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RESEARCH ARTICLE

Natural occurrence of entomophthoroid fungi (Entomophthoromycota) of aphids (Hemiptera: Aphididae) on cereal crops in Argentina

R.G. Manfrino[1,2], J.L. Hatting[3], R. Humber[4], C.E. Salto[1] & C.C. Lopez Lastra[2]

1 Instituto Nacional de Tecnología Agropecuaria (INTA), Área Investigación Agronomía, Protección Vegetal, Ruta Nacional 34, Km. 227, Rafaela 2300, Santa Fe, Argentina
2 Centro de Estudios Parasitológicos y de Vectores (CEPAVE), UNLP-CONICET, Calle 2, nro 584, La Plata 1900, Buenos Aires, Argentina
3 South African Agricultural Research Council, Small Grain Institute, Bethlehem 9700, South Africa
4 USDA-ARS Biological IPM Research, Robert W. Holley Center for Agriculture and Health, New York, NY 14853, USA

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Correspondence
R.G. Manfrino, Instituto Nacional de Tecnología Agropecuaria (INTA), Área Investigación Agronomía, Protección Vegetal, Ruta Nacional 34, Km. 227, Rafaela (2300), Santa Fe, Argentina. Email: manfrino.romina@inta.gob.ar

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Abstract

The spectrum of entomophthoroid fungal species parasitising aphids on cereal crops and a study of the phenology and prevalence of these pathogens were investigated in Argentina. The studies were conducted at six different sites cultivated with crops of *Triticum aestivum* (wheat), *Avena sativa* (oats) and *Sorghum bicolor* (sorghum) during two consecutive years. Entomopathogenic fungi from the new phylum Entomophthoromycota were recorded from six aphid species on cereals in Argentina: *Rhopalosiphum maidis, Rhopalosiphum padi, Rhopalosiphum rufiabdominalis, Schizaphis graminum, Sitobion avenae* and *Sipha maydis*. Three species of entomophthoroid fungi were found infecting these aphid species: *Pandora neoaphidis, Zoophthora radicans* (Entomophthorales: Entomophthoraceae) and *Neozygites fresenii* (Neozygitales: Neozygitaceae). Entomophthoroid fungal infections occurred mostly in autumn–winter seasons (March–August), and coincided with periods of high relative humidity and comparatively low temperatures. This study represents the first base-line characterisation of entomophthoroid fungi infecting aphids on cereal crops in Argentina.

Introduction

More than 5000 species of aphids have been described worldwide (Keller, 2006). Most species are serious pests affecting crop yields through virus transmission (Lucio-Zavaleta *et al.*, 2001; Zwiener *et al.*, 2005) and/or direct physiological damage (Franzen *et al.*, 2008). Aphids are especially damaging in temperate climatic zones (Minks & Harrewijn, 1988). More than 165 aphid species worldwide have been identified from cereal crops (Blackman & Eastop, 2000). In Argentina, Delfino (1983) reported the following species as the most prevalent: *Metopolophium dirhodum* (Walker), *Rhopalosiphum maidis* (Fitch), *Rhopalosiphum padi* (Linnaeus), *Rhopalosiphum rufiabdominalis* (Sasaki), *Schizaphis graminum* (Rondani), *Sitobion avenae* (Fabricius) and *Tetraneura nigriabdominalis* (Sasaki). Botto & Hernández (1989) underscored the economic impact of these species during cereal crop production in Argentina.

Although control of aphids has been based predominantly on the use of chemical insecticides, this practice may lead to human health and environmental problems as well as adverse effects on associated non-target fauna. Another problem is the development of resistance to chemical insecticides, as has been recorded for species such as *Aphis gossypii* (Glover) (Moores *et al.*, 1996), *Myzus persicae* (Sulzer) (Foster *et al.*, 2000), and *Sch. graminum* (Shufran *et al.*, 1997).

