Research Article

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Prevalence of entomophthoralean fungi (Entomophthoromycota) of aphids in relation to developmental stages

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Abstract

BACKGROUND: Transmission of fungal pathogens of aphids may be affected by the host developmental stage. *Brassica* and *Lactuca sativa* L. crops were sampled in Santa Fe, Argentina, to determine the prevalence of fungal-diseased aphids and investigate the differences between developmental stages of aphids.

RESULTS: The fungal pathogens identified were *Zoophthora radicans* (Bref.) A. Batko, *Pandora neoaphidis* (Remaud. & Hennebert) Humber and *Entomophthora planchoniana* Cornu. Their prevalence on each crop was calculated. The numbers of infected aphids were significantly different between the different developmental stages on all crops except *B. oleracea* var. *botrytis* L.

CONCLUSIONS: The entomophthoralean fungi identified are important mortality factors of aphids on horticultural crops in Santa Fe. The numbers of infected nymphs and adults were significantly different, nymphs being the most affected developmental stage.

Keywords: aphids; entomophthoralean fungi; developmental stages; Argentina

1 INTRODUCTION

Aphids are one of the most important factors limiting horticultural crop production in Argentina.[1] *Brevicoryne brassicae* (L.) and *Nasonovia ribisnigri* (Mosley) are the main species of aphids on *Brassica* crops and on *Lactuca sativa* L., respectively, in the world.[2] Aphids are controlled mainly by chemical insecticides, but this practice creates human health and environmental problems and has adverse effects on non-target fauna.

Entomophthoralean fungi have been found to be important antagonists of aphids under field conditions[3] and have the potential to induce epizootics that drastically reduce aphid densities.[4,5] Entomophthoralean fungi have been recorded infecting aphids on horticultural crops worldwide. MacLeod[6] found infections of *Pandora neoaphidis* (Remaud. & Hennebert) Humber of 76.1% on *Macrosiphum pisi* Kaltenbach on horticultural crops in Canada. MacLeod *et al.*[7] recorded the fungus *Entomophthora planchoniana* Cornu destroying a population of *Aphis rumicis* Linnaeus in England. McLeod *et al.*[8] also recorded epizootics of *P. neoaphidis* on *Myzus persicae* (Sulzer) on *Spinacia oleracea* L. in Arkansas. In Spain, Díaz *et al.*[9] identified *P. neoaphidis* on *Macrosiphum euphorbiae* (Thomas), *N. ribisnigri*, *Aphis fabae* Scopoli and *Aphis gossypii* Glover on horticultural crops, and *Conidiobolus coronatus* (Constantin) on *A. fabae* on *Beta vulgaris* var *cicla* (chard) during spring and on *M. euphorbiae* on lettuce crops during autumn. In Argentina, Scorsetti *et al.*[10] cited epizootiological studies of *P. neoaphidis* on *N. ribisnigri* (Mosley) on lettuce crops. Prevalence of entomophthoralean fungi on aphids has also been recorded on cereal crops,[11] as well as on *Capsicum annuum* L. and *Solanum melongena* L.[12]

Generally, the development of epizootics depends on the host population dynamics[13] and on the host developmental stages susceptible to infection. Because aphids are hemimetabolous, nymphs live and feed on colonies together with adults, resulting in the entire population being susceptible to attack by fungal pathogens.

The aim of this study was to determine the fungal infection levels in aphid populations according to their developmental stage (nymphs and adults, apterae and alatae).

2 MATERIALS AND METHODS

2.1 Field surveys

Surveys were conducted in conventional crop fields in Monte Vera (31° 32′ 58.21″ S, 60° 41′ 34.74″ W), Santa Fe Province, Argentina. Studies were conducted on *Lactuca sativa* L., *Brassica oleracea* var.

