OCCURRENCE OF ENTOMOPHTHORALES ON SPITTLEBUGS PESTS OF PASTURE IN EASTERN SÃO PAULO STATE, BRAZIL

L.G. Leite[1], S.B. Alves[2], H.M. Takada[1], A. Batista Filho[1], D.W. Roberts[3]

[1]Centro Experimental Central do Instituto Biológico, Instituto Biológico, CP 70, CEP 13001-970, Campinas, SP, Brazil.

ABSTRACT

Spittlebugs (Hemiptera: Cercopidae) are the most important pests of pasture in Brazil. Nymphal behavior, i.e. residing in the soil, makes their control with insecticides difficult. Although Entomophthorales fungi occasionally have been found at epizootic levels in spittlebug populations, they have not been cultured, had their incidence levels determined, nor evaluated for pest control potential. The research reported here aimed to evaluate the occurrence of Entomophthorales species on spittlebug pests of pasture in Pindamonhangaba County, São Paulo State, Brazil. Evaluations were carried out in 2 adjacent fields with *Brachiaria decumbens* and *Pennisetum purpureum* grasses, respectively, of 5 ha each. Evaluations were done every 4 days from January through February by capturing spittlebug adults on leaves with an entomological sweep net, and the insect samples were kept frozen until examined. Insect abdomens were dissected and observed by microscopy for the presence of hyphae and resting spores. Insects cadavers with sporulating fungus were collected in the field and taken immediately to the lab to isolate the pathogen. Scanning electron microscopy pictures were taken of conidophores, and primary and secondary conidia of both fungi. *Furia* sp. was found at epizootic levels on *Deois schach* in the *Brachiaria* pasture, reaching 80% infected followed by a fall in the insect population. *Batkoa* sp. was found at enzootic levels (< 10% infected) on *Mahanarva fimbriolata* in the *P. purpureum* pasture. *Furia* sp. also was found to infect another important spittlebug, *Deois flavopicta,* indicating that it has good potential as a bioinsecticide. Heavy rainfall adversely affected the occurrence of *Batkoa* sp. in *M. fimbriolata* populations in *P. purpureum* pasture.

KEY WORDS: Entomophthorales, *Batkoa* sp., *Furia* sp., spittlebugs, *Mahanarva fimbriolata*, *Deois* spp., pasture.

RESUMO

OCORRÊNCIA DE ENTOMOPHTHORALES EM POPULAÇÕES DAS CIGARRINHAS DAS PASTAGENS. As cigarrinhas das pastagens (Hemiptera: Cercopidae) estão entre as principais pragas da cultura. Devido à localização das ninfas no solo, inseticidas químicos não são eficientes no controle da praga. Embora os fungos da Ordem Entomophthorales causem epizootias ocasionais em populações das cigarrinhas das pastagens e cana-de-açúcar, tais patógenos não têm sido estudados para o controle desses insetos. Esse trabalho objetivou avaliar a ocorrência de fungos Entomophthorales na população das cigarrinhas das pastagens no Município de Pindamonhangaba, SP. O experimento foi realizado em duas áreas de pastagens próximas, com 10 ha cada. As avaliações foram realizadas a cada 4 dias, entre janeiro e fevereiro de 1998, sendo feitas com a captura de insetos adultos e o transporte para o laboratório. Para avaliar a infecção, os conteúdos do abdome e tórax dos insetos foram retirados, observando-se em microscópio óptico a presença de hifas ou esporos de resistência do patógeno. Nesse estudo também foram fotografados conidióforos e conídios de cada fungo utilizando microscopia eletrônica de varredura. *Furia* sp. foi encontrado em níveis epizoóticos atacando *Deois schach* na pastagem de *Brachiaria*, provocando até 80% de infecção com consequente queda na população do inseto. *Batkoa* sp. foi encontrado em nível enzoótico (< 10% de infecção) atacando *Mahanarva fimbriolata* na pastagem de napier (*Pennisetum purpureum*).

