphidis* (Remaudière & Hennebert) Humber, *Zoophthora radicans* (Brefeld) Batko and *Zoophthora* sp. *Pandora neoaphidis* was the most predominant pathogen of aphids and was found throughout the summer (December–March) 2004. The recovery of *C. obscurus*, *N. fresenii* and *P. neoaphidis* represent first records of these fungi for South America.

Key words: aphididae, biological control, entomophthorales, entomopathogenic fungi, insect pest, vegetable crops, zygomycetes

Introduction

Aphids are among the most significant pest insects in forestry, agriculture and horticulture in the temperate climatic zones (Minks and Harrewijn, 1988). Control of aphids has been done predominantly by using chemical insecticides, however this practice has caused problems for the environment. Aphids are one of the most important factors

limiting horticultural crops production in Argentina (Botto, 1999). In ''Gran La Plata'', Buenos Aires, Argentina, there is one of the most important horticultural areas in this country.

Entomopathogenic fungi are considered to be among the best candidates for biological control of aphids (Latgé and Papierok, 1988). Because fungi invade mainly through the cuticle, these pathogens are especially important for the control of insects with sucking mouthparts such as aphids since they cannot ingest pathogens (Tanada and Kaya, 1993; Hajek and St. Leger, 1994).

Entomophthoralean fungi (Zygomycota: Zygomycetes) are a unique group of entomopathogens that cause natural epizootics among aphid populations (Feng et al., 1990; Wraight et al., 1993; Hatting et al., 1999). Several studies were reported about Entomophthorales infecting aphids from all over the world (Feng et al., 1990; Steinkraus et al., 1995; Hatting et al., 1999; Nielsen et al., 2001). To date, the species of entomophthoralean fungi identified from aphids belong to eight genera: *Conidiobolus, Entomophthora, Batkoa, Pandora, Erynia, Neozygites, Tarichium* and *Zoophthora* (Bałazy, 1993; Keller, 1987, 1991). Nevertheless very few reports of these fungi have dealt with collection sites in South America (Aruta et al., 1984; Méndez Sánchez et al., 2001, 2002a, b; López Lastra and Scorsetti, 2006).

The overall objective of the present paper was to identify and to isolate entomophthoralean fungi from aphid pests of vegetable crops in Argentina.

Materials and methods

Collection of insects

Surveys were conducted in organic and conventional horticultural crops in greenhouses and open fields in Buenos Aires province, Argentina. A total of six locations were surveyed: Colonia Urquiza (La Plata county) 34°56′19.2″ S / 58°06′3.8″ W; Pereyra Iraola (Berazategui county) (two close sites) 34°50′ 36.5″ S / 58°05′33.2″ W; El Peligro (La Plata county) 34°56′ S / 58°10′ W; Arana (La Plata county) 35°0′19.8″ S / 57°55′30″ W; General Mansilla (Magdalena county) 35°05′30.7″ S / 57°45′20.6″ W.

The vegetable crops sampled included *Lactuca sativa* (lettuce), *Lycopersicon esculentum* (tomato), *Brassica oleracea* (cabbage), *Capsicum annuum* (pepper), *Solanum melongena* (eggplant), *Beta vulgaris* var. *cicla* (Swiss chard), *Beta vulgaris* var. *conditiva* (red beet), *Cynara scolymus* (artichoke) and other horticultural crops.

Sampling was conducted over a 3-year period from Jan 2002 to Dec 2004 at weekly intervals. Twenty plants of each species were selected and checked each week from each field on a purely random basis. For immature instars and adults of the host aphids, two leaves of each level (upper, mid, and bottom) of the canopy of each plant were checked. In young plants or leafy vegetables the whole plant was checked. In plants that were found to be infested with aphids, all insects were collected. Living aphids were placed individually in plastic cups with lids (150 cm^3). Dead aphids with or without externals signs of mycosis were collected with the piece of plant material onto which they adhered and were placed in sterilized individual plastic containers (100 cm^3) for transport to the laboratory for the analyses.

Aphids without external signs of mycoses were put in Petri dishes of 60 mm diam with a filter paper moistened with few drops of distilled water and maintained at 20° C for 24–72 h to allow the more complete development of any fungi present in them. Healthy and infected aphids were preserved in 70% ethanol for further identification by the specialized entomologist.

