BIOLOGICAL CONTROL

Natural Occurrence of the Entomopathogenic Fungi *Metarhizium*, *Beauveria* and *Paecilomyces* in Soybean Under Till and No-till Cultivation Systems

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Ocorrência Natural de Fungos Entomopatogênicos *Metarhizium*, *Beauveria* e *Paecilomyces* na Cultura da Soja sob Plantio Direto e Convencional

RESUMO - A ocorrência de fungos entomopatogênicos *Metarhizium*, *Beauveria* e *Paecilomyces* foi estudada em condições de semeadura direta e convencional da soja. Foi determinada a densidade de unidades formadoras de colônia por g de solo e por cm^2 de folíolos de soja. Verificou-se que no solo sob semeadura direta ocorreu maior incidência dos entomopatógenos, mas sobre os folíolos essa diferença não ocorreu, proporcionando as mesmas possibilidades de infecção nos insetos da parte aérea suscetíveis que ocorrem nas duas condições de cultivo.

PALAVRAS-CHAVE: Epizootiologia, Deuteromicetos, Moniliaceae, controle microbiano.

ABSTRACT - The occurrence of the entomopathogenic fungi *Metarhizium*, *Beauveria* and *Paecilomyces* in soybean under till and no-till cultivation systems was studied. The number of colony forming units per g of soil and per cm^2 of leaf area was determined. Soil under no-till system showed a higher incidence of entomopathogenic fungi, but no difference in fungal density was observed on leaves. Thus the probability of infection for susceptible leaf inhabiting insects should be similar in both systems.

KEY WORDS: Epizootiology, Deuteromycetes, Moniliaceae, microbial control.

In Brazil, the most prevalent pathogens that attack insects and mites in soybean (*Glycine max* (L.) Merrill) agroecosystems are the entomopathogenic fungi. In addition to the most common species referred by Sosa-Gómez & Moscardi (1994), *Paecilomyces amoenoroseus* (Hennings) Samson occurs on *Lagria villosa* Fabr. (Coleoptera: Lagriidae); *Neozygites* sp. on the two-spotted mite, *Tetranychus urticae* Koch; *Paecilomyces fumosoroseus* (Wize) Brown & Smith, *Aschersonia aleyrodis* Webber, and an undescribed species of Entomophthorales attack whiteflies, probably *Bemisia argentifolii* Bellows & Perring (D.R. Sosa-Gómez, unpub.). The occurrence and persistence of entomopathogenic fungi can be affected by cultural practices, such as planting date (Sprenkel *et al.* 1979), pesticide applications (Horton *et al.* 1980), and fertilizers (Rosin *et al.* 1996). The soil populations of some fungi are affected by the soybean cultivation system (Sosa-Gómez & Moscardi 1994), but it is unknown if this effect will also occur on aerial parts of the soybean plant, where insects rest, bask or feed and may come in contact with soil-borne infectious fungal inocula. Our objective was to study the effect of till and no-till cultivation practices on the natural density of the entomopathogenic fungi *Metarhizium*, *Paecilomyces* and *Beauveria* on soybean leaves, as related to their occurrence in the soil.

Material and Methods

The experiment was set up in two contiguous plots of 22 x 312 m, in Londrina, State of Paraná, Brazil. One plot was under no-till cultivation and the other under conventional tillage (ploughed once and disc harrowed twice) during the growing seasons of 1992/93 and 1997/98. Soybean in the summer, and wheat or oat in the winter, had been planted on both areas since 1985. Each area was divided into 15 subplots of 20 m x 20.8 m.

One leaflet from each subplot was sampled weekly at half of plant height (15 replicates) in both areas. Soil samples were taken in a similar manner, but at each random sampling point (0.36 m^2) five subsamples of approximately 0.5-0.8 g were collected inside this area and mixed thoroughly in the laboratory. Using a spoon, these samples were taken within the top 2 cm of soil, because the majority of fungal inocula are usually concentrated in this soil layer (Ignoffo *et al.* 1977, Storey *et al.* 1989). One-gram aliquots were taken per sample (the five subsamples were pooled) and suspended in 9 ml of sterile distilled water plus Tween 80 at 0.01%. The resulting suspensions were serially diluted (10^{-2} and 10^{-3}) and plated on dodine-based selective medium (Chase *et al.* 1986) with dodine at 290 µg per ml. After three weeks incubation at 26±1°C, in darkness, the fungal growth was assessed to quantify the number of colony forming units (CFU). Other one-gram aliquots were used to determine the soil dry weight, estimated after heating at 110°C until constant weight.