[2]Departamento de Entomologia e Fitopatologia, ESALQ / USP, Piracicaba, SP.
[3]Department of Biology, Utah State University, Logan, USA.

Furia sp. foi também encontrado atacando outra importante cigarrinha, *Deois flavopicta*, indicando ser um bom agente para ser avaliado como bioinseticida. Chuva intensa pode ser um fator adversso para a ocorrência de *Batkoa* sp. na população de *M. fimbriolata* na pastagem de napier.

PALAVRAS-CHAVE: Entomophthorales, *Batkoa* sp., *Furia* sp., cigarrinhas das pastagens, *Mahanarva fimbriolata*, *Deois* spp.

INTRODUCTION

Spittlebugs (Hemiptera: Cercopidae) are among the most important pests of pastures, causing yield losses of 10% to 100%. These insects suck plant sap and inject toxic substances which cause a typical symptom known as "burned pasture" (ALVES, 1985). The most important species are: *Deois flavopicta* (Stal, 1854), *Deois schach* (Fabr., 1787), *Zulia entreriana* (Berg, 1879) and *Mahanarva fimbriolata* (Fabr., 1787). The latter species is the main pest of the napier grass (*Pennisetum purpureum* Schumach) in eastern São Paulo State, and it is the most important cercopid cane sugar pest in the entire state.

The nymphal behavior of residing on roots below the surface of soil makes their control with insecticides difficult. Farmers prefer alternative methods of control in order to better deal with the pest and reduce chemical pesticide pollution, thereby avoiding intoxication problems with humans and animals. The fungus *Metarhizium anisopliae* is one of the most commonly used non-chemical control measures against these pests. Results have been very dependent on pasture management and weather (RAMIRO & COTTAS, 1979; COTTAS & RAMIRO, 1981; ALVES, 1986; ALVES & LECUONA, 1996).

Although Entomophthorales occasionally have been found at epizootic levels in spittlebug pests of pasture and sugarcane (LEPAGE & MONTE, 1942; GUAGLIUMI, 1969; GUAGLIUMI, 1972; VALERIO & KOLLER, 1982; BATISTA FILHO, 1997; ALVES, 1998), they have not been researched for the control of these insects. To carry out this study, it is important to know, initially, the species of Entomophthorales that occur in the insect populations, as well as the biotic and non biotic factors which can affect the epizootics of these pathogens. This research aimed to evaluate the incidence of Entomophthorales species in populations of spittlebugs pests of two species of pasture grass in Pindamonhangaba County, São Paulo State, Brazil.

MATERIALS AND METHODS

Evaluations were carried out in 2 adjacent fields with *Brachiaria decumbens* and napier (*Pennisetum purpureum*) grasses, respectively, and a total area of 10 ha on a private farm in Pindamonhangaba County, São Paulo State, Brazil. *B. decumbens* had an average height of 0.5 m at the beginning of the experiment, while napier was 0.8 m high.

Evaluations were done at 4-day intervals during January and February by capturing spittlebug adults on leaves and transporting them to the laboratory. Capture was done with a 30 cm diameter entomological net, with 50 uninterrupted sweeps per replication and 4 replications per evaluation. The insects were stored in a freezer (-10° C) to preserve the integrity of the hosts and fungi until the counting of insects and identification of those infected by the fungus could be carried out. Due to the predominance of *M. fimbriolata* in napier pasture and *D. schach* in *Brachiaria*, only these two insect species were included in the evaluations.

In order to identify infection, the contents of the insect's abdomen and thorax were dissected out, put on a slide with a drop of water, covered with a cover slip, squashed, and observed by optical microscopy for the presence of hyphae and resting spores. The results allowed for calculation of the incidence of Entomophthorales infections in these areas and, based on these infection levels, estimate the inoculum density. Inoculum density was estimation of from the average area (0.3 m^2) covered by each sweep [0.3 m net diameter times the average length (1 m) covered by each sweep], the insect population density, and the percentage of insects that would die from infection by the pathogen.

