Temporal changes in the prevalence of three species of Trichomycetes (Zygomycota: Zygomycotina) in Dipteran aquatic larvae from Argentina

C.C. López Lastra[1,2*], M.G. Mazzucchelli[1] and V. Dikgolz, [1,3]

[1]CEPAVE (Centro de Estudios Parasitológicos y de Vectores) (CONICET-UNLP) Calle 2 Nº 584 (1900) La Plata Provincia de Buenos Aires, ARGENTINA
[2]Scientific Researcher of National Research Council of Argentina (CONICET)
[3]Research fellow from Scientific Research Committee of Buenos Aires Province (CIC)

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Trichomycetes (fungi) inhabit the digestive tracts of insects and other arthropods. Three species of Harpellales (Zygomycotina: Trichomycetes) were collected in the field from Dipteran larvae at La Plata, Argentina every 15 days for 1.5 years from 1999 to 2000. Records of the occurrence of the insect larval hosts, *Dasyhelea necrophila* (Ceratopogonidae), *Chironomus* sp. (Chironomidae) and *Culex pipiens* (Culicidae), and the prevalence of their associated Harpellales, *Carouxella coemeteriensis*, *Stachylina platensis* and *Smittium culisetae,* are presented. The fungi were somewhat seasonal, being present during fall, winter and spring. Their abundance appeared to depend on host density.

Key words: aquatic insects, Argentina, fungi, seasonality, Trichomycetes

Introduction

Fungi in the class Trichomycetes are cosmopolitan obligate inhabitants of the gut of arthropods, including aquatic insects such as flies (Diptera), mayflies (Ephemeroptera) and stoneflies (Plecoptera) (Lichtwardt, 1986).

Although several reports have focused on hosts and their geographic range and species richness (e.g. Cafaro, 2002), little attention has been given to temporal changes in levels of infection of Trichomycetes in host populations and the factors relating to transmission of the fungi. There are only a few publications on seasonality and prevalence of Trichomycetes in arthropods (Mattson, 1988; Grigg and Williams, 1989, 1990; Maciá *et al.*, 1995, 1997;

*Corresponding author: Claudia Lopez Lastra; email: ccll@museo.fcnym.unlp.edu.ar

Campos *et al*., 1995; Labeyrie *et al*., 1996; Taylor *et al*., 1996; Beard and Adler, 2002).

In the present paper we report changes in levels of infestation in three species of Trichomycetes in the order Harpellales, *Carouxella coemeteriensis*, *Stachylina platensis* and *Smittium culisetae,* in three species of aquatic Diptera larvae from water in flower vases at the La Plata cemetery, Buenos Aires province, Argentina. The Argentinean fungi, *Carouxella coemeteriensis* Lichtwardt, López Lastra & Ferrington, were described from *Dasyhelea necrophila* larvae, and *Stachylina platensis* López Lastra, Lichtwardt & Ferrington from Chironomidae, whereas the geographically widespread species, *Smittium culisetae* Lichtwardt, has previously been reported from mosquito larvae in Argentina (López Lastra, 1997; Lichtwardt *et al*., 1999, 2000).

Material and methods

Larvae of aquatic dipterans *Dasyhelea necrophila* Spinelli & Rodríguez (Ceratopogonidae), *Chironomus* sp. (Chironomidae) and *Culex pipiens* Wiedemann, (Culicidae) were collected every 15 days for 1.5 years from flower vases at the La Plata cemetery, Argentina. Forty flower vases were randomly selected and the number of larvae determined for each species. They were carried to the laboratory on ice where the larvae were dissected and checked for the presence of Trichomycetes. The minimal number of insects dissected was 50, and the percentage of infection was recorded. The larvae were dissected under a stereomicroscope by pulling away the abdomen and thorax from the head, and separating the hindgut and peritrophic membrane of the midgut and microscopic preparations under an Olympus microscope (CH 30) with phase contrast. The contents of the peritrophic membranes were removed in a drop of sterile distilled water on a glass slide. Semipermanent slide mounts of the peritrophic membranes (for *Chironomus* sp.) and the hindguts (for *Dasyhelea necrophila* and *Culex pipiens*) were prepared using lactophenol-cotton blue and sealed with nail polish. Slides have been deposited in the CEPAVE fungal culture collection and at the Spegazzini Mycological Herbarium (La Plata). Unstained preparations were first observed in water under an Olympus phase-contrast microscope (CH 30) before the final slides were made. Photomicrographs were taken using an Olympus (SC 35) camera adapted to the microscope.