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italica Plenck, *B. oleracea* var. *botrytis* L. and *B. oleracea* var. *capitata* L. The periods of observations on each crop were: *L. sativa* from 16 June to 7 September 2010; *B. oleracea* var. *italica* Plenck from 28 December 2012 to 31 May 2013 (spring–summer in Argentina) (designated *B. oleracea* var. *italica* 1) and from 25 March to 24 July 2013 (autumn–winter in Argentina) (designated *B. oleracea* var. *italica* 2); *B. oleracea* var. *botrytis* from 1 June to 16 July 2010 and from 8 April to 20 July 2011; *B. oleracea* var. *capitata* from 18 May to 5 August 2010.

The daily data of average temperatures and relative humidity were obtained from the Faculty of Hydrological Sciences of the Universidad Nacional del Litoral.

2.2 Aphid sampling

Aphid populations were sampled weekly. To this end, 20 plants of each crop species were randomly selected and checked for the presence of aphids. All aphids were quantified but in the count were not discriminated by species. Quantification was done *in situ*. For identification of aphid species, healthy living aphids were collected and transferred into plastic cups with lids (150 cm^3), and subsamples were then transferred to Eppendorf microcentrifuge tubes (1.5 cm^3). These subsamples were preserved in 70% ethanol. Aphids were identified to species level using the keys of Blackman and Eastop.[2]

2.3 Identification of fungal pathogens

Dead aphids with evidence of external fungal growth (mycelia growth or sporulation) were examined under a stereomicroscope and a compound microscope for the presence of rhizoids, cystidia and/or spores. Dead aphids without external signs of mycosis were placed on 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 (infection is evidenced by colour change of the aphid and limited mobility) 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 (1:1) or stained with 1% aceto-orcein plus glycerine for semi-permanent mounts. Fungal structures from dead infected aphids were measured to allow species identification. Fungal species were identified according to taxonomic keys and monographs of Bałazy,[14] Humber[15] and Keller.[16]

2.4 Statistical analyses

The numbers of healthy and infected aphids in each development stage (nymphs, apterae and alate adults) were compared by parametric ANOVA and Tukey's (HSD) *post hoc* test with $P = 0.05$ after log transformation of data. The risk of infection at the different developmental stages was compared with logistic regression. The Mann–Whitney *U*-test was used to compare the density of healthy and infected aphids between crops. Analyses were performed using InfoStat[17] statistical software.

3 RESULTS

Brevicoryne brassicae L., *M. persicae*, *Myzus ascalonicus* Doncaster, *Myzus* sp., *Lipaphis erysimi* Kaltenbach, *Lipaphis pseudobrassicae* (Davis), *Pemphigus populitransversus* Riley, *A. gossypii* and *N. ribisnigri* were the aphid species found infesting the crops studied (Table 1). *Brevicoryne brassicae* was the predominant species on *Brassica* crops, while *N. ribisnigri* was the main species found on *L. sativa*.

The averages of healthy and infected aphids were calculated per sample date and are shown in Table 2. To calculate the averages, all species of aphids were considered together (aphids not discriminated by species).

The fungal species recorded from aphids on *Brassica* crops were *Zoophthora radicans* (Bref.) A. Batko, *P. neoaphidis* and *E. planchoniana*, whereas those recorded from aphids on *L. sativa* were *P. neoaphidis* and *E. planchoniana*.

3.1 Population variations

On *B. oleracea* var. *capitata*, we observed high population densities of both healthy and infected aphids during the first week of sampling (18 May 2010). The number of infected aphids significantly decreased in the next and subsequent weeks, and then started to increase again after the fourth week. The highest percentage of infection was 75.6% ($n = 45$). On *L. sativa*, the level of infection was variable throughout the period of observations. The highest percentage of infection was 60.9% ($n = 87$). On both crops, the highest percentages of infection were observed on 16 July 2010 (Table 2).

When we studied and compared the prevalence of entomophthoralean fungi of aphids on *B. oleracea* var. *botrytis* during the 2 years of study, we found that during 2010 infected aphids were recorded throughout the period of observations. The highest percentage of infection in 2010 (64.4%, $n = 73$) was observed on 8 July. During 2011, the density of healthy aphids remained low during the period of observations, and the proportion of infected aphids varied widely among sampling dates. The highest percentage of infection in 2011 (96%, $n = 75$) was recorded on 24 June. The number of infected aphids significantly decreased in the following week and decreased to zero in the last week of sampling, coinciding with the end of the crop cycle (Table 2).