Identification of fungal pathogens

Infected aphids with evidence of external fungal growth were examined with a dissecting stereomicroscope for evidence of fungal structures (rhizoids, cystidia, and spores). Fungal structures were mounted in lactophenol-aceto-orcein (LPAO) (1:1) or stained with 1% aceto–orcein plus glycerine for semipermanent mounts and observed with phase contrast using an Olympus CH3 microscope. Fungal preparations were photographed using a Nikon Optiphot microscope equipped with differential interference contrast (DIC) and fitted with a Canon Power Shot A80 camera.

Measurements of fungal structures from fresh infected cadavers were made to enable specific identification. Fungal species were identified according to taxonomic keys and monographs of Waterhouse and Brady (1982); Keller (1987, 1991); Bałazy (1993) and Humber (1989).

Isolation of fungal pathogens

Infected aphids with no externally apparent mycelium were surface sterilized by dipping them successively in 70% ethanol (10–15 s), 0.5% sodium hypochlorite solution (1 min), and sterile distilled water (1 min, two successive baths) (Lacey and Brooks, 1997). Insects were placed on a moistened piece of sterile filter paper attached with

double coated tape to the lid of a sterile 60 mm Petri dish, and then was inverted over the bottom of a sterile Petri dish containing SEMA (80% Sabouraud dextrose agar + 1% yeast extract and a 20% of a mixture of egg yolk and skim milk) (Papierok and Hajek, 1997) plus 40,000 units/ml penicillin G (Merck, Germany) and 80,000 units/ml streptomycin (Parafarm, Argentina). This assembly was left 12 h in darkness at 22 ± 1° C. A sterile lid replaced the lid with the attached aphid after 12 h. All isolates were incubated at 22 ± 1° C with a photoperiod of 16:8 (L:D).

Isolates were deposited in the Mycological Collections at the Centro de Estudios Parasitológicos y de Vectores (CEP, La Plata, Argentina), and in the USDA-ARS Collection of Entomopathogenic Fungal Cultures (ARSEF, Ithaca, New York). Herbarium materials such as dried infected specimens and microscope slides were deposited in the Mycological Collections of the Instituto de Botánica Spegazzini (LPSC, La Plata, Argentina).

Results

Nine species of aphids were observed to have been infected by entomophthoralean fungi: *Aphis fabae* Scopoli, *Aphis gossypii* Glover, *Brevicoryne brassicae* (L.), *Lipaphis erysimi* (Kaltenbach), *Macrosiphum euphorbiae* (Thomas), *Myzus* sp., *Myzus persicae* (Sulzer), *Nasonovia ribisnigri* (Mosley) and *Capitophorus elaeagni* (del Guercio) (Table 1).

Six species of Entomophthorales were identified from these aphids (Figure 1a–n) but only three of these species were successfully isolated and maintained in pure cultures

Conidiobolus obscurus (Hall & Dunn) Remaudière & Keller

Host: *Nasonovia ribisnigri, Brevicoryne brassicae, Myzus* sp.

Primary conidia 32.6 ± 1.1 × 24.9 ± 0.8 μm (31.8–33.4 × 24.3–25.5 μm; $n = 20$, 1 series) generally globose unitunicate, with basal hemispherical papilla emerging abruptly from the spore. Conidial cytoplasm presented a granular appearance in aceto-orcein but the nuclei remain unstained (Figure 1a, b). Secondary conidia morphologically similar to primary, 30.2 ± 1.3 × 20.5 ± 1.8 μm (28.5–32.1 × 19.1–23.2 μm; $n = 20$, 1 series). Conidiophores simple and unbranched. Resting spores spherical to subspherical, 33.5 ± 2.3 μm (30.6–39.3 μm; $n = 15$, 1 series), observed in field at July 03.