Entomophthoroid fungi are important mortality factors of aphids in the field (Keller, 2006). Furthermore, intensive studies on entomophthoroid fungi contribute to the understanding of their epizootiological potential, phenology and management in agricultural crops (Pell *et al.*, 2001). Entomophthoroid fungi often cause

epizootics that may dramatically reduce aphid densities (Steinkraus *et al.*, 1995), with approximately 30 species known to cause mycoses in aphid populations (Humber, 1991; Keller, 1991, 1997, 2006; Bałazy, 1993). Unlike several fungal–cereal aphid associations reported from other countries (Feng *et al.*, 1992; Hatting *et al.*, 1999; Barta & Cagáň, 2003*a*,*b*) such associations remain largely unexplored in South America (Lázzari, 1985), with most reports limited to other insect hosts (Scorsetti & López Lastra, 2007; Sosa-Gómez *et al.*, 2010). In Argentina, six species of entomophthoroid fungi have been reported from aphids on horticultural crops (Scorsetti *et al.*, 2007) while on cereal crops (rice) only one fungal species attacking a hemipteran pest has been reported (Toledo *et al.*, 2008) in Gran La Plata area, Buenos Aires province. The overall goal of this study was to survey and to identify entomophthoroid fungi pathogenic to cereal aphids and to focus on the phenology and prevalence of these fungi in the Pampeana Region of Santa Fe province, Argentina. No such information had been documented prior to this study.

The taxonomy of entomophthoroid fungi used here follows the new, molecularly based classification of these fungi in a newly described phylum *Entomophthoromycota* (Humber, 2012*a*) that recognised three classes (*Basidiobolomycetes*, *Neozygitomycetes* and *Entomophthoromycetes*), three orders (*Basidiobolales*, *Neozygitales*, and *Entomophthorales*) and the same six families accepted by Humber (1989).

Materials and methods

Field surveys

The surveys covered the west of the province of Santa Fe in the Argentinean Pampa. The Argentinean Pampas (situated between 28–40°S and 68–57°W), one of the most important areas for agricultural production in the world, is a vast region of ca 52 million ha of suitable agricultural land (Hall *et al.*, 1992; Viglizzo & Roberto, 1998). The annual average rainfall is 1050 mm (variation WE = 125 mm), distributed with an isohydro regime, with 70% of the rainfall occurring in spring–summer, 23% during autumn and only 7% in winter. The annual mean temperature is 18.0°C (variation NS = 1.0°C) with the extreme means of 26.0 and 12.7°C during January and July, respectively (Panigatti, 1980; Panigatti *et al.*, 1982).

The crops sampled here included *Triticum aestivum* L. var. Klein Guerrero (wheat), *Avena sativa* L. var. Calen (oats) and *Sorghum bicolor* (L.) Moench var. ADV 114 (sorghum). Sampled areas did not exceed an area of 500 m^2 per site. No insecticides or fungicides were applied to the parts of the fields where collections were made during the course of the study. Six sites were surveyed (Table 1) throughout the entire period of crop cultivation.

Table 1 Description of study sites

Site	Crop	Town/City	Latitude S	Longitude W
1	Wheat	Monte Vera	31°32′49.79″	60°41′33.89″
2	Oats	Rafaela	31°11′10.16″	61°30′19.09″
3	Wheat	Rafaela	31°11′16.02″	61°30′20.40″
4	Wheat	Rafaela	31°11′59.32″	61°29′59.29″
5	Sorghum	Rafaela	31°12′6.62″	61°30′11.14″
6	Oats	Rafaela	31°11′32.64″	61°30′3.90″

Aphid sampling

Aphids were sampled weekly from April 2010 to November 2011. Sampling was conducted from planting until harvest and covered 11, 7, 6, 13, 10 and 12 sampling dates in the sites 1, 2, 3, 4, 5 and 6, respectively. On each sampling date, one tiller was examined at each of 10 equally spaced points (spaced 1 m) along five parallel transects (spaced 10 m) within the field for a total of 50 tillers per site. Transects were not permanently marked and distances were measured by pacing. Therefore, although located in the same area, tillers were never sampled at the same point locations. All aphids on these tillers were quantified as described by Hatting *et al.* (1999). Quantification of aphids was done *in situ*.