Also, insect cadavers with sporulating fungus were collected in the field and taken to the laboratory to isolate the pathogen. The fungi were cultured from their conidial phase using 2% dextrose plus 2% yeast extract solid (1.5% agar) medium. Some cadavers with fungal infections were shipped to Dr. Richard W. Humber, entomophthorales specialist of USDA in Ithaca, New York, for identification of the fungi.

In order to know the degree of relationship between the diseases, the host populations and abiotic factors (weather) the data were compared by Pearson and multiple regression methods, using SPSS programs. Infection levels in each evaluation to be dependent on the climatic factors which occurred during the previous 3 or 4 days, so correlations were made using averages of temperature and relative humidity as well as the sum of precipitation data recorded during these periods.

The isolated fungi were examined by scanning electron microscopy to record in detail their conidiogenesis, conidia and secondary conidia. The

fungi were grown on solid media [yeast extract (2%), dextrose (2%) and agar (1.5%)] in petri dishes. The fungi, after covering more than 50% of the surface area, were cut into 0.3 x 0.3 cm squares and transferred to other petri dishes containing water-agar. The mycelial squares were then incubated for at least 12hs at 23º C for the fungi to sporulate. The mycelial squares were taken out together with the water-agar, cutting the substrate around the pathogens at a distance of 0.5 cm from the colony. The samples were placed on the inverted base of a petri dish (9 cm diameter) inside a bell flask containing formaldahyde (16%) plus glutaraldahyde (10%) and exposed to the fixative vapor for at least 24 hs. The samples then were quickly frozen in liquid nitrogen or Freon 113 and lyophilized for 24 hs. The dried samples were mounted on aluminum stubs and coated with gold-paladium. Finally, they were observed with an Hitachi S-4000 scanning electron microscopy.

RESULTS AND DISCUSSION

There was found the occurrence of *Batkoa* sp. attacking *M. fimbriolata* and *Furia* sp. attacking *D. Schach.*

Taxonomic aspects - *Batkoa* sp. and *Furia* sp. showed many taxonomic differences, some of which contributed to the epizootic potential differences between the species. *Batkoa* sp. has simple conidiophores, with globose primary and secondary conidia (Fig. 1). It had thick rhizoids with terminal discoid holdfasts that fix the host onto the substrate. Resting spores were found inside the host and on solid media made of yeast extract (1%) plus dextrose (2%). *Batkoa* sp. is similar to *Entomophaga* genus, but differs especially in the formation of rhizoids, which are not formed by *Entomophaga* (HUMBER, 1989).

Furia sp. has branched conidiophores and obovoid primary and secondary conidia (Fig. 2). It makes numerous rhizoids, with no discoid terminal holdfast. This genus is close to *Erynia*, differing especially in making more rhizoids than *Erynia* (HUMBER, 1989).

The methodology used for observing the fungi by scanning electron microscopy efficiently fixed the samples, and avoided destruction during electron emission of weak structures, like the conidiophores that support the secondary conidia. It is a relatively easy, safe and inexpensive methodology compared with that traditionally used (BOZZOLLA & RUSSELL, 1992). It is easier because it does not use solutions of osmium tetroxide and ethanol, but requires only leaving the samples inside a sealed chamber exposed to the vapor phase only of formaldehyde plus glutaraldehyde for 24 hrs. It is safer because the solutions used are less toxic than osmium tetroxide. It is less expensive since specialized and sophisticated equipment (e.g. critical point drier) is not needed. The only equipment used is a lyophilizor, which is frequently available in general laboratories.

Occurrence of *Batkoa* sp. – *Batkoa* sp. was found at enzootic levels (< 10% infected) in the *M. fimbriolata* population (Fig. 3). The infection level of 100% observed at the final evaluation does not represent true pathogen incidence, since the insect population was too low at this date for analysis (4 insects captured). There was a significant and inverse correlation (Pearson method) between the disease and the insect population (Table 1), suggesting the pathogen, although occurring at low infection level, was one of the factors responsible for decrease of the insect population. Precipitation, as well as the other climatic factors, did not significantly correlate (Pearson method) with disease development. In fact, rainfall had a negative effect since it was inversely correlated with the infection level.