The relation between percentage of infection and environmental variables (temperature and rainfall averages) were analysed by multiple regression. Percentage of infection was analysed by “backward elimination analysis" and was used to select the environmental variables that had significant effect on the per cent of infection (Zar, 1996). Percentage of infection of the three species of Trichomycetes were transformed (arcsin-square root method) prior to analyses.

Results

Carouxella coemeteriensis, Stachylina platensis and *Smittium culisetae* from their respective Dipteran hosts collected from water vases at the La Plata cemetery are shown in Figure 1.

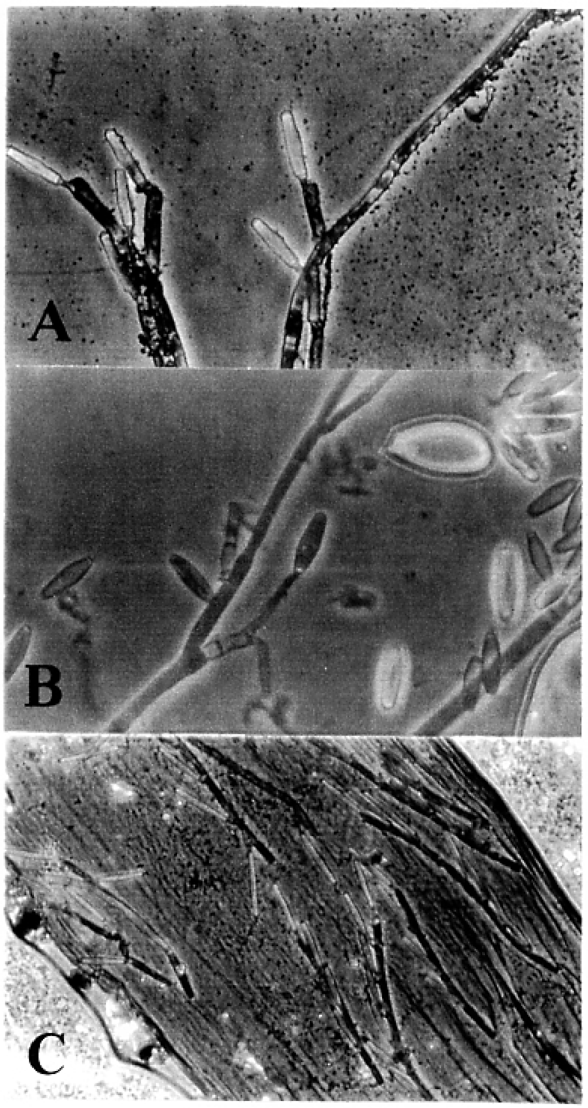

Fig. 1. A. *Carouxella coemeteriensis* thallus with trichospores from a *Dasyhelea necrophila* larva. **B.** *Smittium culisetae* from a *Culex pipiens* larva, thallus with trichospores. **C.** *Stachylina platensis*, thallus with trichospores from a *Chironomus* sp. larva. Bars A, C = 15 µm; B = 13 µm.

