International Journal of Pest Management
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ttpm20

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Published online: 17 Dec 2013.

To cite this article: R.G. Manfrino, L. Zumoffen, C.E. Salto & C.C. López Lastra (2013) Potential plant-aphid-fungal associations aiding conservation biological control of cereal aphids in Argentina, International Journal of Pest Management, 59:4, 314-318, DOI: 10.1080/09670874.2013.869372

To link to this article: http://dx.doi.org/10.1080/09670874.2013.869372

International Journal of Pest Management, 2013
Vol. 59, No. 4, 314–318, http://dx.doi.org/10.1080/09670874.2013.869372

Potential plant–aphid–fungal associations aiding conservation biological control of cereal aphids in Argentina

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(*Received 22 April 2013; final version received 21 November 2013*)

The overall aim of this study was to identify potential associations between aphids and plants as reservoirs of entomophthoralean fungi. A survey of weeds associated with wheat field borders in two different localities was performed over two years, from April 2010 to April 2012 in the Pampeana central region, Argentina. On each sampling date, five individuals of each plant species were randomly selected, and healthy and infected aphids were collected and quantified once a month. The taxonomic identities of both aphid hosts and entomophthoralean fungi were established. Aphid–fungus associations identified as favorable for inclusion in conservation biological control strategies in borders of wheat crops are the following: *Sonchus oleraceus* (L.)/*Hyperomyzus carduellinus* (Theobald), *Uroleucon sonchi* (L.)/*Pandora neoaphidis* (Remaudière & Hennebert) Humber; *S. oleraceus* (L.)/*H. carduellinus* (Theobald)/*Zoophthora radicans* (Brefeld) Batko; *Lamium amplexicaule* (L.)/*Cryptomyzus korschelti* Bôrner/*P. neoaphidis*, *Z. radicans*, *Entomophthora planchoniana* Cornu; *Foeniculum vulgare* (Miller)/*Dysaphis apiifolia* (Theobald)/*Z. radicans*; *Morrenia brachystephana* Griseb/*Aphis nerii* Boyer de Fonscolombe/*P. neoaphidis* and *Brassica rapa* L./*Brevicoryne brassicae* (L.)/*P. neoaphidis.*

Keywords: conservation biological control; entomophthoralean fungi; non-crop vegetation; aphids; cereal crops

Introduction

Conservation biological control (CBC) is a biological control strategy in which farming management practices are altered to enhance the living conditions for natural enemies of pests (Barbosa 1998; Eilenberg et al. 2001). A common CBC practice is the diversification of the agroecosystem through the establishment and conservation of weeds bordering the crop. Non-crop plants can be a key factor in promoting the survival, reproduction and activity of natural enemies of aphids, and they may also act as a reservoir for entomopathogenic fungi (Wratten et al. 2002; Albrecht 2003; Wackers 2004; Asteraki et al. 2004; Pell et al. 2010).

To date, the majority of examples of CBC relate to arthropod natural enemies (Landis et al. 2000; Gurr et al. 2004; Wade et al. 2008; Zumoffen et al. 2012) with few studies relating to entomopathogens (Fuxa 1998; Ekesi et al. 2005). CBC with entomopathogenic fungi includes the manipulation of both the crop environment and habitats situated outside the crop (Pell et al. 2010). Wheat production is generally characterized by large cultivated areas where crop protection input costs are a constraint upon profit realization. Non-crop plants growing alongside the borders of wheat fields can act as alternative hosts for non-pest aphids (Shah & Pell 2003), which indirectly provide an ecosystem service by enhancing natural enemy impact on the pest aphids.

Among entomopathogenic fungi, the Entomophthoromycota offer high potential for exploitation in CBC strategies against aphids (Keller & Suter 1980; Powell et al. 1986; Steenberg & Eilenberg 1995; Barta & Cagán 2003; Shah & Pell 2003; Ekesi et al. 2005; Steinkraus 2006). Entomophthoralean fungi have been found to be important antagonists of aphids under field conditions (Latgé & Papierok 1988) and have the potential to induce spectacular epizootics which drastically reduce aphid population densities (Wilding & Perry 1980; Steinkraus et al. 1995; Nielsen 2002). The importance of these organisms as natural enemies of aphids resides with their ability to suppress aphid populations under cool humid conditions which contrasts with the activity requirements of arthropod natural enemies (Pell 2007). Studies on the role of alternative aphid hosts as sources of fungal inoculum have been conducted in Europe (Carruthers & Soper 1987; Eilenberg 1988; Nielsen et al. 2001; Powell et al. 2003; Pell 2007) and Africa (Hatting et al. 1999). To date, however, there have been no studies relating to CBC with entomopathogens in South America.