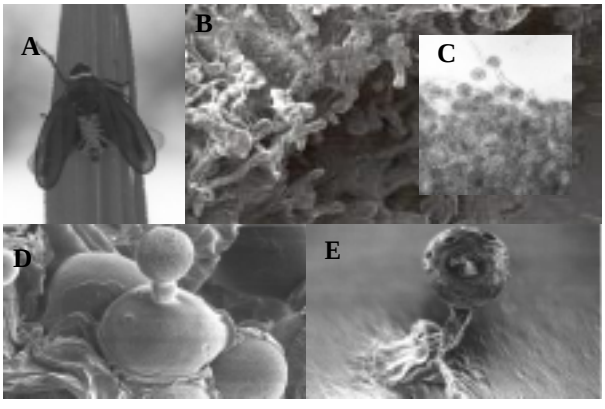

Fig. 1 – *Batkoa* sp. on *Mahanarva fimbriolata* (A). Fungal conidiogenesis (B), resting spores (C) and secondary conidia (D and E). Bars = 30 µm.

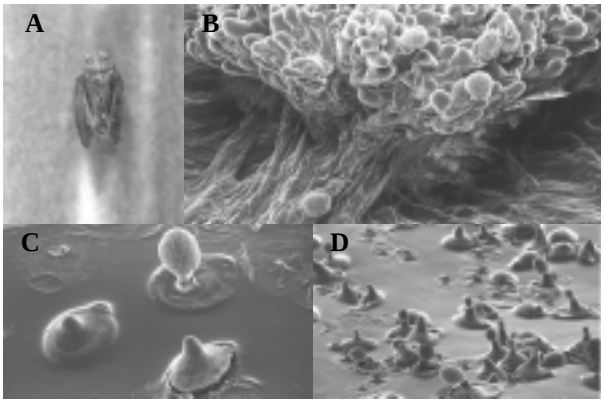

Fig. 2 – *Furia* sp. on *Deois schach* (A). Fungal conidiogenesis (B) and secondary conidia (C and D). Bars = 37.5 µm.

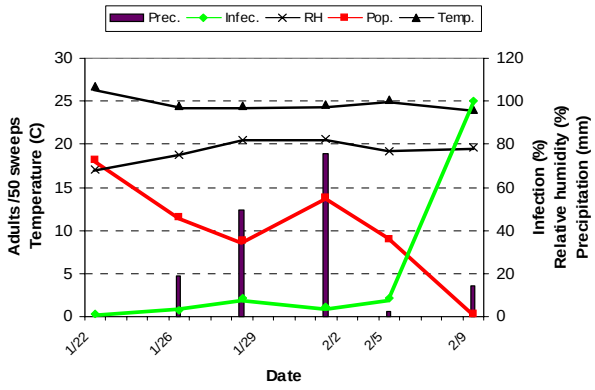

Fig. 3 – Occurrence of *Batkoa* sp. infections in the *Mahanarva fimbriolata* population in napier grass, *Pennisetum purpureum*, and climatic data recorded during the evaluation period. Pindamonhangaba, SP, Brazil.

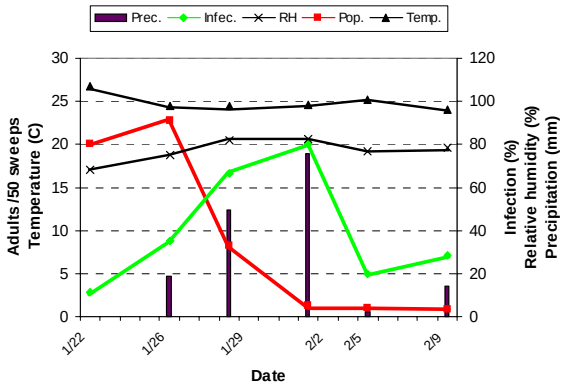

Fig. 4 – Occurrence of *Furia* sp. infections in the *Deois schach* population in brachiaria grass, *Brachiaria decumbens*, and climatic data recorded during the evaluation period. Pindamononhangaba, SP, Brazil.

