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Prevalence of *Pandora neoaphidis* (Zygomycetes: Entomophthorales) infecting *Nasonovia ribisnigri* (Hemiptera: Aphididae) on lettuce crops in Argentina

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ABSTRACT

Lettuce crops, *Lactuca sativa*, organically produced in La Plata, Argentina, were sampled in order to determine the prevalence of fungal diseased aphids. *Nasonovia ribisnigri* was the only aphid detected and its occurrence was highly variable. The fungal pathogen *Pandora neoaphidis* (Entomophthoromycotina: Entomophthorales) was the only pathogen detected. We recorded a maximum of 34.2 aphids per plant and the highest rate of fungal prevalence was 56.6% ($n = 30$) (aphids infected/total aphids). Infected aphids were observed in all sampling sites. No differences of infection rates were detected between the center and the edge of crops. Host density was an important factor determining infection. The majority of host population was comprised of nymphs which were the most infected in terms of individuals per habitat unit (lettuce plant), but considering the proportion of infected aphids per stage of development, the prevalence of infection in nymphs and adults was similar.

1. Introduction

Aphids are serious insect pests throughout the world and are one of the most important factors limiting horticultural crop production in Argentina (Botto, 1999). The aphid *Nasonovia ribisnigri* (Mosley) (Hemiptera: Aphididae) is a major pest of lettuce in Europe, Canada (Mackenzie et al., 1988; Rufingier et al., 1997), New Zealand (Stufkens and Teulon, 2003) and United States (Palumbo and Hannan, 2002). In South America it was first reported in 1963 (Remaudiére, 1963), however, there have been few reports of this aphid from Argentina (Delfino, 1983; Vasicek et al., 2000). *N. ribisnigri* is a vector of virus diseases, including "cucumber mosaic virus" and "lettuce mosaic virus" (Davis et al., 1997). Because of this aphid species prefers to feed in the "heart" of lettuce plants, it is difficult to control using contact insecticides. In spite of this, control of aphids, in general, has been done predominantly by using chemical insecticides, but this practice creates human health and environmental problems and adverse effects on the non-target fauna. Ideally, control tactics against aphids should be harmless to non-target fauna to protect their natural enemies, enhancing natural control by predators and parasitoids.

Entomopathogenic fungi are considered to be among the best candidates for biological control of aphids (Latge and Papierok, 1988). Fungi generally invade hosts through their integument, and therefore they are especially important for the control of insects with sucking mouthparts such as aphids since they cannot ingest other pathogens such as bacteria, viruses, or nematodes (Tanada and Kaya, 1993; Hajek and St. Leger, 1994).

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 of these fungi have been reported from South America (Aruta et al., 1984; Sánchez et al., 2001, 2002a,b) and there are only a few citations for Argentina (López Lastra and Scorsetti, 2006; Scorsetti et al., 2007).

Pandora neoaphidis (Remaudière and Hennebert) Humber (Entomophthoromycotina: Entomophthorales) (Hibbett et al., 2007), is an aphid-specific fungal pathogen and is the most common entomopathogen of aphids in temperate regions. It is the most common agent causing epizootics in natural field populations of aphids (Nielsen et al., 2003) and has, therefore, received considerable attention as a potential biological control agent (Steenberg and Eilenberg, 1995). Epizootics in aphids caused by fungi are frequent (Pell et al., 2001), and have been reported on alfalfa, *Medicago sativa* L. (Pickering and Gutierrez, 1991), wheat, *Triticum avenae* L. (Feng et al., 1991), barley, *Hordeum vulgare* L. (Feng et al., 1992) and spinach, *Spinacia oleracea* L. (McLeod et al., 1998). In South America there is little information about prevalence of these fungi in agricultural pests. Delalibera et al. (2000) reported an epizootic caused by *Neozygites tanajoe* Delalibera, Hajek & Humber sp. nov. (Entomophthoromycotina: Entomophthorales)

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on *Mononychellus tanajoa* (Bondar) (Acari: Tetranychidae) in Brazil and Alzugaray et al. (1998) cited epizootics of Entomophthorales on Lepidoptera in Uruguay.

Understanding the local species composition and distribution of entomopathogenic fungi is important to assist the control of pest insect populations within an organic agroecosystem.

In the present study, the occurrence and spatial distribution of entomopathogenic fungi in an organically grown agroecosystem is reported for South America for first time. We also give information about infection levels in host populations, according to density and stage of development (nymphs and adults, apterae and alatae), in order to explore the relationship between abundance of healthy and infected aphids.