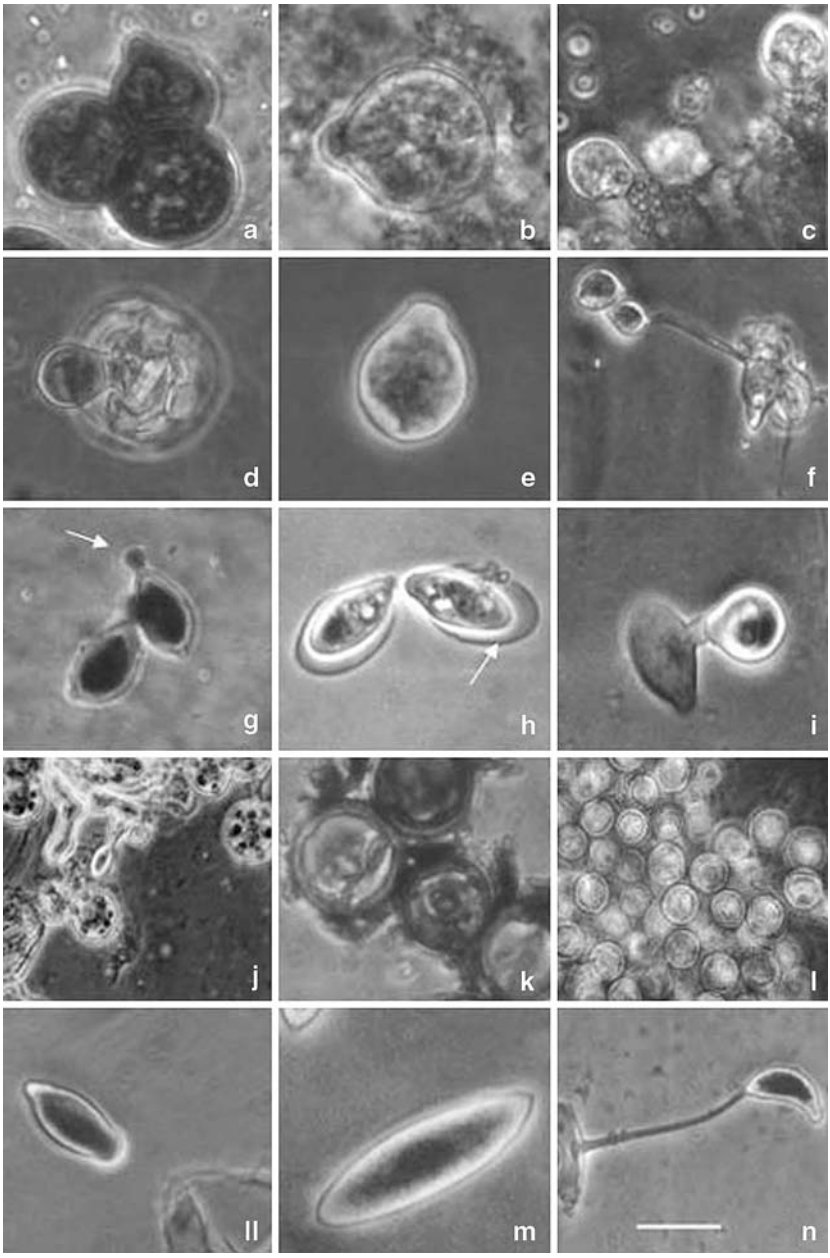
Table 1. Entomophthoralean fungi from aphids (Hemiptera: Aphididae) of vegetables crops in Buenos Aires province, Argentina

Fungal species	Host	Plant host	Locality	Date of collection
Conidiobolus obscurus (Hall & Dunn) Remaudière & Keller	*Nasonovia ribisnigri*	*Lactuca sativa*	P. P. Iraola (site 1)	Nov 02
			El Peligro	Jul 03
	Brevicoryne brassicae	*Brassica oleracea*	Colonia Urquiza	Apr 03
	Myzus sp.	*L. sativa*	Colonia Urquiza	Oct 03
Entomophthora planchoniana Cornu	*N. ribisnigri*	*Capsicum annuum*	P. P. Iraola (site 1)	Oct 02; Nov 02
	Aphis fabae	*Solanum melongena*	Colonia Urquiza	Apr 03
	Myzus sp.	*C. annuum*	Colonia Urquiza	May 03
	Myzus persicae	*Beta vulgaris var. conditiva*	Colonia Urquiza	May 03
	Capitophorus elaeagni	*Cynara scolymus*	Arana	Oct 03; Nov 03; Dec 03; Aug 04
			Colonia Urquiza	Aug 04
Neozygites fresenii (Nowakowski) Remaudière & Keller	*B. brassicae*	*B. oleracea*	Colonia Urquiza	Apr 03
			Arana	Mar 04
	Myzus sp.	*Beta vulgaris var. cicla*	Colonia Urquiza	May 03
	A. fabae	*S. melongena*	Colonia Urquiza	Apr 04
Pandora neoaphidis (Remaudière & Hennebert)	*N. ribisnigri*	*L. sativa*	Parque Pereyra Iraola	Sep 02; Oct 02; Nov 02; Sep 03; Oct 03; Apr 04; May 04