Regarding the comparison of the prevalence of entomophthoralean fungi of aphids between seasons, on *B. oleracea* var. *italica* 1 we observed a gradual increase in the population of healthy aphids from December to 14 February 2013. On this date, we observed a peak in the aphid population density ($\bar{x} = 259.6$ aphids $plant^{-1}$) and then a decrease in the number of healthy aphids. The first colonies of infected aphids were recorded on 15 March 2013, and the peak of infection level was observed on 8 April 2013 (25.4%; $n = 503$). After this date, the number of healthy and infected aphids decreased (Table 2).

On *B. oleracea* var. *italica* 2, the density of healthy and infected aphids varied widely. The peak in the density of healthy aphids was 21.1 aphids $plant^{-1}$ and was recorded on 7 June 2013. The infection levels varied between 6.1% ($n = 196$) and 58.2% ($n = 67$) throughout the period of observations. The highest percentage of infection was 58.2% ($n = 67$), recorded on 24 June 2013 (Table 2).

The density of healthy aphids was higher on *B. oleracea* var. *italica* 1 than in *B. oleracea* var. *italica* 2 ($P < 0.05$; Mann–Whitney $U = 5637.0$), and the occurrence of infected aphids was more common on *B. oleracea* var. *italica* 2, where infected aphids were recorded throughout the period of observations (Table 2). On *B. oleracea* var. *italica* 1, the occurrence of infected aphids was restricted to March and April, when the temperature decreased and the percentage humidity increased (more than 65%). The percentage of infection was higher on *B. oleracea* var. *italica* 2 than on *B. oleracea* var. *italica* 1 (Table 2). The number of infected

Table 1. Entomophthoralean fungi identified on aphids on *Brassica* and *L. sativa* crops in Argentina

Crop	Host	Fungal species	Date
Brassica oleracea var. *capitata*	*Myzus persicae*	*Z. radicans*	18-05-2010
		E. planchoniana	01-06-2010
	Myzus sp.	*Z. radicans*	16-06-2010
	B. brassicae	*Z. radicans*	24-06-2010
			08-07-2010
			16-07-2010
	Lipaphis erysimi		
	Lipaphis pseudobrassicae		
	Pemphigus populitransversus		
	Myzus ascalonicus		
Lactuca sativa	*Aphis gossypii*	*P. neoaphidis*	16-06-2010
	Myzus persicae	*P. neoaphidis*	24-06-2010
		E. planchoniana	08-07-2010
	Myzus sp.	*P. neoaphidis*	16-07-2010
	Nasonovia ribisnigri	*P. neoaphidis*	05-08-2010
		E. planchoniana	
Brassica oleracea var. *botrytis*	*Myzus persicae*	*P. neoaphidis*	01-06-2010
	Lipaphis pseudobrassicae		16-06-2010
	Myzus sp.	*P. neoaphidis*	24-06-2010
	B. brassicae	*Z. radicans*	08-07-2010
			16-07-2010
			05-05-2011
			13-05-2011
			19-05-2011
			24-06-2011
			01-07-2011
			08-07-2011
Brassica oleracea var. *italica*	*B. brassicae*	*P. neoaphidis*	15-03-2013
		Z. radicans	25-03-2013
	M. persicae	*P. neoaphidis*	05-04-2013
		Z. radicans	08-04-2013
			19-04-2013
			22-04-2013
			23-04-2013
			07-05-2013
			17-05-2013
			22-05-2013
			31-05-2013
			07-06-2013
			14-06-2013
			24-06-2013
			16-07-2013
			24-07-2013

aphids was significantly higher on *B. oleracea* var. *italica* 2 ($P < 0.05$; Mann–Whitney $U = 641$).