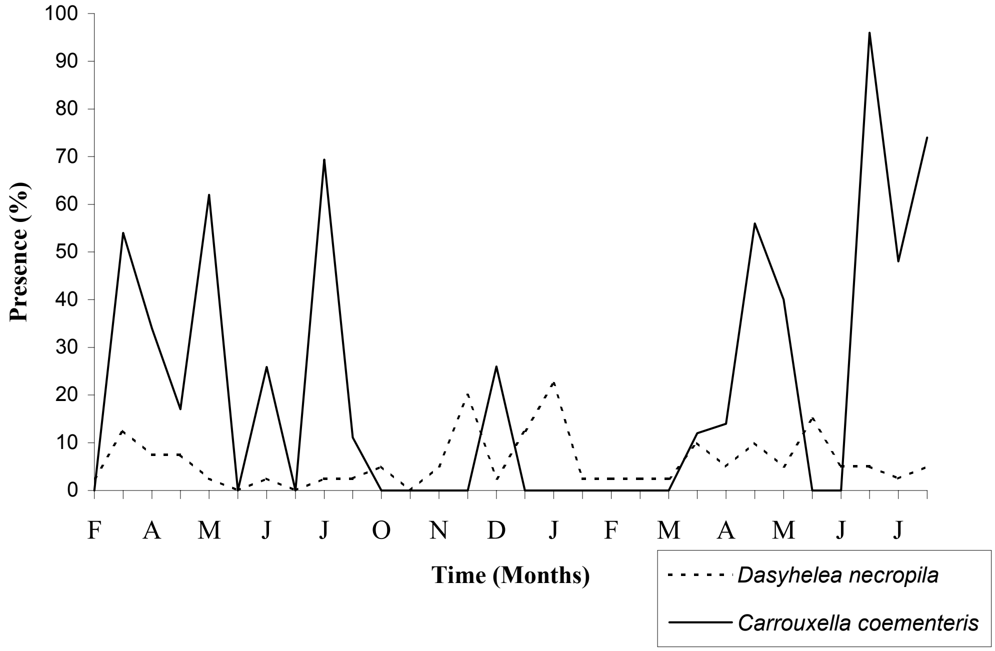

Fig 2. Seasonal variation of *Dasyhelea necrophila* larvae and prevalence of *Carouxella coemeteriensis* in La Plata, Argentina.

Figure 2 demonstrates that *Carouxella coemeteriensis* was present in *Dasyhelea necrophila* larvae from February 1999 to July 2000 with a highest peak of infection of 68% (n = 25) in July and then it appeared again in December, and during the next year from March to July with a peak in July [98% infection (n = 50)], while the host was present over the entire 1.5 years.

Stachylina platensis was present in *Chironomus* sp. larval guts essentially during the entire period surveyed, from February 1999 to May 1999 (Fig. 3), having a peak of infection of 98% (n = 50) in July, which then decreased in October of the same year. During the year 2000 there appeared a very small number of infestations (2.5%) (n = 2) in January, and then again in May with a maximum level of infestation in July (20 %) (n = 10). *Stachylina platensis* was also present mostly during the intermediate seasons (fall and spring), and also showed a maximum infection during winter.

Smittium culisetae was present only at three intervals during the entire time of the survey, and this was in April-May 1999, December 1999, and May-June 2000, the last with the maximum infection percentage of 32 % (n = 30) in June 2000 (Fig 4).

The results of the multiple regression analysis showed that there was only a negative correlation between temperature and fungal infections by *Carouxella coemeteriensis,* and no correlation for annual precipitation (Fig. 5). The equation values of multiple regression analyses are shown in Table 1. Annual precipitation and average temperatures during the period surveyed showed many fluctuations.

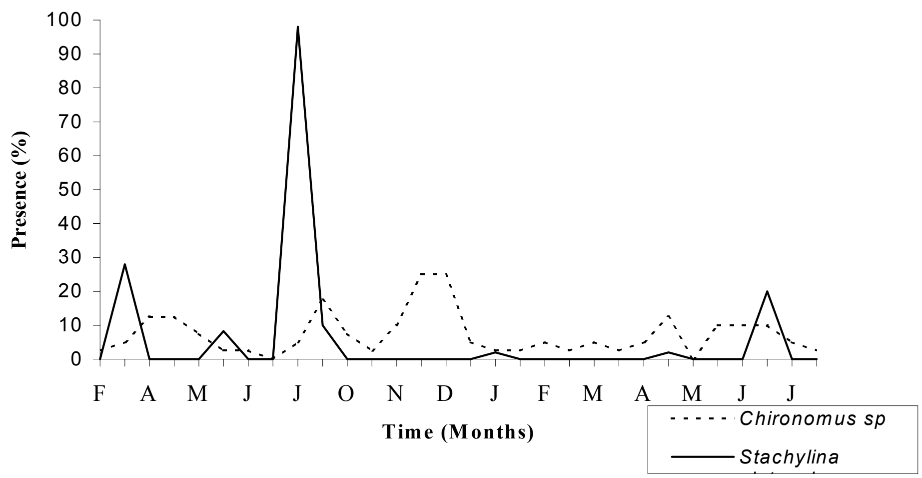

Fig. 3. Seasonal variation of *Chironomus* sp. larvae and prevalence of *Stachylina platensis* in La Plata, Argentina.