Table 1. Continued

Fungal species	Host	Plant host	Locality	Date of collection
Humber			P. P. Iraola (site 2)	Aug 04
			Colonia Urquiza	Nov 02; May 03; Jun 03; Jul 03; Aug 03; Nov 03; Apr 04
			El Peligro	Jun 03; Jul 03
			Arana	Nov 03; Dec 03; Mar 04; Aug 04
			General Mansilla	Aug 04
	A. fabae	*S. melongena*	Colonia Urquiza	Mar 03; Apr 03
	B. brassicae	*B. oleracea*	Colonia Urquiza	Apr 03; May 03
			El Peligro	Oct 03
			Arana	Nov 03
		B. oleracea var. botrytis	Colonia Urquiza	Nov 03
			P. Pereyra Iraola	Apr 04
	Myzus sp.	*B. vulgaris var. cicla*	Colonia Urquiza	Apr 03; May 03
			P. Pereyra Iraola	Jul 04
		L. sativa	Colonia Urquiza	May 03; Oct 03
	M. persicae	*B. vulgaris* var. *conditiva*	Colonia Urquiza	Apr 03; May 03
		C. annuum	General Mansilla	Apr 04
	Lipaphis erysimi	*B. oleracea*	Colonia Urquiza	May 03
	Macrosiphum euphorbiae	*L. sativa*	Colonia Urquiza	Jun 03
	C. elaeagni	*C. scolymus*	Arana	Aug 04

Zoophthora sp.	*C. elaeagni*	*C. scolymus*	General Mansilla	Apr 04; May 04
Zoophthora radicans	*N. ribisnigri*	*L. sativa*	P. Pereyra Iraola	Oct 02
(Brefeld) Batko	*M. euphorbiae*	*Lycopersicon esculentum*	P. Pereyra Iraola	Dec 02; Jan 03
	A. fabae	*S. melongena*	Colonia Urquiza	Mar 03; Apr 03
	M. persicae	*B. vulgaris var. conditiva*	Colonia Urquiza	Apr 03; May 03
		B. vulgaris var. cicla	Colonia Urquiza	Apr 03
		L. sativa	Colonia Urquiza	Oct 03
	B. brassicae	*B. oleracea*	Colonia Urquiza	May 03
		B. oleracea var. botrytis	Arana	Mar 04
	C. elaeagni	*C. scolymus*	Arana	Nov 03; Aug 04
			General Mansilla	Aug 04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◀*Figure 1.* (a, b) *Conidiobolus obscurus*, primary conidia with basal hemispherical papillae; (c, d) *Entomophthora planchoniana*; (c) primary conidium apiculate; (d) secondary conidium; (e, g) *Neozygites fresenii*; (e) primary conidium; (f) secondary capilliconidia; (g) secondary capilliconidia with mucoid apical haptor (arrow); (h, l) *Pandora neoaphidis*; (h) bitunicate primary conidia with separate external wall layer (arrow); (i) secondary conidium; (j) structures like formation resting spores; (k, l) structures like mature resting spores; (ll) *Zoophthora* sp., primary conidium; (m, n) *Zoophthora radicans*; (m) primary conidia; (n) capilliconidium forming on capillary conidiophore. Scale bar: a = 21 μm; b, i = 15 μm; c, h, ll, = 17 μm; d = 13 μm; e = 10 μm; f = 35 μm; g, n = 20 μm; j = 42 μm; k = 26 μm; l = 68 μm; m = 8 μm.

Locations: Pereyra Iraola, Colonia Urquiza, El Peligro.

Attempts to isolate cultures were not successful due the scarce of the material collected. Herbarium material LPSC 47461

Entomophthora planchoniana Cornu

Host: *N. ribisnigri, Aphis fabae, Aphis gossypii, Myzus* sp., *Myzus persicae*.

Aphid cadavers typically reddish initially becoming brownish, attached to the substrate by numerous and isolated thin rhizoids. Primary conidia $15.8 \pm 1 \times 13.4 \pm 0.8$ μm (13–18.9 × 11.8–15.4 μm; $n = 85$, 7 series), multinucleate spherical with a relatively broad, flat basal papilla (Figure 1c). Secondary conidia $11.9 \pm 1.8 \times 9.7 \pm 1.5$ μm (7.1–15.8 × 7.1–14.8 μm; $n = 55$, 5 series), budding from primary conidia, slightly smaller, nonapiculate (Figure 1d). Conidiophores simple and unbranched. Cystidia and resting spores not observed.

Locations: Pereyra Iraola, Colonia Urquiza, Arana.