2. Materials and methods

2.1. Field survey

Surveys were conducted in organic horticultural crop production in Buenos Aires province, Argentina. No insecticides or fungicides were applied during the course of the study. Three sites were surveyed, during time of crop production in all sites. Site 1: open field production. Abasto, La Plata county (34°57′35.7″S, 57°46′34.8″W) survey was made from May to August 2005. Site 2: greenhouse production. Olmos, La Plata county (34°56′36.4″S, 58°07′43.6″W) survey was made from June to September 2005. Site 3: open field production. Pereyra Iraola, Berazategui county (34°57′55.4″S, 57°52′58.6″W) survey was made from September to November 2005.

2.2. Aphid sampling

Aphids were sampled weekly from May to November 2005. Sampling was conducted from planting to harvest period. To evaluate whether differences in colonization of crop and percentage of infection of the entomopathogenic fungi does exist between the border and the middle of the crop, each site was divided into a border and a central area. One hundred plants were randomly selected (50 plants located at the edge and 50 at the center of the crop fields). We defined "edge" as the first 3 m of rows around the crop. In plants at early state of "rosette" (up to 10 leaves), the whole plant was sampled. In mature plants, only leaves from the center of the plant were sampled because the majority of the aphids are found in the innermost leaves (Liu, 2004).

All aphids found on leaves were individually placed in sterilized plastic vials (30 ml), held in cool ice chests, and transported to the laboratory for later analysis of fungal infection. Temperature and humidity data at the time of the survey were registered using a TFA® (Wertheim, Germany) thermo-hygrometer.

Aphids were inspected in the laboratory with a dissecting stereomicroscope and classified as adults (apterae or alates), or nymphs, and dead from fungal mycoses or alive. Living aphids were maintained in plastic vials at 25 ± 1 °C, 75% HR, and 12:12 h (L:D) for 3 days for diagnosis of infections acquired at the field. Healthy aphids were preserved in 70% ethanol for identification.

2.3. Identification of fungal pathogens

Aphids with signs of external fungal growth were examined under a dissecting stereomicroscope. Fungal structures were mounted in lactophenol–aceto-orcein (LPAO) (1:1) or stained with 1% aceto-orcein plus glycerine for semipermanent mounts, and examined with a phase contrast microscope. 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 Keller (1987, 1991), Bałazy (1993) and Humber (1989).

2.4. Isolation of fungal pathogens

Infected aphids with no external 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 consecutive baths) (Lacey and Brooks, 1997). Insects were placed on moist sterile filter paper attached with a double coated tape to the lid of a sterile 60 mm Petri dish, and then inverted on 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 U/ml penicillin G (Merck, Darmstadt, Germany) and 80,000 U/ml streptomycin (Parafarm, Buenos Aires, Argentina). This assembly was left 12 h in darkness at 22 ± 1 °C. A Petri dish lid with the attached aphid was replaced by a sterile new one, after 12 h. All the 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 (CEPAVE, La Plata, Argentina), and at ARSEF USDA-ARS Collection of Entomopathogenic Fungal Cultures (Ithaca, New York).

2.5. Healthy and infected aphid population trends

T-tests were used to test for differences between total number of aphids of center and edge of the crops. 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 ordered from the lowest to highest density values). 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. Homogeneity of variances was checked with Levene's test. Comparisons among mean proportion of diseased aphids in each development stage were performed on raw data after testing homogeneity of variances, followed by parametric ANOVA. To estimate the spatial aggregation of healthy and fungal-infected aphids, the negative binomial exponent for a clumped distribution, k, was calculated (Krebs, 1989) using GenStat software (GenStat, 2003).

3. Results

The aphid *N. ribisnigri* was the only aphid species found in the survey of lettuce crops and *P. neoaphidis* was the only fungal species recorded in the present study. From a total of 17,058 aphids detected in surveys, 2171 (12%) were infected with *P. neoaphidis*. A similar trend was observed in each survey. Although aphids were detected during all sampling dates, the lowest numbers generally occurred at the beginning of the sampling period. During the time of transplanting to ground, plants were colonized naturally by *N. ribisnigri* and by the "whitefly" *Trialeurodes vaporarorium* (Westwood), however, by the end of the third week whiteflies were no longer observed in the crops. Infestations of *Lactuca sativa* plants with aphids were registered from the beginning to end of cultivations with ample fluctuations. Furthermore these infestations were observed in both edge and center sections of the field.

Aphids killed by *P. neoaphidis* were typically white in color and bodies were tightly held to the leaf surface by fungal rhizoids.

No significant differences were found in the density of mean number of infected aphids between edge and center of fields at a