Discussion

There have been previous studies on seasonality and prevalence of Trichomycetes in aquatic insects. Grigg and Williams (1989) reported that in 1986 and 1987 in Nebraska, USA, *Smittium culisetae* was found in mosquito larvae with a prevalence of 14.1% and 16.1%, while *S. culicis* had a prevalence of 1.8% and 3.2%. Taylor *et al*. (1996) reported that *Harpella melusinae* was present throughout the year in *Simulium ornatum* larvae in a stream in England, with very high percentages of larval infection from May to December (100% in May, November, and December)

In a study concerning trichomycete prevalence on blackfly larvae and adults, several species of simuliids were sampled in New York State streams (Labeyrie *et al*., 1996). They reported ranges of larval infection from 9 to

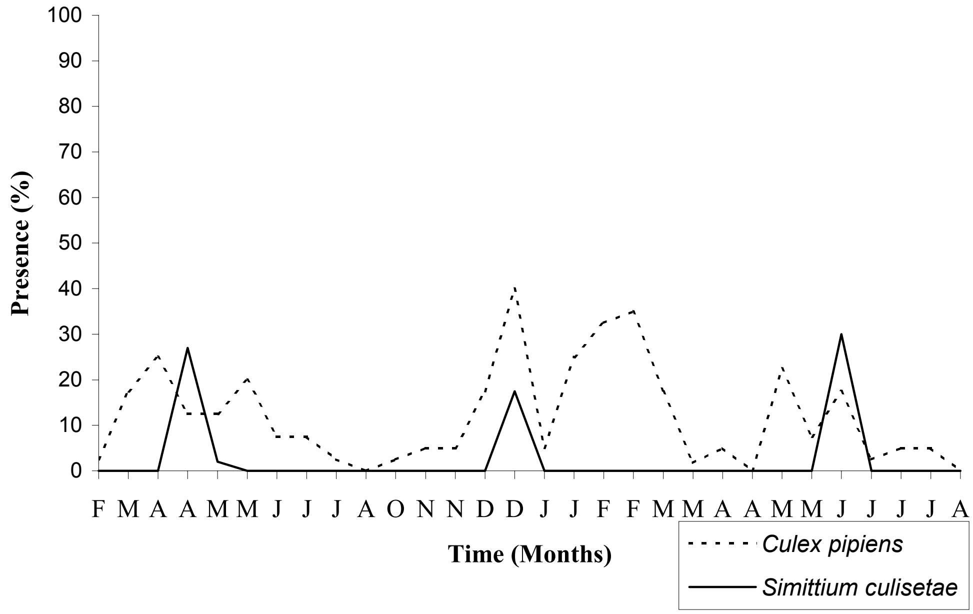

Fig 4. Seasonal variation of *Culex pipiens* larvae and prevalence of *Smittium culisetae* in La Plata, Argentina

100% for *Harpella melusinae,* and 9 to 75% for *Genistellospora homothallica,* for blackfly larvae, depending on the host species and time of year. Maciá *et al*. (1995) studied the presence of pathogens and parasites of Culicidae larvae in Argentina and reported that *Smittium morbosum* infected *Aedes albifasciatus* and *Ae*. *crinifer* larvae at the rate of 0.2 to 87% in 1990 and 0.3 to 60% in 1991. Campos *et al*. (1995) reported that *Smittium morbosum* infected *Psorophora fe*rox larvae from October 1990 to March 1991 with a maximum of 100% infection in October and January.