Attempts to obtain fungal isolates were not successful. Herbarium material LPSC 47458

Neozygites fresenii (Nowakowski) Remaudière & Keller

Host: *B. brassicae, Myzus* sp., *A fabae*

Rhizoids absent. Primary conidia $18.3 \pm 1.6 \times 15.7$ μm (15.7–19.6 μm; $n = 20$, 1series) forcibly discharged, multinucleate, with a flattened basal papilla (Figure 1e). Secondary conidia similar to or smaller than primary, or capilliconidia, $26 \pm 2.1 \times 13.3 \pm 1.4$ μm (23.5–27.5 × 11.8–14.5 μm; $n = 20$, 1 series) almond-shaped with a disk-like haptor at the apex on slender capillary arising from primary conidia (Figure 1f, g). Conidiophores simple, unbranched. Resting spores were not observed. Cystidia absent.

Locations: Colonia Urquiza, Arana.

Attempts to isolate cultures were not successful. Herbarium material LPSC 47468.

Pandora neoaphidis (Remaudière & Hennebert) Humber

Hosts: *N. ribisnigri, A. fabae, B. brassicae, Myzus* sp., *M. persicae, Lipaphis erysimi, Macrosiphum euphorbiae, Capitophorus elaeagni.*

Aphids killed by *P. neoaphidis* were pale brown turning red after desiccation. Cadavers attached to the substrate by a few single rhizoids generally emerging from the host abdomen or thorax. Primary conidia $23.1 \pm 1.8 \times 11.1 \pm 0.8$ μm (19.8–27.5 × 9.4–12.4 μm; $n = 442$, 29 series), generally ovoid to clavate, bitunicate, uninucleate, with a basal papilla rounded (Figure 1h). Forcibly discharged, creating a white halo around the cadaver. Secondary conidia $15.1 \pm 1.5 \times 11.1 \pm 1.2$ μm (13–19 × 7.8–13.4 μm; $n = 260$, 15 series), produced singly on primary conidia similar to them or more nearly globose than primary ones (Figure 1i). Conidiophores branched at their apices. Cystidia 14.6 ± 1.4 μm diameter. Structures suggestive of the formation of resting spores and of mature resting spores 31.4 ± 2.4 μm (24.8–37.2 μm) of diameter (Figure 1j–l), were observed in aphids collected in open fields in June-03 and May-04.

Locations: Pereyra Iraola (sites 1 and 2), Colonia Urquiza, El Peligro, General Mansilla, Colonia Urquiza, Arana.

Isolates in pure cultures were established on SEMA. Access numbers: CEP 045; CEP 164; CEP 166; CEP 167; CEP 170; CEP 171. Herbarium material LPSC 47466, 47459.

This is the major species infecting the surveyed aphid species, and was recorded from eight aphid host species in six locations.

Zoophthora sp.

Host: *C. elaeagni.*

Numerous and individual rhizoids. Primary conidia $26.7 \pm 2.3 \times 9.1 \pm 0.9$ μm (21.1–29.7 × 7.4–9.9 μm; $n = 75$, 3 series), elongate, apex rounded, papilla distinct, rounded, uninucleate, bitunicate (Figure 1ll). Secondary capilliconidia $21.8 \pm 0.6 \times 8.3 \pm 0.4$ μm (21.1–22.3 × 7.2–9.2 μm; $n = 50$, 2 series), asymmetrically fusoid to falciform, length of capillaries 44.64 ± 4.96 μm (37.2–52.08 μm; $n = 15$, 1 series). Conidiophores digitately branched. Resting spores and cystidia were not observed.

Location: General Mansilla.

Culture isolated: CEP 127 (ARSEF 7454)

Zoophthora radicans (Brefeld) Batko

Host: *N. ribisnigri, M. euphorbiae, A. fabae, M. persicae, B. brassicae, C. elaeagni.*

Aphids killed by *Z. radicans* turned pale orange brown to orange pink with abdominal rhizoids, numerous, individual or fasciculate. Primary conidia $24 \pm 2.3 \times 7.5 \pm 0.9$ μm (19.7–27.3 × 6.7–9.5 μm; $n = 135$, 8 series), ellipsoidal, uninucleate, basal papilla rounded (Figure 1m). Secondary conidia similar to primary or capilliconidia $18.6 \pm 3.9 \times 7.5 \pm 0.7$ μm (13.6–22.3 × 4.9–8.7 μm; $n = 55$, 3 series) fusiform and formed laterally on slender capillary conidiophores arising from primary conidia (Figure 1n). Conidiophores digitately branched. Cystidia 3.72 μm of diameter at base tapering uniformly. Resting spores not observed.