In Switzerland, *Pandora neoaphidis* (Remaudière & Hennebert) Humber and *Conidiobolus obscurus* (Hall & Dunn) Remaud. & Keller were reported to multiply in economic unimportant aphid species in meadows adjacent to annual crops (Keller & Suter 1980). Powell et al. (1986) found that entomophthoralean fungi were more common at the edges of fields, since alternative aphid hosts were present and the weed canopy afforded a better environment for fungal transmission than the wheat crop alone. Aphid pathogenic species also overwinter in aphid hosts in hedges and forest borders (Hall et al. 1992).

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The overall aim of this study was to identify potential aphid–fungus combinations for use as reservoirs of entomophthoralean fungi that are useful for suppressing populations of aphids that are pests of wheat in Argentina.

Material and methods

Field survey

The survey covered the west of the province of Santa Fe, in the Argentinean Pampeana Region. The Argentinean Pampas (situated between 28 and 40°S and 68 and 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 land for agriculture and cattle production (Hall et al. 1992; Viglizzo & Roberto 1998). Two sites were selected for surveys: Rafaela (31°11′16.02″S/61°30′20.40″W) and Monte Vera (31°32′49.79″S/60°41′33.89″W). The surveys were conducted on a weekly basis from April 2010 to April 2012 and the border plants around wheat crops were sampled. On each sample date, five individuals of each plant species were randomly collected. Samples of ostensibly healthy living aphids were collected and transferred into plastic cups with lids (150 cm^3); from those, subsamples were transferred to microcentrifuge tubes (Eppendorf; 1.5 cm^3). The subsamples were preserved in 70% ethanol for further identification to species level according to the keys of Blackman and Eastop (2000, 2006a, 2006b). Voucher specimens were deposited into the entomological collections of Instituto Nacional de Tecnología Agropecuaria (INTA). To calculate the prevalence of entomophthoralean fungi in the aphid populations, healthy and infected aphids were quantified once a month. Dead aphids with evidence of external fungal growth (showing sporulation) were examined under a stereo microscope and an optic 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 (70% RH) and maintained under the same conditions detailed above to facilitate the development of infection. The plant species were identified by specialists at the INTA (see Acknowledgements).

Identification of fungal pathogens

Fungal structures (rhizoids, cystidia and/or spores) were mounted in lactophenol-aceto-orcein (LPAO) (1:1) or stained with 1% aceto-orcein plus glycerin for semipermanent mounts. Fungal species were identified using the taxonomic keys and monographs of Keller (1987, 1991), Bałazy (1993) and Humber (1989, 1997).

Results

Thirteen plant species were identified at both of the surveyed localities (Table 1). Eight aphid species were identified from these plants. *Hyperomyzus carduellinus* was the most abundant aphid species and it was recorded from *Sonchus oleraceus* (L.) (Table 2). At Rafaela, fungal-infected aphids were recorded on the following plant species: *Lamium amplexicaule* L. (Lamiaceae), *Morrenia brachystephana* Griseb (Apocynaceae), *Foeniculum vulgare* (Miller) (Apiaceae), *S. oleraceus* (Asteraceae) and *Malva sylvestris* L. (Malvaceae), while at Monte Vera the host plant species included the following: *Brassica rapa* L. (Brassicaceae), *S. oleraceus*, *Anoda cristata* (L.), (Malvaceae) *M. sylvestris* and *Capsella bursa-pastoris* (L.) (Brassicaceae) (Table 2). The fungal species identified from the aphids residing on these plants included the following: *P. neoaphidis*, *Zoophthora radicans* (Brefeld) Batko and *Entomophthora planchoniana* Cornu. Permanent microscopic slides and preserved dried material were deposited with CEPAVE Mycological Collection of Entomopathogenic Fungi.

P. neoaphidis was recorded from the following: *Brevicoryne brassicae* (L.), *Aphis* L., *Aphis gossypii* Glover, *H. carduellinus* (Theobald), *Uroleucon sonchi* (L.), *Cryptomyzus korschelti* Bôrner, *Aphis nerii* Boyer de Fonscolombe and *Myzus persicae* (Sulzer) (Table 2). *P. neoaphidis* was recorded causing infection of up to 70% on *H. carduellinus*. The highest percentage of infection was recorded on this aphids species on 21 October 2011 (Table 2). *Z. radicans* was identified among the aphid species such as *C. korschelti*, *H. carduellinus*, *Dysaphis apiifolia* (Theobald), *M. persicae*, and *Aphis* sp. The highest percentages of infection from *Z. radicans* were recorded from *Aphis* sp. and *C. korschelti* on *C. bursa-pastoris* and *L. amplexicaule*, respectively (Table 2). *E. planchoniana* was identified only from *C. korschelti* on *L. amplexicaule* and was recorded only during September 2010. This fungus was the most prevalent at 24% ($n = 75$) (Table 2).