For further identification of aphid species healthy living aphids were collected and transferred into plastic cups with lids (150 cm^3) from where subsamples were transferred to microcentrifuge tubes (Eppendorf; 1.5 cm^3), and these subsamples were preserved in 70% ethanol. The identification to species level was done according to the keys of Blackman & Eastop (2000).

Identification of fungal pathogens

Dead aphids with evidence of external fungal growth (showing sporulation) were examined under a stereo microscope and an optical microscope for the presence of rhizoids, cystidia and/or spores. Dead aphids without external signs of mycosis were placed in Petri dishes (60 mm diameter) with a filter paper moistened with a few drops of distilled water (humid chambers) and maintained at 20°C for 24–72 h to allow the development of overt mycoses. Living aphids with apparent symptoms of infection were also transferred to humid chambers and maintained under the same conditions detailed above to facilitate the development of infection.

Fungal structures were mounted in lactophenol-aceto-orcein (LPAO) (1:1) or stained with 1% aceto–orcein plus glycerine for semi-permanent mounts. Fungal

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Table 2 Number of aphids by species (percentages of total population in parentheses) and total number of healthy and infected aphids per each sampling site

	Insect species							Total aphids	
Site	*Schizaphis graminum*	*Rhopalosiphum padi*	*Rhopalosiphum rufiabdominalis*	*Rhopalosiphum maidis*	*Metopolophium dirhodum*	*Sitobion avenae*	*Sipha maydis*	Healthy	Infected
1	32 (4.0)	4 (0.5)	3 (0.3)	0 (0.0)	89 (10.6)	599 (72.9)	93 (11.2)	641	179 (21.8)[a]
2	920 (55.9)	57 (3.5)	9 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	659 (40.5)	1626	19 (1.2)[a]
3	1704 (29.4)	265 (4.6)	236 (4.1)	3100 (53.5)	0 (0.0)	75 (1.3)	416 (7.2)	5451	345 (6.0)[a]
4	8 (3.1)	0 (0.0)	11 (4.3)	11 (4.3)	71 (27.4)	118 (45.6)	40 (15.4)	253	6 (2.3)[a]
5	20349 (93.0)	0 (0.0)	0 (0.0)	1532 (7.0)	0 (0.0)	0 (0.0)	0 (0.0)	21708	173 (0.007)[a]
6	90 (15.3)	11 (1.9)	352 (59.9)	0 (0.0)	55 (9.4)	34 (5.8)	46 (7.8)	561	27 (4.8)[a]

[a]Percentage infection in parentheses (number infected/total number of aphids × 100).

preparations were photographed using an Olympus BX51 microscope fitted with an Olympus DP71 camera.

Measurements of fungal structures from freshly infected cadavers were made to enable specific identification. Fungal species were identified according to taxonomic keys and monographs of Bałazy (1993); Humber (2012*b*) and Keller (1991).

Statistical analyses

The Kruskal–Wallis one-way analysis of variance by ranks (Siegel, 1956) test was used to determine, at each site, if there were differences in the rate of infection among the sampling weeks (all sampling dates included in the analyses). Where differences were detected at $P < 0.05$, the U-test of Mann–Whitney was used to determine which weeks showed such differences. Analyses were performed using InfoStat (InfoStat, 2004) statistical software.

Results

Seven species of aphids were identified from cereal crops (Table 2), of which six were found to be infected by entomophthoralean fungi. These aphids comprised *Sch. graminum*, *Sipha maydis* Passerini, *Sit. avenae*, *R. maidis*, *R. padi* and *R. rufiabdominalis*. Three species of entomophthoroid fungi were identified from these aphids, belonging to the families Neozygitaceae, *Neozygites fresenii* (Nowakowski) Remaudière & Keller (Fig. 1) and Entomophthoraceae, *Pandora neoaphidis* (Remaudière & Hennebert) Humber and *Zoophthora radicans* (Brefeld) Batko (Figs 2 and 3, respectively). Permanent microscopic slides and preserved dried material were deposited with CEPAVE Mycological Collection of Entomopathogenic Fungi. In addition, samples were stored at 4°C in 96% alcohol for further molecular studies.

