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Short Communication

Prevalence of entomophthoralean fungi (Entomophthoromycota) of aphids (Hemiptera: Aphididae) on solanaceous crops in Argentina

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ABSTRACT

Solanum melongena L. and *Capsicum annuum* L. were sampled in Argentina to determine the prevalence of fungal diseased aphids. The pathogens identified were *Pandora neoaphidis* (Remaudière & Hennebert) Humber and *Zoophthora radicans* (Brefeld) Batko (Entomophthorales: Entomophthoraceae) on aphids from eggplants; and *P. neoaphidis* and *Entomophthora planchoniana* Cornu (Entomophthorales: Entomophthoraceae) on aphids from peppers. The highest fungal prevalence was 45.5% ($n = 2296$) and 98.1% ($n = 3212$) from aphids on eggplants and peppers, respectively. In both crops, significant differences were found on number of infected aphids among developmental stages. *P. neoaphidis* and *E. planchoniana* caused epizootics in *M. persicae*.

1. Introduction

Aphids are one of the most important factors limiting horticultural crop production in Argentina (Botto, 1999). *Myzus persicae* Sulzer, *Macrosiphum euphorbiae* (Thomas), *Aphis gossypii* Glover, *Aphis fabae* Scopoli, *Aphis nasturtii* Scopoli and *Aulacorthum solani* (Kaltenbach) are the main species of aphids on *Solanum melongena* L. (eggplant) and on *Capsicum annuum* L. (pepper) in the world (Blackman and Eastop, 2000). Control of aphids has been predominantly by using chemical insecticides, but this practice creates human health, environmental problems and adversely effects non-target fauna.

Entomophthoralean fungi have been found to be important antagonists of aphids under field conditions (Latgé and Papierok, 1988) and they have the potential to induce epizootics that drastically reduce aphid densities (Nielsen, 2002; Pell et al., 2001).

Generally, the development of epizootics depends on host population dynamics (Anderson and May, 1981) and on host developmental stages susceptible to infection. Because aphids are hemimetabolous, the nymphs live and feed in colonies with the adults, resulting in the entire population being susceptible to attack by fungal pathogens. Furthermore, the transmission of aphid fungal pathogens is affected by environmental factors (Steinkraus, 2006), among them, temperature, RH and agricultural chemicals.

We provide information about infection levels in host populations, according to density and stage of development (nymphs and adults, apterae and alatae). And we try to explain the environmental factors that influence the development of epizootics under field conditions.

2. Materials and methods

2.1. Field survey

Surveys were conducted in conventional crop production in two localities in Santa Fe province, Argentina. In Monte Vera (31°32′58.21″S/60°41′34.74″W) we monitored a crop of *S. melongena* L. in open field production (one crop), from October 08, 2010 to May 05, 2011. In Recreo (31°33′28.80″S/60°43′38.60″W) studies were conducted on *C. annuum* L. in a greenhouse (one greenhouse), from March 29 to December 21, 2011.

2.2. Aphid sampling

Aphid populations were sampled weekly. Twenty plants of each species were randomly selected and checked from each crop.

For identification of aphid species healthy living aphids were collected and transferred into plastic cups with lids (150 cm^3);

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then subsamples were transferred to microcentrifuge tubes (Eppendorf; 1.5 cm^3). These subsamples were preserved in 70% ethanol. Identification to species level was made using the keys of Blackman and Eastop (2000).

2.3. Identification of fungal pathogens

Dead aphids with evidence of external fungal growth (mycelial growth or sporulation) were examined under a stereo microscope and a compound 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. 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 (2012b) and Keller (1991).

2.4. Statistical analyses

In order to assess the relationship between abundance of the host population and percentage of fungal infection, we performed a linear regression on natural logarithms of both variables (total number of healthy and infected aphids per sampling date). Comparisons among number of healthy and infected aphids in each development stage (nymphs, apterae and alate adults) were performed by parametric ANOVA and Tukey's (HSD) *post hoc* test with $p = 0.05$ after log transformation of data. We used logistic regression to compare the risk of infection among developmental stages. The Kruskal–Wallis one-way analysis of variance by ranks (Siegel, 1956) test was used to determine 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.

3. Results

M. persicae, *M. euphorbiae*, *A. fabae*, *A. gossypii* and *Toxoptera odinae* (van der. Goot) were the aphid species we found infesting eggplant. On pepper we found *M. persicae* and *M. euphorbiae*. *M. persicae* was the predominant species in both crops, while the other species were recorded only occasionally.