Table 1. Weeds associated with wheat field borders in Rafaela and Monte Vera.

Plant family	Species	Rafaela	Monte Vera
Brassicaceae	*Brassica rapa* L.	x	x
	Capsella bursa-pastoris (L. Medik)	x	x
Asteraceae	*Sonchus oleraceus* (L.)	x	x
	Conyza bonariensis (L.)	x	x
	Cichorium intybus L.	x	x
	Taraxacum officinale Weber	x	x
Apiaceae	*Ammi majus* (L.)	x	x
	Foeniculum vulgare (Miller)	x	
Lamiaceae	*Lamium amplexicaule* L.	x	x
Scrophulariaceae	*Verbascum* L.	x	
Apocynaceae	*Morrenia brachystephana* Griseb	x	
Malvaceae	*Malva sylvestris* L.		x
	Anoda cristata (L.)		x

Note: x = present.

Table 2. Occurrence and prevalence of entomophthoralean fungi identified from aphid species hosts on weeds associated with wheat field borders in Rafaela and Monte Vera.

Fungal species	Aphid host	Date of collection	Prevalence (%) and *n* total (in parentheses)	Location	Plant species
Pandora neoaphidis	*Brevicoryne brassicae*	9 May 2011*	20.5 (161)	Monte Vera	*Brassica rapa*
		8 July 2011	–		
	Aphis sp.	24 June 2011	–		*Anoda cristata*
		9 May 2011*	19.1 (47)		*Capsella bursa-pastoris*
		1 July 2011*	11.8 (17)		
		8 July 2011	–		
	Aphis gossypii	24 June 2011*	19.2 (26)		
	Hyperomyzus carduellinus	27 May 2011	–		*Sonchus oleraceus*
		24 June 2011*	43.1 (350)		
		1 July 2011*	39.3 (247)		
		21 October 2011*	73.9 (88)		
		27 May 2010*	15.7 (70)	Rafaela	
		12 May 2011	–		
		13 October 2011			
		14 October 2011	–		
		18 October 2011*	58 (100)		
	Uroleucon sonchi	24 June 2011*	10.3 (39)	Monte Vera	
		1 November 2010*	12.5 (40)	Rafaela	
	Cryptomyzus korschelti	9 June 2010*	17. 6 (34)	Rafaela	*Lamium amplexicaule*
		5 July 2010	–		
		20 September 2010	–		
		26 May 2011	–		
		23 June 2011*	35.6 (101)		
		12 July 2011	–		
		17 August 2011*	11.7 (128)		
	Aphis nerii	9 June 2010*	0.6 (164)		*Morrenia brachystephana*
	Myzus persicae	17 August 2011*	15.2 (66)		*Malva sylvestris*
Zoophthora radicans	*C. korschelti*	5 July 2010*	21.8 (55)	Rafaela	*Lamium amplexicaule*
		17 August 2011*	20.2 (89)		
	Myzus persicae	17 August 2011*	4 (50)	Monte Vera	*Malva sylvestris*
		10 June 2012*	4.6 (43)		
	H. carduellinus	27 May 2010*	14.4 (201)	Rafaela	*Sonchus oleraceus*
		17 July 2011	-		
	Dysaphis apiifolia	21 July 2010*	0.6 (170)		*Foeniculum vulgare*
	Aphis sp.	1 July 2011*	22.4 (58)	Monte Vera	*C. bursa-pastoris*
Entomophthora planchoniana	*C. korschelti*	14 September 2010*	24 (75)	Rafaela	*L. amplexicaule*

*Sampling dates when aphids were quantified.

No fungal infections were recorded from aphids collected from either wheat or non-crop plants at Monte Vera during 2010. However, during the second year of observations, infected aphids were recorded from weeds associated with wheat field borders from May to July, while on wheat, the only record of entomophthoralean infection was that of *Z. radicans* in October 2011 (Manfrino et al., unpublished data). At Rafaela, natural infections of aphids on wheat and on non-crop plants occurred from May to October in 2010 and 2011.