The leaflets were washed individually in 10 ml of water plus Tween 80 at 0.01%, and then plated for CFU determination on the same selective medium. The series of means (CFU per g of dry soil or per mm^2 of foliar surface) obtained weekly from till and no-till plots were compared by the non-parametric Mann-Whitney rank sum test, using the Sigmastat statistical software (Siegel 1975, Jandel Scientific 1994). When the postulates of normality and homogeneity were fulfilled, the "t" test was applied. Throughout both seasons, precipitation was recorded in the experimental site once a day.

Results and Discussion

The fungal species isolated were *Metarhizium anisopliae*, *Beauveria bassiana* and *Paecilomyces lilacinus*. The number of samples collected were too large to permit the identification of all colonies to species, so they were only identified to genera. In the soil samples higher CFU densities of *Metarhizium* spp. (T = 376.5, P = 0.007, Fig. 1) and *Paecilomyces* spp. (t test =1.71, P= 0.12, Fig.1 and t test = 3.08, P=0.004, Fig. 2) were observed under the no-till system.

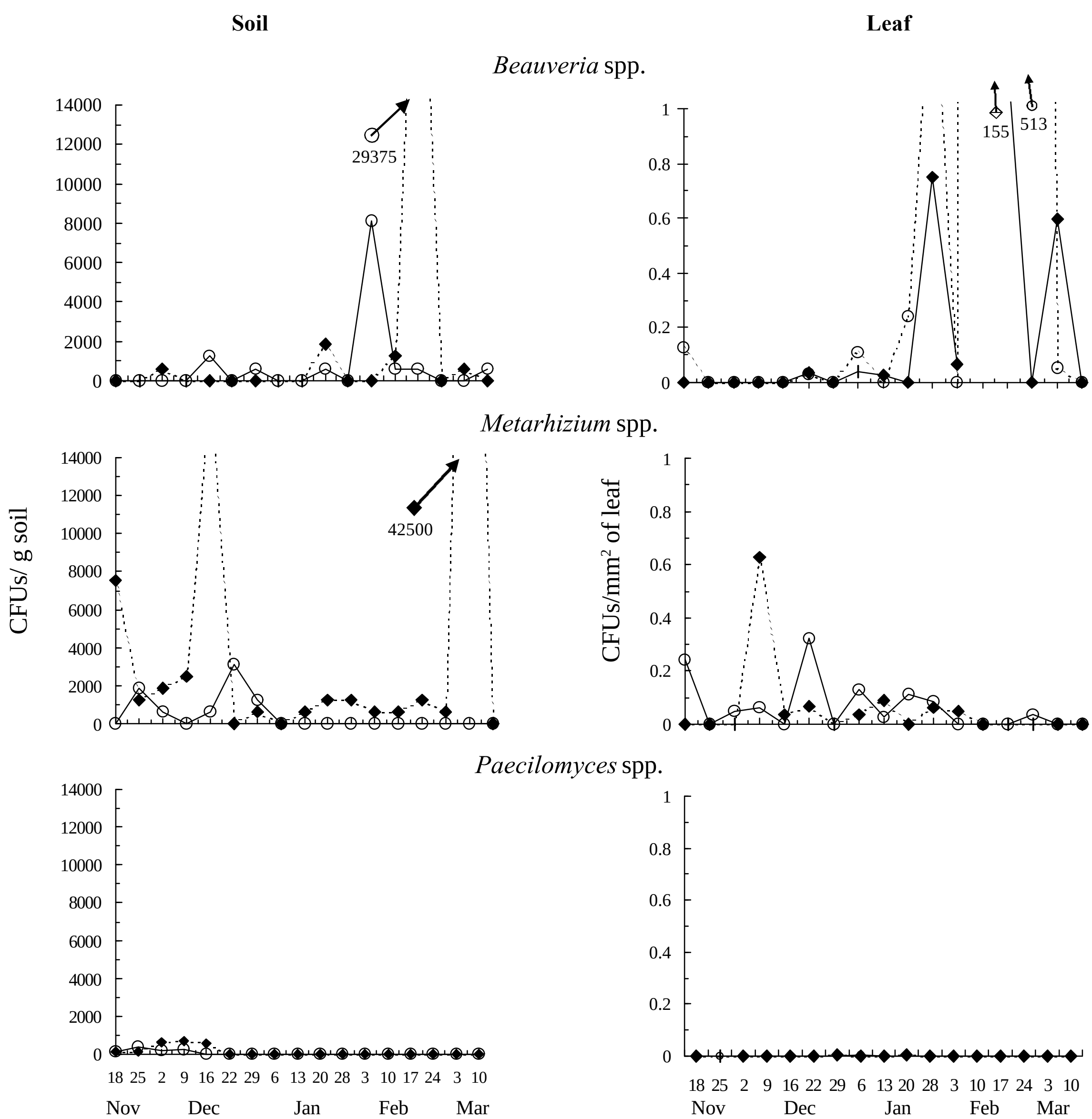

Figure 1. Colony forming units (CFUs) of *Metarhizium*, *Beauveria* and *Paecilomyces* per g of dry soil and mm^2 of leaf area under till and no-till cultivation. 1992-93 soybean growing season (—○— Till ···◆··· No till).