Table 1 – Correlations of the development of *Batkoa* sp. disease (infection level) with the population of its host, *Mahanarva fimbriolata*, and climatic data. Pindamonhangaba, SP, Brazil.

Variable	Multiple regression*			Pearson correlation	
	Coefficient	Standard error	P	Coefficient	P
Constant	1.327	1.700	0.578	-	-
Population	-0.990	0.038	0.024	-0.991	0.000
Precipitation	0.004	0.001	0.187	-0.199	0.353
Temperature	0.051	0.044	0.455	-0.459	0.180
Humidity	-0.021	0.010	0.275	0.131	0.348

*Coefficient of determination (r^2) = 0.999

Table 2 – Correlations of the development of *Furia* sp. disease (infection level) with the population of its host, *Deois schach*, and climatic data. Pindamonhangaba, SP, Brazil.

Variable	Multiple regression*			Pearson correlation	
	Coefficient	Standard error	P	Coefficient	P
Constant	-2.06	0.289	0.089	-	-
Population	0.051	0.006	0.073	-0.184	0.364
Precipitation	0.009	0.000	0.015	0.994	0.000
Temperature	0.029	0.006	0.137	-0.525	0.143
Humidity	0.020	0.002	0.062	0.825	0.022

Coefficient of determination (r^2) = 1.0

The low incidence of *Batkoa* sp. probably was due to the low inoculum density at the beginning of evaluations, relative humidity below 80% during most of the evaluations, and, especially, high precipitation during the experiment. Few infected insects (1.4%) were found in the first evaluation, which indicated a low inoculum density, estimated at about 1 cadaver per each 60 m^2. The minimal inoculum density, enough to allow the development of *Zoophthora radicans* epizootics in *Empoacsa* sp. populations in beans, is between 0.4 to 0.5 cadavers per single bean plant (GALAINI-WRAIGHT et al., 1991).

Several factors can explain the low inoculum density of *Batkoa* sp. at the beginning of the survey. One is probably related to napier pasture, which is renewed or replaced by another crop more often than

is *Brachiaria* grass, contributing to reduction or even deletion of the fungal inoculum. Also, after grazing, the crop offers few leaves protecting the soil, which is the main storage site for resting spores, allowing the exposure of these propagules to the deleterious solar radiation and high temperatures. Organic matter, humidity, protection from solar radiation and moderate temperatures in the subsoil are factors favorable for the development and perpetuation of entomopathogenic agents, and are very important for the storage and release of conidia responsible for the primary foci of disease (ALVES, 1998).

Daily mean relative humidity (RH), during most of the evaluation period remained below 80%, which is unfavorable for many Entomophthorales fungi (CARRUTHERS & HAYNES, 1986; LE-RU & IZIQUEL, 1990; YU et al., 1995; ODUOR et al., 1996). The RH in the napier microenvironment may be less favorable for the fungus compared to other pasture grasses, since this crop has less foliage covering the soil, which contributes to keeping higher humidity in the microenvironment around the insects and, consequently, allows development of the disease.

Rainfall is favorable to Entomophthorales epizootics if it occurs at low intensity. Heavy rainfall, like the 67 mm that occurred at date 2/1, can decrease infection rates by washing conidia from dead insects and, worse, knocking the cadavers from leaves to the soil, thereby decreasing inoculum density. This high precipitation was the primary explanation for the reduction of infection level from 8.6 to 4.3% during the dates of 1/29 to 2/2, since all other climatic factors in this period were favorable for the fungus, including RH with most averages higher than 80%. This adverse effect is emphasized with *Batkoa* sp. by the negative correlation (Pearson method) between precipitation and infection level. According to ALVES (1998), continuous and high precipitations during the occurrence of primary focus of disease (pre-epizootic phase) suppress pathogen dissemination and epizootic development. The epizootic development of *Neozygites fumosa* on *Phenacoccus manihoti* appeared to be more closely related to the frequency of rainfall than with total rainfall (LE-RU & IZIQUEL, 1990).