From a total of 30 989 aphids detected in surveys, 749 (2.4%) were recorded as fungus-infected. At Site 1, there were six species of aphids (Table 2). The only pathogen recorded at this site was *Z. radicans* (Table 3), and the only species of aphid infected with this pathogen was *Sit. avenae*. At Site 2, there were four species of aphids (Table 2). There were two fungal species present, *P. neoaphidis* and *Z. radicans*. *Pandora neoaphidis* was recorded infecting *Sch. graminum* and *R. rufiabdominalis* while *Z. radicans* was found on *Sch. graminum* and *Sip. maidis* (Table 3). At Site 3, there were six species of aphids (Table 2). The same two species of entomophthoroid fungi found at Site 2 were recorded here. *Pandora neoaphidis* was the predominant pathogen and was recorded from *Sch. graminum*, *R. maidis* and *R. padi*, while *Z. radicans* was found infecting *Sip. maydis*, *R. maidis* and *R. padi* (Table 3). Six species of aphids were present at Site 4 (Table 2). *Pandora neoaphidis* and *Z. radicans* were the pathogens recorded and were found in three aphid cadavers each (Table 3). At this site, *P. neoaphidis* was found infecting *R.*

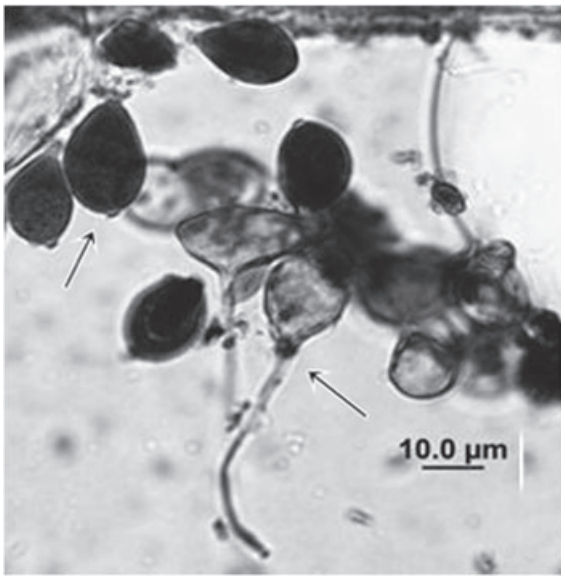

Figure 1 Primary and secondary conidia of *Neozygites fresenii* from *Schizaphis graminum*.

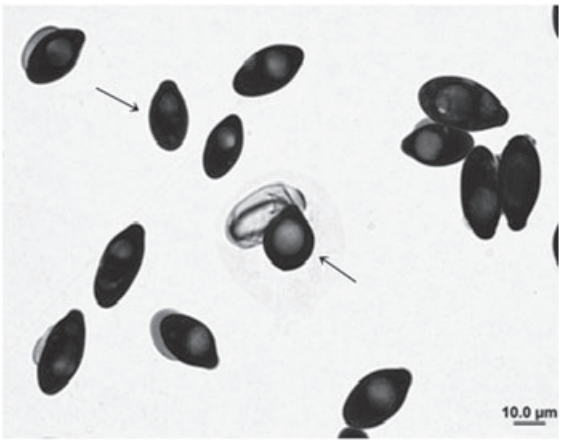

Figure 2 Primary and secondary conidia of *Pandora neoaphidis* from *Rhopalosiphum rufiabdominalis*.