The fungal species recorded from aphids on eggplant were *P. neoaphidis* and *Zoophthora radicans* (Brefeld) Batko. On peppers, we found aphids infected with *P. neoaphidis* and *Entomophthora planchoniana*. *Pandora neoaphidis* and *E. planchoniana* were recorded causing epizootics on *M. persicae* on both eggplant and pepper.

In both crops, wide fluctuations in the densities of aphid populations occurred among sampling dates (Table 1). On eggplants the first colonies of aphids were recorded on November 01, 2010 and the peak in populations density ($\bar{x} = 525.2$ aphids/plant) was recorded on February 09, 2011. Infected aphids were found from December 30, 2010 to February 09, 2011 with an average monthly temperature of 26.1 °C. The highest prevalence of fungal infection was 45.5% ($n = 2296$) recorded on January 10, 2011.

On pepper, the first aphid colonies were observed on September 15, 2011. The peak in population density ($\bar{x} = 123.7$ aphids/plant) occurred on October 11, 2011 and coincided with the appearance of the first colonies of infected aphids. The number of infected aphids significantly increased into the next week (October 21) ($p < 0.05$) (Mann–Whitney test). The largest number of infected aphids was recorded on October 28, 2011 (3152 total aphids) while the highest percentage of infection was observed on November 4, 2011 (100%, $n = 15$). The average temperature during October and November was 21 °C. After this date there was a sharp decline in the number of healthy and infected aphids (Table 1), which coincided with the end of the cultivation cycle.

On eggplant, nearly 83% of the variation was explained by the regression model. The number of aphids that died from fungal infections increased as the insect population increased. On pepper, however, only 37% of the variation was explained by the regression model.

Table 1
Mean number of healthy and infected aphids by sampling date.

Eggplant			Pepper		
Sample date	Healthy aphids	Infected aphids	Sample date	Healthy aphids	Infected aphids
08-10-2010	0.00 (0.0) a*	0.00 (0.0) a	29-03-2011	0.00 (0.0) a	0.00 (0.0) a
01-11-2010	0.10 (0.4) a	0.00 (0.0) a	08-04-2011	0.00 (0.0) a	0.00 (0.0) a
12-11-2010	0.00 (0.0) a	0.00 (0.0) a	13-05-2011	0.00 (0.0) a	0.00 (0.0) a
26-11-2010	1.35 (3.5) a	0.00 (0.0) a	19-05-2011	0.00 (0.0) a	0.00 (0.0) a
09-12-2010	0.35 (0.9) a	0.00 (0.0) a	27-05-2011	0.00 (0.0) a	0.00 (0.0) a
17-12-2010	4.20 (5.5) a	0.00 (0.0) a	24-06-2011	0.00 (0.0) a	0.00 (0.0) a
30-12-2010	209.65 (298.5) b	9.15 (19.1) a	01-07-2011	0.00 (0.0) a	0.00 (0.0) a
04-01-2011	21.70 (18.5) a	14.00 (9.4) a	08-07-2011	0.00 (0.0) a	0.00 (0.0) a
10-01-2011	65.10 (68.3) a	52.20 (53.1) b	20-07-2011	0.00 (0.0) a	0.00 (0.0) a
18-01-2011	82.20 (128.1) a	53.35 (35.4) b	27-07-2011	0.00 (0.0) a	0.00 (0.0) a
09-02-2011	525.20 (492.9) c	66.65 (88.1) b	05-08-2011	0.00 (0.0) a	0.00 (0.0) a
04-03-2011	0.10 (0.3) a	0.00 (0.0) a	19-08-2011	0.00 (0.0) a	0.00 (0.0) a
29-03-2011	0.00 (0.0) a	0.00 (0.0) a	26-08-2011	0.00 (0.0) a	0.00 (0.0) a
08-04-2011	0.10 (0.4) a	0.00 (0.0) a	15-09-2011	55.25 (45.8) b	0.00 (0.0) a
05-05-2011	0.00 (0.0) a	0.00 (0.0) a	11-10-2011	123.75 (148.2) c	4.10 (12.5) a
			21-10-2011	3.70 (2.9) a	53.00 (26.7) b
			28-10-2011	3.00 (7.9) a	157.60 (105.1) c
			04-11-2011	1.50 (4.5) a	0.75 (1.9) a
			25-11-2011	0.00 (0.0) a	0.00 (0.0) a
			05-12-2011	0.00 (0.0) a	0.00 (0.0) a
			21-12-2011	0.00 (0.0) a	0.00 (0.0) a

* Points show mean number (±SE) of healthy and infected aphids. Means sharing the same letter are not statistically different ($p > 0.05$).