However, no significant differences in *Beauveria* spp. densities were observed between till and no-till systems, in both seasons. Sosa-Gómez & Moscardi (1994) observed that no-till cultivation favored the prevalence of *Metarhizium*, *Beauveria* and *Paecilomyces* in the soil compared with areas under conventional tillage. In several cases, the temporal occurrence of CFUs of *Metarhizium* spp. in the soil, under no till, was higher than that observed for *Beauveria* spp. (1992/93 season, T = 374.5, P = 0.008, Fig. 1, and 1997/98 season, T= 218, P=0.006, Fig. 2), that means higher probabilities of fungal infection with *M. anisopliae*, for insects equally susceptible to both pathogens.

At the canopy level, for soybean conventional tillage in the 1992/3 season, higher numbers of CFUs of *Beauveria* spp. (T=366, P=0.02) and *Metarhizium* spp. (T=366, P=0.02) were observed than for *Paecilomyces* spp. (Fig. 1). These differences were not so evident in the 1997/8 season, since the Mann Whitney tests were significant at P=0.09 and P=0.17, respectively (Fig. 2). The differential recovery of CFU could be due to different responses to the culture media by the fungal species. On leaves, the number of CFU was similar in both cultivation systems for both soybean seasons. Thus, the differences observed between the no-till and tillage systems regarding incidence of fungi in the soil, in this study or previously by Sosa-Gómez & Moscardi (1994), did not occur on soybean leaves, indicating the same probability of infection of susceptible insects on the plants, for both cultivation systems. The higher densities of fungi observed in the soil could be due to extensive saprophytic growth (Callot *et al.* 1996) favored by the microclimatic conditions