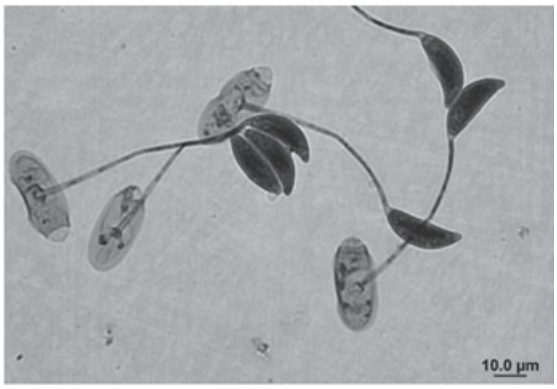

Figure 3 Capiliconidia of *Zoophthora radicans* from *Rhopalosiphum maidis*.

rufiabdominalis and *Sit. avenae*, whereas *Z. radicans* was recorded from *R. rufiabdominalis*, *Sit. avenae* and *Sch. graminum*. At Site 5, only two species of aphids were recorded, *Sch. graminum* and *R. maidis* (Table 1). The only pathogen recorded from a single sampling date (3 March 2011) was *N. fresenii* (Table 3). Finally, at Site 6, six species of aphids were present (Table 2). *Pandora neoaphidis* and *Z. radicans* (Table 3) were the only pathogens recorded. *Pandora neoaphidis* was found infecting only *R. rufiabdominalis*, while *Z. radicans* was observed on *R. rufiabdominalis* and *Sit. avenae*.

Prevalence and seasonality

In our study *P. neoaphidis* was the most important pathogen recorded from *R. rufiabdominalis* at Sites 2 and

Table 3 Identification of Entomophthoralean fungi on cereal aphids collected in Argentina during 2010–2011

Site	Sample date[a] (Field Site)	Aphid species	No. of dead aphids examined	Percentage of dead aphids with		
				Pandora neoaphidis	*Zoophthora radicans*	*Neozygites fresenii*
1	21 Oct 2011	*Sit. avenae*	172	0	100	0
1	28 Oct 2011	*Sit. avenae*	7	0	100	0
2	15 Apr 2010	*Sch. graminum*	4	0	100	0
2	12 May 2010	*Sch. graminum*	3	100	0	0
2	20 May 2010	*Sch. graminum*	5	100	0	0
2	02 Jul 2010	*R. rufiabdominalis*	2	100	0	0
2	05 Jul 2010	*Sip. maidis*	5	0	100	0
3	12 May 2010	*S. graminum*	15	100	0	0
3	20 May 2010	*S. graminum*	37	100	0	0
3	09 Jun 2010	*Sch. graminum*	251	100	0	0
3	24 Jun 2010	*R. maidis*	17	58.8	41.2	0
3	06 Jul 2010	*R. padi*	18	50.0	50.0	0
3	20 Jul 2010	*Sip. maidis*	7	0	100	0
4	06 Jul 2011	*Sch. graminum*	1	0	100	0
4	12 Jul 2011	*R. rufiabdominalis*	1	100	0	0
4	20 Jul 2011	*R. rufiabdominalis*	1	0	100	0
4	24 Aug 2011	*Sit. avenae*	1	0	100	0
4	14 Oct 2011	*Sit. avenae*	2	100	0	0
5	03 Mar 2011	*Sch. graminum*	173	0	0	100
6	23 Jun 2011	*R. rufiabdominalis*	11	100	0	0
6	07 Jul 2011	*R. rufiabdominalis*	6	50.0	50.0	0
6	12 Jul 2011	*R. rufiabdominalis*	8	100	0	0
6	21 Jul 2011	*R. rufiabdominalis*	1	0	100	0
6	17 Aug 2011	*Sit. avenae*	1	0	100	0

[a]There were only included the sampling dates when fungal infections were recorded.