Maciá *et al*. (1996-1997) reported the presence of *Smittium morbosum* in three other species of mosquitoes, *Culex dolosus*, *Cx*. *intrincatus* and *Cx*. *maxi*, with maximum infection levels of 28.6% (September 1990), 7.4% (April 1991) and 29% (April 1991). In the present study we observed that the prevalence of *Carrouxella coemeteriensis* in *Dasyhelea necrophila* larvae ranged from 12% to almost 100% (in the winter), showing its presence over most of the year (Fig

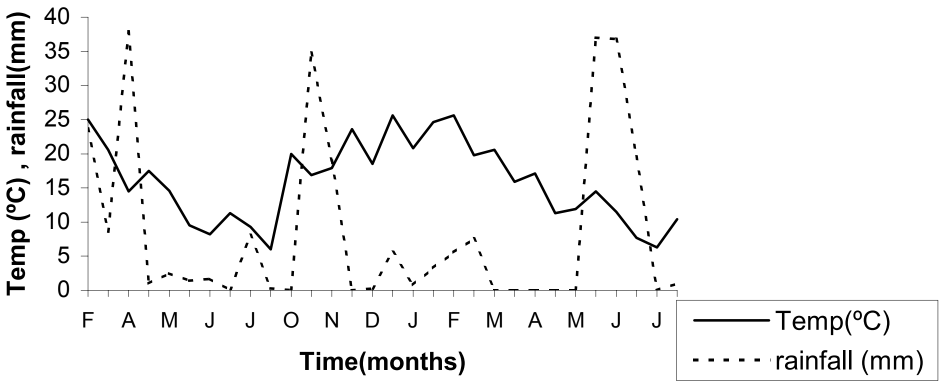

Fig. 5. Annual variation of temperature and rainfall during the period 1999-2000.

2). *Stachylina platensis* occurring in *Chironomus* sp larvae had a prevalence of between 2.5% to 95% with a maximum level of infection during the winter of 1999 (Fig 3). *Smittium culisetae* was present for a determined and restricted period of time, during the fall of 1999, a short period of time at the end of spring and beginning of summer 2000 and finally during the fall of 2000, showing a lower percentage of infection (15% to 30%) as compared with the other two fungal species in the present study (Fig. 4).

This study has illustrated a seasonal occurrence among Trichomycetes with highest occurrence during fall, spring and winter. This is similar to the results obtained by Maciá *et al*. (1995, 1997). Campos *et al*. (1995) reported trichomycete infections throughout October to March (which includes spring, summer and fall for the Southern Hemisphere). Beard and Adler (2002) reported a higher infection level of *Smittium culisetae* in blackfly larvae during the summer in South Carolina at USA. The higher prevalence of *S. culisetae* during the summer might be due to a greater tolerance of this species to higher temperatures. In our present study we have not detected such results for *S. culisetae* from mosquito larvae. This difference could be due to the low host density and differences in organic matter contents as well as water temperatures in the flowerpots we sampled.

The greater presence of the Trichomycetes seems to be related to density of the hosts at different seasons. Although the larval rearing flower vases were

Table 1. Results of the multiple regression analysis between presence of Trichomycetes species with temperature and rainfall.

Species	Multiple regression analysis	R2	F	df	p
Carouxella coemeteriensis	arcsen√p1= 53,94 - 0,52.Tº - 0,03.log (pLL+1)	0.278	5.2	2.27	0.012
	arcsen√p1= 53,46 - 0,53.Tº	0.277	10.74	1.28	0.027
Stachylina platensis	arcsen√p2= 17,15 - 0,32.Tº + 0,195.log (pLL+1)	0.126	1.95	2.27	0.16
Smittium culisetae	arcsen√p3= 0,15 + 0,11.Tº + 0,041.log (pLL+1)	0.014	0.2	2.27	0.81

p1 = proportion of *Carouxella coemeteriensis*
p2 = proportion of *Stachylina platensis*
p3 = proportion of *Smittium culisetae*
pLL =rainfall average
Tº = temperature

distributed some distance from each other and selected randomly, *Dasyhelea necrophila* and *Stachylina platensis* were present in their larval hosts generally throughout the year. However, *S. culisetae* was absent during certain months. This might suggest that there might be some method of dispersal of fungal inoculum by adults, as was demonstrated for Simuliidae (Labeyrie *et al*., 1996). This is an interesting topic for further investigation.

Acknowledgments

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