3.2 Association between infection levels and developmental stages

On all crops except *B. oleracea* var. *botrytis* (2011), the proportions of infected aphids were significantly different between developmental stages ($P < 0.05$). Between nymphs and apterous adults there were significant differences ($P < 0.05$) on all crops except *B. oleracea* var. *botrytis* (2010). When comparing nymphs and alate adults, we found significant differences on all crops except *B. oleracea* var. *italica* 1 and *L. sativa* ($P > 0.05$). There were significant differences between apterous adults and alate adults on all crops except *B. oleracea* var. *botrytis* (2011) ($P > 0.05$) (Table 3).

Regarding the infection risk between developmental stages (Table 3), we found that on *L. sativa* and *B. oleracea* var. *capitata* the risk that nymphs would become infected was greater than the risk that apterous adults would become infected (Table 3). Also, on *L. sativa*, *B. oleracea* var. *capitata*, *B. oleracea* var. *botrytis* (2010) and *B. oleracea* var. *italica* 2, the risk that alate adults would be infected was greater than the risk that nymphs would be infected (Table 3). On *B. oleracea* var. *botrytis* (2010) and on both crops of *B. oleracea* var. *italica*, the risk that apterous adults would be infected was greater than the risk that nymphs would be infected (Table 3). On *B. oleracea* var. *italica* 1, the risk that nymphs would be infected was greater than the risk that alate adults would be infected (Table 3). On *B. oleracea* var. *botrytis* (2010), *L. sativa*, *B. oleracea* var. *capitata* and *B. oleracea* var. *italica* 2, the risk that alate adults would be infected was greater than the risk that apterous adults would be infected (Table 3). *B. oleracea* var. *italica* 1 was the only crop where the risk that apterous adults would be infected was greater than the risk that alate adults would be infected.

4 DISCUSSION AND CONCLUSIONS

The prevalence of entomophthoralean fungi in aphids has been previously studied. In Argentina, we have previously recorded *P. neoaphidis* causing mortalities of up to 97% ($n = 257$) on aphids on cereal crops.[11] On horticultural crops, Scorsetti *et al.*[10] recorded

Table 2. Mean number of healthy and infected aphids and percentage of infection by sampling date[a]