Table 4 Prevalences of Entomophthoralean fungi in populations of cereal aphids infesting cereal crops in Argentina during 2010–2011[a]

Site	Sampling date[b]	% SU[b] with infected aphids	Mean no. of infected aphids per SU	Percentage of infection of							
				S. g	R. p	R. r	R. m	M. d	S. a	S. m	All aphids
1	21 Oct 2011	38.0 (19)[c]	9.05	0 (11)[d]	–	–	–	0 (54)	83.9 (205)	–	63.7 (270)
	28 Oct 2011	6.0 (3)	2.3	–	–	–	–	0 (2)	5.4 (128)	–	5.3 (130)
2	15 Apr 2010	4.0 (2)	2.0	0.58 (689)	–	–	–	–	–	–	0.58 (689)
	12 May 2010	4.0 (2)	1.5	6.12 (49)	–	–	–	–	–	0 (2)	5.8 (51)
	20 May 2010	6.0 (3)	1.6	11.1 (45)	0 (5)	0 (11)	–	–	–	–	8.2 (61)
	02 Jul 2010	2.0 (1)	2.0	–	–	28.5 (7)	–	–	–	0 (395)	0.49 (402)
	05 Jul 2010	4.0 (2)	2.5	–	–	–	–	–	–	3.75 (133)	3.75 (133)
3	12 May 2010	10.0 (5)	3.0	3.72 (403)	–	–	0 (9)	–	–	–	3.6 (412)
	20 May 2010	16.0 (8)	4.6	9.3 (396)	–	–	0 (19)	–	–	–	8.9 (415)
	09 Jun 2010	70.0 (35)	7.17	97.6 (257)	–	–	0 (21)	–	–	–	90.2 (278)
	24 Jun 2010	12.0 (6)	2.8	0 (696)	–	–	0.61 (2760)	–	–	–	0.5 (3456)
	06 Jul 2010	16.0 (8)	2.2	0 (99)	6.6 (270)	0 (222)	0 (125)	–	–	–	2.5 (716)
	20 Jul 2010	4.0 (2)	3.5	0 (53)	–	–	–	–	0 (68)	1.75 (398)	1.3 (519)
4	06 Jul 2011	2.0 (1)	1	50.0 (2)	–	–	–	–	–	–	50.0 (2)
	12 Jul 2011	2.0 (1)	1	0 (4)	–	16.6 (6)	–	–	–	–	10.0 (10)
	20 Jul 2011	2.0 (1)	1	–	–	25.0 (4)	–	–	–	–	25.0 (4)
	24 Aug 2011	2.0 (1)	1	0 (1)	–	–	–	–	12.5 (8)	–	11.1 (9)
	14 Oct 2011	4.0 (2)	1	–	–	–	–	0 (33)	2.0 (98)	0 (35)	1.2 (166)
5	03 Mar 2011	24 (12)	14.4	18.3 (947)	–	–	–	–	–	–	18.3 (947)
6	23 Jun 2011	8.0 (4)	2.7	0 (57)	–	26.1 (42)	–	–	0 (1)	0 (1)	10.9 (101)
	07 Jul 2011	6.0 (3)	2.0	0 (9)	–	11.5 (52)	–	–	–	–	9.8 (61)
	12 Jul 2011	8.0 (4)	2.0	– (5)	– (3)	44.4 (18)	–	–	–	–	30.7 (26)
	21 Jul 2011	2.0 (1)	1.0	–	–	2.2 (46)	–	–	–	–	2.2 (46)
	17 Aug 2011	2.0 (1)	1.0	0 (1)	–	0 (34)	–	0 (11)	7.6 (13)	–	1.7 (59)

[a]There were only included the sampling dates when fungal infections were recorded.
[b]Sample units (aphid infested).
[c]Number of infested sample units examined in parentheses.
[d]Number of aphids in sample in parentheses.