The low incidence of the pathogen allowed high *M. fimbriolata* populations (over 8 adults / 50 sweeps) during most of the evaluation period. The decrease in population during the last two evaluations was probably due to the end of insect cycle.

Occurrence of *Furia* sp. – *Furia* sp. was found at epizootic levels in *D. schach* populations (Fig. 4), reaching 80% infected. Precipitation and relative humidity were the most important climatic factors for disease development, showing a significant and direct correlation (Pearson method) with the entomopathogen (Table 2).

The high density of *Furia* sp. inoculum at the beginning of the experiment, the high epizootic potential of this fungus, and the suitable conditions for infection offered by the *B. decumbens* crop probably explains the high incidence of this entomopathogen. Several cadavers with fungus were found at the first survey, which may explain the relatively high infection level (11.5%) found at this date. The inoculum density resulting from this infection level was estimated as at least 1 cadaver for each 6 m^2. This is an inoculum density much higher than the one estimated for *Batkoa* sp. (1 cadaver / 60 m^2). This inoculum density is much lower than the one estimated for *Z. radicans* (0.4 to 0.5 cadavers / plant) as the minimum necessary for epizootic development in *Empoasca* sp. populations in beans (GALAINI – WRAIGHT et al., 1991). Nevertheless, these inoculum densities may be closer than the numbers indicate since the *Furia* sp. host is much larger than that of the *Z. radicans* host which allows production of a much larger number of conidia / cadaver.

Furia sp. demonstrates high epizootic potential. It grows much faster on solid medium and produces larger numbers of conidia than *Batkoa* sp. (Figs. 1 and 2). One of its main attributes is the production of a high density of rhizoids that attach the host to the substrate (HUMBER, 1997). Holding the cadaver on the leaf decreases loss of inoculum due to dropping to the soil as a consequence of rain or other factors.

The *B. decumbens* field had very suitable conditions for this entomopathogen. This pasture had been managed for three years without rotation, which was very helpful for inoculum preservation. The shorter *Brachiaria* height (0.5 m average) compared to napier (1.0 m average) allowed *D. schach* insects to keep close proximity to each other, favoring pathogen dissemination in the host population. In addition, *Brachiaria* had an extremely dense canopy, and produced an 8-cm-thick layer of organic matter over the soil. This helped to keep humidity high on the soil surface, allowing the pathogen to attack the recently-emerged adults attached to the plant root with complete protection against rainfall and solar radiation. Thus, the heavy precipitation that occurred during the experiment was not harmful to *Furia* sp., but rather increased the RH in the spittlebug environment, providing good conditions for the fungus infection. This is confirmed by the significant and direct correlation (Pearson method) between these two climatic factors and the infection level (Table 2). The mean daily precipitation (7.6 mm) that occurred during the observation period was in the range of 3.6 to 7.0 mm, which promoted epizootics of *Zoophthora phytonomi* in the curculionid *Hypera posticata* according to GILES et al. (1994).

The redution of the infection level to 20% at the next-to-last evaluation probably was due to the decrease of inoculum density and the crashing of the insect population. It is clear that *Furia* sp.was very important to the control of *D. schach* in 1998. Few adults of *D. flavopicta* were found during the surveys. This fungus also was evaluated regarding infection level, showing levels of 28% (23 adults evaluated) and 100% (2 adults) at the third and fourth evaluation. Accordingly, *Furia* sp. has potential for use against at least two spittlebugs, *D. schach* and *D. flavopicta.*

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