Crops	Sample date	Healthy aphids	Infected aphids	Percentage of infection
Brassica oleracea var. *capitata*	18-05-2010	11.80 (10.2) c	3.60 (5.7) b	23.4
	01-06-2010	7.20 (8.2) b	1.05 (1.3) a	12.7
	16-06-2010	0.80 (2.7) a	0.95 (1.7) a	54.3
	24-06-2010	0.85 (2.5) a	0.55 (0.9) a	39.3
	08-07-2010	1.58 (3.8) a	0.84 (0.9) a	34.8
	16-07-2010	0.55 (1.3) a	1.70 (3.1) a	75.6
	05-08-2010	0.00 (0.0) a	0.00 (0.0) a	0
Lactuca sativa	16-06-2010	1.50 (2.1) b	0.60 (0.8) ab	26.8
	24-06-2010	3.20 (4.1) c	0.75 (1.2) ab	19
	08-07-2010	1.55 (1.3) b	1.85 (1.9) bc	54.4
	16-07-2010	1.70 (2.3) b	2.65 (4.0) c	60.9
	05-08-2010	0.00 (0.0) a	0.30 (0.0) a	100
	07-09-2010	0.00 (0.0) a	0.00 (0.0) a	0
Brassica oleracea var. *botrytis* (2010)	01-06-2010	10.40 (5.5) b	1.85 (1.8) ab	14
	16-06-2010	4.10 (6.5) a	4.00 (4.0) b	49.4
	24-06-2010	4.40 (7.9) a	2.85 (3.8) ab	39.3
	08-07-2010	1.30 (2.6) a	2.35 (3.9) ab	64.4
	16-07-2010	0.95 (2.6) a	0.95 (1.9) ab	50
Brassica oleracea var. *botrytis* (2011)	08-04-2011	0.85 (3.5) a	0.00 (0.0) a	0
	05-05-2011	1.25 (2.3) a	0.05 (0.2) a	3.8
	13-05-2011	4.10 (8.2) b	0.65 (2.4) a	13.7
	19-05-2011	2.30 (4.9) ab	0.25 (0.7) a	9.8
	27-05-2011	0.00 (0.0) a	0.00 (0.0) a	0
	13-06-2011	0.00 (0.0) a	0.00 (0.0) a	0
	24-06-2011	0.15 (0.6) a	3.60 (9.1) b	96
	01-07-2011	0.75 (2.4) a	0.65 (1.6) a	46.4
	08-07-2011	0.00 (0.0) a	0.20 (0.4) a	100
	20-07-2011	0.25 (1.1) a	0.00 (0.0) a	0
Brassica oleracea var. *italica* 1	28-12-2012	0.00 (0.0) a	0.00 (0.0) a	0
	04-01-2013	0.00 (0.0) a	0.00 (0.0) a	0
	08-01-2013	3.05 (3.4) a	0.00 (0.0) a	0
	15-01-2013	1.95 (8.0) a	0.00 (0.0) a	0
	23-01-2013	13.40 (17.2) ab	0.00 (0.0) a	0
	08-02-2013	73.15 (165.9) c	0.00 (0.0) a	0
	14-02-2013	259.60 (191.9) e	0.00 (0.0) a	0
	22-02-2013	121.10 (146.1) d	0.00 (0.0) a	0
	15-03-2013	55.40 (59.1) b	1.00 (4.2) a	1.8
	25-03-2013	15.80 (14.6) ab	0.50 (1.8) a	3.1
	05-04-2013	16.65 (16.3) ab	1.00 (3.0) a	5.7
	08-04-2013	18.75 (10.7) ab	6.40 (12.0) b	25.4
	19-04-2013	3.05 (7.4) a	0.00 (0.0) a	0
	23-04-2013	6.65 (5.6) ab	0.40 (0.8) a	5.7
	07-05-2013	0.75 (0.4) a	0.00 (0.0) a	0
	17-05-2013	0.10 (0.4) a	0.00 (0.0) a	0
	22-05-2013	1.00 (3.8) a	0.00 (0.0) a	0
	31-05-2013	0.50 (2.2) a	0.00 (0.0) a	0
Brassica oleracea var. *italica* 2	25-03-2013	1.45 (2.1) a	0.10 (0.4) a	6.5
	05-04-2013	11.05 (6.4) c	2.10 (3.7) cd	16
	08-04-2013	5.40 (4.3) ab	1.50 (1.1) abcd	21.4
	19-04-2013	1.25 (3.0) a	0.45 (0.7) abc	26.5
	22-04-2013	5.05 (12.0) ab	0.35 (1.1) ab	6.5
	07-05-2013	3.55 (6.3) ab	0.40 (0.5) ab	10.1
	17-05-2013	4.75 (7.1) ab	0.75 (1.3) abcd	13.6
	22-05-2013	9.20 (13.7) bc	0.60 (2.2) abc	6.1
	31-05-2013	4.30 (11.8) ab	0.55 (1.7) abc	11.3
	07-06-2013	21.35 (20.5) d	2.30 (3.7) d	9.8
	14-06-2013	3.60 (6.9) ab	1.50 (2.9) abcd	29.4
	24-06-2013	1.40 (3.2) a	1.95 (4.7) bcd	58.2
	16-07-2013	1.25 (3.9) a	0.25 (0.4) ab	16.7
	24-07-2013	0.65 (2.0) a	0.30 (0.9) ab	31.6

[a] Entries show the mean number (± SE) of healthy and infected aphids. Means sharing the same lower-case letter are not statistically different ($P > 0.05$).