6 (oats) with a maximum prevalence of 28.5% ($n = 7$) and 44.4% ($n = 18$) on 2 July 2010 and 12 July 2011, respectively (Table 4). At Site 2, mycosis of *Sch. graminum* reached 11.1% ($n = 45$) (Table 4) while at Site 6 no infections by *P. neoaphidis* were observed in other aphid species, suggesting an apparent low level of susceptibility to this pathogen by the other aphid species on oats. Furthermore, at both sites a low number of aphids was recorded infected with *Z. radicans* (Table 3). Regardless of the fungal species at Site 2, the rate of infection between weeks of study did not differ significantly ($\chi^2 = 5.51$; df = 6; $P > 0.05$). At site 6, significant differences were recorded ($\chi^2 = 27.38$; df = 11; $P < 0.05$), being detected during the weeks of 23 June and 12 July 2011, the dates with the highest percentages of infection (Table 4). Similarly, *P. neoaphidis* was the most prevalent species within populations of aphids at Site 3 with a peak prevalence of 90.2% ($n = 278$) on 9 June 2010 (Table 4) which decreased significantly two weeks later ($P < 0.05$) (Mann–Whitney test) (Table 4). At this site, infections by *Z. radicans* in aphid populations were less frequent than infections by *P. neoaphidis* (Table 3). *Zoophthora radicans* was the only pathogen recorded at Site 1, where it affected only *Sit. avenae* with a peak prevalence of 83.9% ($n = 205$) observed on 21 October 2011. Seven days later, on 28 October 2011, the incidence of infected aphids had decreased significantly to 5.4% ($n = 128$) (Table 4) ($P < 0.05$) (Mann–Whitney test). No cadavers of the other five aphid species were found to display signs of fungal infection at this site.

Similarly, at Site 4, *Z. radicans* was the most prevalent species within populations of *Sch. graminum* (50%; $n = 2$) on 6 July 2011; *R. rufiabdominalis* (25%; $n = 4$) on 20 July 2011 and *Sit. avenae* (12.5%; $n = 8$) on 24 August 2011, while infections of *P. neoaphidis* in *R. rufiabdominalis* and *Sit. avenae* were 16.6% ($n = 6$) and 2.0% ($n = 98$) on 12 July 2011 and 14 October 2011, respectively (Table 4). The number of infected aphids at this study site was low. There were no significant differences between study weeks on the percentage of infection ($\chi^2 = 11.4$; df = 12; $P > 0.05$). At Site 5, natural infection of *Sch. graminum* by *N. fresenii* reached a level of 18.3% ($n = 947$) (Table 4) on 3 March 2011. This week was differentiated significantly from the rest ($\chi^2 = 110.17$; df = 9; $P < 0.05$). The fact

that there were no fungal infections of *R. maidis* during the 2 years of this survey seems to suggest that this aphid species could be much less susceptible to *N. fresenii* than is *Sch. graminum*.

In this study, there was a seasonal distribution, with entomophthoroid fungi generally being observed in host populations from March through August (75.8%) and a lower rate between the months of September through February (24.1%). *Pandora neoaphidis* was recorded during the months of May 2010, June and July in both years of observations and October 2011. *Zoophthora radicans* was recorded during the months of April and June in 2010, July in both years, as well as August and October in 2011. Finally, *N. fresenii* was recorded only during the month of March in 2011.

Discussion

We found three species of entomophthoroid fungi pathogenic to aphid pests on cereal crops in Argentina. These species are first records for cereal aphids in the Neotropical region of the Pampa. The only previous report of such fungi for this region was that of *Pandora* sp. (Entomophthoraceae) and *Conidiobolus coronatus* (Costantin) Batko (Ancylistaceae) in a hemipteran pest, *Oliarus dimidiatus* Berg. (Hemiptera: Fulgoromorpha: Cixiidae), on rice (Toledo *et al.*, 2008). Otherwise other species of entomophthoroid fungi were recorded from other horticultural crop pests (López Lastra & Scorsetti 2006; Scorsetti *et al.*, 2007, 2012). *Pandora neoaphidis* has been reported from more than 70 aphid species (Pell *et al.*, 2001) and noted as the most prevalent pathogen among natural aphid populations in several cropping systems (Feng *et al.*, 1992; Hatting *et al.*, 1999, 2000; Barta & Cagáň, 2006; Díaz *et al.*, 2010; Scorsetti *et al.*, 2010). A similar phenomenon is reported here with almost 50% of aphid cadavers infected with *P. neoaphidis*. *Pandora neoaphidis* is the most frequent and common causal agent of epziootics (Nielsen *et al.*, 2003). Steenberg & Eilenberg (1995) reported prevalence of up to 60%. In our study infection of *Sch. graminum* (Site 3) reached 97.6% ($n = 257$) on 9 June 2010. Furthermore, at Sites 2 and 6 levels of infection on *R. rufiabdominalis* reached 28.5 ($n = 7$) and 44.4% ($n = 18$) on 2 July 2010 and 12 July 2011, respectively, which is consistent with observations by Wraight *et al.* (1993). Likewise, a recent study by Scorsetti *et al.* (2010) reported *P. neoaphidis* infecting *Nasonovia ribisnigri* on lettuce crops in Buenos Aires, Argentina, with a peak prevalence of 56.6%. McLeod *et al.* (1998) reported epizootics of *P. neoaphidis* on *Myzus persicae* on crops of spinach during the winter months. Furthermore, Steinkraus (2006) argued that *P. neoaphidis* is important for the control of aphids because it appears in early spring, just as aphid populations are establishing throughout various crops. In Slovakia, Barta & Cagáň (2006) identified *P. neoaphidis* as a sporadic species infecting *Brevicoryne brassicae*, *M. persicae* and *Chaitophorus leucomelas* Koch (Hemiptera: Aphididae) thus underscoring the wide aphid host range of this pathogen.