Locations: Pereyra Iraola, Colonia Urquiza, Arana, General Mansilla.

Fungal cultures from infected aphids were established on SDA + 1% yeast extract. Accession numbers: CEP 052 (ARSEF 7208); CEP 130 (ARSEF 7455); CEP 172 (ARSEF 7788); CEP 173 (ARSEF 7789). Herbarium material LPSC 47467, 47460.

Discussion

We found a considerable diversity of entomophthoralean fungi from the aphid pests of horticultural crop systems in Argentina. Among the six species of Entomophthorales recorded here, it appears that *P. neoaphidis, C. obscurus*, and *N. fresenii* are new records for the Neotropic region.

Zoophthora radicans and *E. planchoniana* were previously reported from *Tuta absoluta* (Lepidoptera: Gelechiidae) and from *Myzus persicae* (Hemiptera: Aphididae), respectively, on vegetable crops from Buenos Aires province, Argentina (López Lastra and Scorsetti, 2006).

There are previous reports of Entomophthorales from USA and South Africa affecting cereal aphids (Feng et al., 1990; Hatting et al., 1999). In the Neotropic region there are some records of entomophthoralean fungi infecting other agricultural insect pests (Fresa, 1979; Aruta et al., 1974; Lange, 1996; Méndez Sánchez et al., 2001, 2002b; Edelstein and Lecuona, 2003) nevertheless there are few reports of these fungi infecting aphids (Aruta et al., 1974; López Lastra and Scorsetti, 2006).

In the present work entomophthoralean fungi from vegetable horticultural crops were collected from March–June through September–December and in a lower rate during summer with its prevailing daily high temperatures of 25–30° C.

Pandora neoaphidis is the species with greatest abundance in the majority of different vegetable crops and aphid species; it was present mostly during autumn and spring even though it was also present but much less prevalent in winter (June–September) and summer (December–March). We observed thickly double-walled structures (Figure 1k, l) in aphids otherwise apparently infected by *P. neoaphidis* during the winter (May 2003 and June 2004); these were similar to the resting spores of other entomophthoralean fungi. However there is only one previous report of *P. neoaphidis* resting spores produced *in vitro* (Uziel and Kenneth, 1986), but those spores were reported to have a diameter of 74 ± 2.6 (52–119 μm), on average more than twice the diameter of the spores found in the Argentinean aphids. Eventhough this report was not corroborated neither was never been replicated and the size of the resting spores were too large for belonging to Entomophthorales, at present time is the only cite in the literature. Nonetheless, we need further evidence and study of these spores and, hopefully further collections before we could confidently state that these structures are, in fact, resting spores of *P. neoaphidis* rather than of some other entomophthoralean pathogen co-infecting the same aphid individuals.

Zoophthora radicans was recorded from six aphid species and from ten different species of vegetable crops; it was present throughout all seasons of the years 2003 and 2004.

Zoophthora sp. was recorded in only one aphid species and in a single vegetable crop; its morphological characteristics clearly differed from those of *Z. radicans*. This fungus resembles *Z. orientalis* Ben-Ze'ev and Kenneth (1981) except that the fungus we collected and isolated has generally smaller primary conidia than those of the Israeli fungus (23.7–34.8 × 6.3–10.3 μm), and the capilliconidia of the Argentinean fungus were also smaller than those of *Z. orientalis* (24.5–29.5 × 5.5–10.3 μm). Otherwise we observed rhizoids but no cystidia whereas *Z. orientalis* was described to have septate cystidia while no rhizoids or pseudorhizomorphs were observed. Albeit, further studies are necessary to identify this fungal species that is possible that could be a new species.

Entomophthora planchoniana was present mostly during spring and autumn, and infected five species of aphid hosts. *Conidiobolus obscurus* was present in autumn, winter and spring, and *N. fresenii* was present only in autumn and affected a narrower host range of three species of aphids.

Temperature appears to be the most significant weather component affecting the presence of these fungal pathogens. Only *P. neoaphidis* was present at summer while the rest of the entomophthoralean fungi were present in more temperate conditions. During winter fungal infections occurred primarily in greenhouses, where the temperatures were higher than in open fields.

Conidiobolus obscurus, N. fresenii and *P. neoaphidis* are new records for South America, and the aphid host ranges for entomophthoralean fungi are also further extended to nine species of aphids as new host records.

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