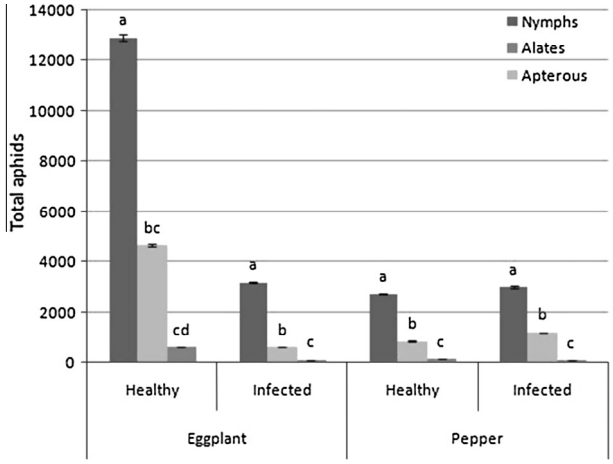

Fig. 1. Abundance of total uninfected and fungal-infected aphid nymphs, apterous and alates in eggplant (right) and pepper (left) crops. Points show mean number (±SE) of healthy and infected aphids. Means sharing the same letter are not statistically different ($p > 0.05$).

On eggplant, when considering healthy aphids, total number of nymphs was significantly different than adult apterae numbers ($p < 0.05$) and alatae ($p < 0.05$), and number of apterous and alate adults were not significantly different ($p > 0.05$). The proportions of infected aphids were significantly different between developmental stages (parametric one-way ANOVA, $F = 69.5$, $df = 2$, $p < 0.05$) (Fig. 1). The likelihood of nymphs becoming infected was 1.8 and 1.4 times greater than the likelihood that apterae or alatae would become infected ($p < 0.05$ for each, respectively).

On pepper, there were significant differences in the abundance of healthy (parametric one-way ANOVA, $F = 18.86$, $df = 2$, $p < 0.05$) and infected aphids (parametric one-way ANOVA, $F = 48.4$, $df = 2$, $p < 0.05$) between developmental stages (Fig. 1). The risk that nymphs will be infected was 0.8 and 1.4 times greater than the risk of apterae will be infected ($p < 0.05$) and alatae ($p < 0.05$), respectively.

4. Discussion

There have been previous studies on prevalence of entomophthoralean fungi in aphids. *P. neoaphidis* has been found causing epizootics in *M. persicae* on spinach in Arkansas (McLeod et al., 1998). In Argentina, Scorsetti et al. (2010) recorded *P. neoaphidis* infecting *Nasonovia ribisnigri* on lettuce crops with a prevalence of 56.6% ($n = 30$). In this study *P. neoaphidis* and *E. planchoniana* were recorded causing epizootics on *M. persicae* reaching levels of 45.5% ($n = 2296$) and 100% ($n = 15$) on eggplant and on pepper respectively. These fungal species appeared to play a role in declining green peach aphid populations. Interestingly, epizootics occurred between the months of October–February (spring and summer in Argentina).

Studies conducted in conventional lettuce crops in Argentina, recorded peak prevalence of 28.5% ($n = 4$) (Scorsetti, unpublished data) compared to organic crops in which recorded a prevalence of 56.6% ($n = 30$) (Scorsetti et al., 2010). The authors also found that the aphid numbers were lower in conventional than in organic crops. Chemicals applied to crops can affect transmission of aphid fungal pathogens (Steinkraus, 2006). It is important to emphasize that in this study, we recorded epizootics of *P. neoaphidis* and of *E. planchoniana* on *M. persicae* in conventional crops, even with applications of insecticides and fungicides; in contrast to previous records that have been cited significant prevalence but in organic crops (Scorsetti et al., 2010). This fact can allow us to presume that chemicals would not completely inhibit fungal activity.

Host population density has been considered to be a major factor controlling infection levels in some crop-aphid-pathogen systems (Feng et al., 1992; Soper and McLeod, 1981). In our study, this factor explained 83% of the variability in aphid infestations on eggplant. However, in pepper only 37% of the variability was due to host population density. Scorsetti et al. (2010) found that 66% of variability in infectivity by *P. neoaphidis* was explained by the density of healthy aphids. In both crops we found differences in infection levels between nymphs and adults. Significantly higher numbers of infected nymphs related to the number of apterae and alatae. Our results showed that most of the host population was nymphs. According to Steinkraus (2006), dense populations of aphid nymphs often result through which fungal pathogens can rapidly spread. Scorsetti et al. (2010) found that *N. ribisnigri* was equally susceptible to the fungal disease throughout its life cycle.

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