Discussion

In agreement with Powell et al. (1986), infections caused by entomophthoralean fungi in aphids from non-crop plants were more frequently observed than in aphids from wheat. Studies on the associations between aphids, their host plants and fungal antagonists are critical aspects of the implementation of CBC. Ideally, these plant species should not be natural hosts of aphids that could constitute a potential danger to the crop. In this study, from the aphid species identified from weeds associated with wheat field borders, the suitable hosts for entomophthoroid fungi were *A. nerii*, *B. brassicae*, *D. apiifolia*,*C. korschelti*, *H. carduellinus*, and *U. sonchi.* The aphids *A. gossypii* and *M. persicae* could not be considered as suitable hosts for use in CBC as both have been reported from a variety of cultivated host plant species such as *Capsicum annuum*, *Helianthus annuus*, *Lactuca sativa*, *Brassica* sp., *Urtica urens*, and *Malva* sp. in Argentina (Nieto Nafría et al.

1994). Barta and Cagán (2003) studied the potential of stinging nettle patches as natural reservoirs for pathogens in the agroecosystem; they identified five pathogenic species from *Microlophium carnosum*, including *P. neoaphidis* and *E. planchoniana*, which were also recorded in this study.

Shah et al. (2001) discussed strategies for habitat manipulation with emphasis on the role of field margins as shelters for fungal pathogens of aphids, especially *P. neoaphidis*. *P. neoaphidis* is the predominant pathogen in natural aphid populations (Feng et al. 1990, 1992; Hatting et al. 2000; Barta & Cagáň 2006; Scorsetti et al. 2007, 2010; Toledo et al. 2008; Díaz et al. 2010) and it has been reported in more than 70 aphid species (Pell et al. 2001). In Switzerland, Keller and Suter (1980) found *P. neoaphidis* and *C. obscurus* on aphid species in meadows adjacent to annual crops. We identified *P. neoaphidis* from five non-pest aphids of wheat with a prevalence of up to 70%. *P. neoaphidis* is the most frequent and common causal agent of epizootics (Nielsen et al. 2003), causing up to 56.6% mortality, thus confirming the capacity of this fungus to be an effective biological control agent (Scorsetti et al. 2010). Steenberg and Eilenberg (1995) reported up to 60% prevalence of entomophthoralean fungi on aphids. In this study, *P. neoaphidis* was observed causing epizootics on *H. carduellinus* from *S. oleraceus*. Interestingly, *H. carduellinus* was the most abundant aphid. Seemingly, epizootics of *P. neoaphidis* could be favored by the high density of the host; a major factor determining infection levels in some crop–aphid–pathogen systems (Wilding & Perry 1980; Feng et al. 1992).

Z. radicans was secondary to *P. neoaphidis* in occurrence and was identified from five aphid species on non-crop plants. The prevalence of *Z. radicans* reached 20% on *C. korschelti* and on *Aphis* sp. (Table 2). *P. neoaphidis* and *Z. radicans* were recorded causing infections on aphids on wheat with prevalences up to 90.2% ($n = 278$) and 83.9% ($n = 205$), respectively (Manfrino et al., unpublished data).

When the annual economically important crop is not growing in the field, the presence of non-crop plants contributes to the presence of the pathogen in the environment. The following associations have been identified for possible inclusion in future CBC of wheat cereal aphids in Argentina (i.e. host plan/aphid/pathogen): *S. oleraceus* (L.)/*H. carduellinus* (Theobald), *U. sonchi* (L.)/*P. neoaphidis* (Remaudière & Hennebert) Humber; *Sonchus oleraceus* (L.)/*H. carduellinus* (Theobald)/*Z. radicans* (Brefeld) Batko; *L. amplexicaule* (L.)/*C. korschelti* Bôrner/*P. neoaphidis*, *Z. radicans*, *E. planchoniana* Cornu; *F. vulgare* (Miller)/*D. apiifolia* (Theobald)/*Z. radicans*; *M. brachystephana* Griseb/*A. nerii* Boyer de Fonscolombe/*P. neoaphidis* and *B. rapa* L./*B. brassicae* (L.)/*P. neoaphidis.*

Aphids that are highly likely to act as alternative hosts for fungi include *H. carduellinus*, *U. sonchi*, *C. korschelti*, *B. brassicae*, *D. apiifolia* and *A. nerii*.

Acknowledgements

The authors thank Dr Justin Hatting, Lic. Carlos Bainotti and Mr Joao Machado for the revision of the manuscript. We also thank Lic. Julieta Merke for the identification of plant species.

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