Table 3. Comparison of the number of infected aphids between developmental stages and between observation dates

	Comparison between developmental stages			Logistic regression		Comparison between observation dates	
Crop	Chi square	Stages[a]	Signif.	Risk	Signif.	Chi square	Signif.
L. sativa	0.000	N & aa	0.002	2.25	0.001	45.79	0.000
		N & al	0.096	0.37	0.002		
		aa & al	0.001	0.16	0.106		
B. oleracea var. *capitata*	0.000	N & aa	0.034	1.55	0.000	88.39	0.000
		N & al	0.000	0.09	0.034		
		aa & al	0.000	0.05	0.000		
B. oleracea var. *botrytis* (2010)	0.000	N & aa	0.063	0.84	0.001	97.14	0.000
		N & al	0.000	0.05	0.393		
		aa & al	0.000	0.06	0.000		
B. oleracea var. *botrytis* (2011)	0.423	N & aa	0.212	–	–	185.65	0.000
		N & al	0.863	–	–		
		aa & al	0.405	–	–		
B. oleracea var. *italica* (summer–spring)	0.000	N & aa	0.000	0.54	0.000	2094.07	0.000
		N & al	0.338	1.62	0.000		
		aa & al	0.026	2.99	0.342		
B. oleracea var. *italica* (autumn–winter)	0.000	N & aa	0.002	0.60	0.000	159.37	0.000
		N & al	0.000	0.16	0.002		
		aa & al	0.000	0.27	0.000		

[a] N: nymphs; aa: apterous adults; al: alate adults.

P. neoaphidis from *N. ribisnigri* on *L. sativa*, with a prevalence of 56.6% ($n = 30$), while we have previously recorded infection levels of *P. neoaphidis* and of *E. planchoniana* on aphids on *S. melongena* and on *C. annuum* and observed the development of epizootics of these fungal species on *M. persicae*.[12] In the present study, we found *P. neoaphidis*, *E. planchoniana* and *Z. radicans* parasitising four aphid species. *Zoophthora radicans* was recorded causing epizootics on *B. brassicae*, reaching levels of 96% ($n = 75$) on *B. oleracea* var. *botrytis* during 2011.

The highest percentages of infection recorded in this study coincided with periods of high relative humidity and comparatively low temperatures. Comparison of the prevalence recorded on *B. oleracea* var. *italica* between the two periods of observations (spring–summer and autumn–winter) allowed variables such as type of crop or species of aphid host to be separated from the analysis, confirming that the conditions of temperature and relative humidity were the most important factors in determining the infection levels. On *B. oleracea* var. *italica* 2 (winter crop), the highest percentage of infection observed was greater than that recorded on *B. oleracea* var. *italica* 1 (summer crop). In Argentina, previous studies on the phenology of entomophthoralean fungi in populations of insects other than aphids have shown that fungal infections are more common in the autumn–winter season (i.e. from March to August in the southern hemisphere).[18,19] The low temperatures and humid conditions prevalent during the winter growing season tend to be propitious for the occurrence of entomophthoralean mycoses.[20]

Regarding the differences between developmental stages, we found significant differences in the number of infected nymphs and adults. Similarly to the results obtained in this study, Scorsetti *et al.*[10] found significant differences between different developmental stages on *L. sativa*, whereas we have previously recorded differences on *S. melongena* and *C. annuum*.[12] In this study, the number of infected nymphs was significantly higher than the number of infected apterae and alatae. Our results showed that most of the host population consisted of nymphs. According to Steinkraus,[14] dense populations of aphid nymphs may allow fungal pathogens to spread rapidly. Scorsetti *et al.*[10] found that *N. ribisnigri* was equally susceptible to fungal disease throughout its life cycle. Kim and Roberts[21] suggested that early instars of cotton aphids can escape from fungal infection owing to the combination of three factors: the low number of conidia attached to their cuticles, the low levels of conidium germination and quick moulting. These factors would promote the removal of conidia before the germ tube penetrates into the haemolymph of the host.

This study represents the first record of prevalence on lettuce and *Brassica* crops in Santa Fe Province, Argentina. It also represents an advance in the study of the epidemiology of entomophthoralean fungi on aphids in relation to developmental stages of aphids.

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