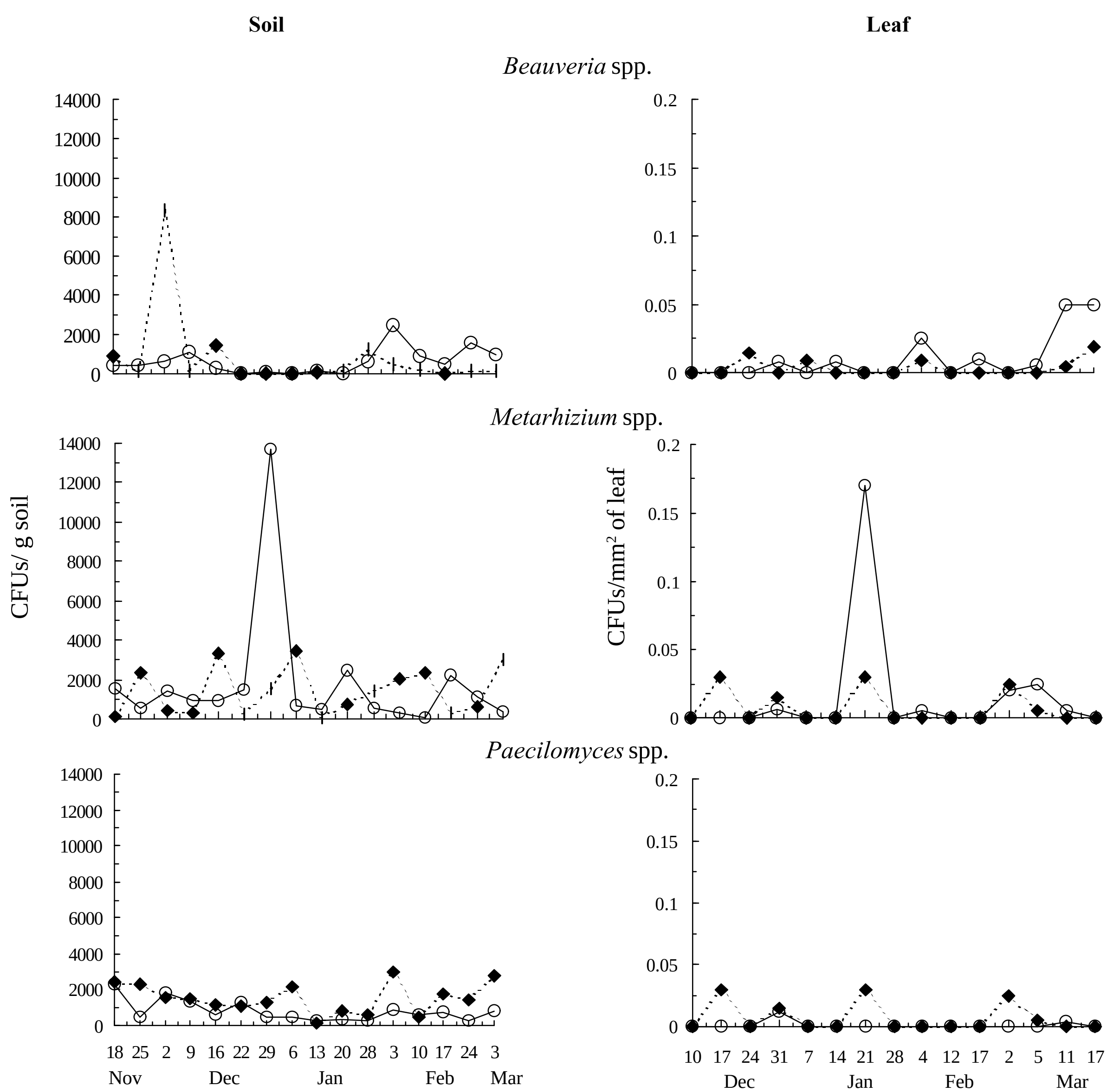

Figure 2. Colony forming units (CFUs) of *Metarhizium*, *Beauveria* and *Paecilomyces* per g of dry soil and mm² of leaf area under till and no-till cultivation. 1997-98 soybean growing season (—o— Till, ···◆··· No till) .

of no-till cultivation. At the canopy level, these conditions are probably the same in both areas not affecting the inocula density. Another reason for the lack of differences between CFU in the aerial part of the plants could be an inefficient mechanism of displacement of fungal propagules from soil to plant, considering that the soil in the no-till conditions remains undisturbed. Young and Yearian (1986) observed that most inoculum of *Anticarsia gemmatalis* nucleopolyhedrovirus on plants was in the lower portion of the soybean canopy, and the tillage appeared to increase the concentration of the pathogen on this part of the plant. Perhaps, rainfall and tillage can increase CFU concentration on leaves close to the soil, but samples at this plant portion were not taken in the present study.

The total amount of rain in the 1992/3 season (807mm, mean = 7.14mm day^{-1}) was higher than in the 1997/8 season (592mm, mean = 6.05mm day^{-1}), and this could account for the higher prevalence of the fungi in the 1992/3 season. On leaves and in soil, *Metarhizium* spp. usually reached the maximum level in December and January. On the other hand, the highest density of *Beauveria* spp. was commonly observed in February and March (Figs. 1 and 2). Significantly, *Beauveria bassiana* is an important cause of mortality of adults of Chrysomelid beetles such as *Cerotoma* sp., *Colaspis* sp. and *Diabrotica* sp., at this time of the year (D.R. Sosa-Gómez, unpubl.). It must be taken into consideration that differences between species of entomopathogenic fungi or even between strains may be attributed to differential recovery in the culture medium. However, Liu *et al.* (1993) observed no evidence of this, at least among *Metarhizium* species. If this effect really occurs it should not be so important because in other seasons, more *B. bassiana* was recovered from the soil than *M. anisopliae* (Sosa-Gómez & Moscardi 1994).

In summary, the differences observed between the CFU densities of entomopathogenic fungi occurring in the soil, under no-till and till conditions, are not reflected at the canopy level. Therefore, the probabilities of phylloplane inhabiting insects becoming infected by these fungi are the same, regardless of the tillage system. Under these experimental conditions, *M. anisopliae* and *B. bassiana* were more prevalent than *P. lilacinus*. A deeper knowledge on the natural densities of entomopathogenic fungi in soil and how they are related to densities on leaves can contribute to a better understanding about the epizootiology of these fungal diseases on insect populations.

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