Zoophthora radicans was secondary to *P. neoaphidis* in ocurrence and identified from six aphid pest species. In contrast, Feng *et al.* (1990) encountered *Z. radicans* on only a few occasions and from two aphid species (*M. dirhodum* and *Sit. avenae*). In this study *Z. radicans* was recorded from four of the six studied sites and was observed causing epizootics in *S. avenae* in wheat (Site 1) with mycosis reaching 83.9% ($n = 205$) during October 2011.

Neozygites fresenii has been recorded on all continents (Bałazy, 1993; Keller, 1997) and exhibits a strong tendency to establish epizootics in dense aphid colonies. In agreement with the findings of Scorsetti *et al.* (2007), *N. fresenii* was detected only in autumn and, in this study, affected only a single species of aphid except in horticultural rather than cereal crops. There are records of *N. fresenii* as a pathogen of other species of aphids such as *Aphis gossypii* on cotton in California (Steinkraus *et al.*, 2002; Steinkraus & Boys, 2005), and on *B. brassicae*, *Myzus* sp. and *A. fabae* on horticultural crops in Buenos Aires province, Argentina (Scorsetti *et al.*, 2007). In South Africa, *N. fresenii* was identified from four aphid species – *Chaitophorus populialbae*, *Hyalopterus pruni*, *M. persicae* and *A. gossypii* (Hemiptera: Aphididae) – on the host plants *Populus canescens*, *Phragmites australis*, *Tropaeolum majus* and *Cuphea melvilla*, respectively (Hatting *et al.*, 1999); in Slovakia this fungus is known from 24 species of aphids (Barta & Cagáň, 2006).

In this study, there was a seasonal distribution with entomophthoroid fungi generally being observed in host populations from March to August, and at a lower rate between the months of September to February (spring and summer in Argentina). Observations of Alzugaray *et al.* (2010) in Uruguay, recorded *P. neoaphidis* as one of the main mortality agents of aphids but emphasised that its action was restricted to autumn and winter. In Argentina, previous studies on the phenology of entomophthoroid fungi in populations of insects other than aphids found that fungal infections were more common in the autumn-winter season (in the southern hemisphere, from March to August) (López Lastra *et al.*, 2006; Toledo *et al.*, 2008). The low temperatures and humid conditions prevalent during the winter growing season tend to be propitious for the occurrence of entomophthoroid mycoses (Wraight *et al.*, 1993).

The aphid host ranges for entomophthoralean fungi are further extended to six species of aphids as new host records for Argentina and for the Neotropical region.

The results of this study conducted in Argentina clearly indicate that entomophthoroid fungi have potential to reduce aphid populations under uncontrolled field conditions. This study further expands the knowledge of entomopathogenic fungi of aphids in cereal crops and their potential as biocontrol agents.

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