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CROP PROTECTION

White grub (Coleoptera: Melolonthidae) mortality induced by *Ophiocordyceps melolonthae*

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ABSTRACT: *The occurrence of white grub roots in soybean crops in the South of Brazil has gradually increased. However, there is not information on the biological control of grubs by entomopathogenic fungi. This study aimed to induce infection by Ophiocordyceps melolonthae and analyze longevity in Cyclocephala modesta and Dyscinetus gagates larvae (Coleoptera: Melolonthidae). In the laboratory, Cyclocephala modesta and Dyscinetus gagates had a mortality rate of 85% and 75%, respectively.*
Key words: *biological control, dissemination, entomopathogen, entomopathogenic fungi, induced infection.*

Mortalidade em corós (Coleoptera: Melolonthidae) induzida por *Ophiocordyceps melolonthae*

RESUMO: *A ocorrência de corós-praga de raízes em lavoura de soja, no Sul do Brasil, tem gradualmente aumentado. Entretanto, não há informação sobre controle biológico de corós por fungos entomopatogênicos. Este estudo tem o objetivo de induzir a infecção por Ophiocordyceps melolonthae e analisar a longevidade em larvas de Cyclocephala modesta e Dyscinetus gagates (Coleoptera: Melolonthidae). Em laboratório, Cyclocephala modesta e Dyscinetus gagates apresentaram uma taxa de mortalidade de 85% e 75%, respectivamente.*
Palavras-chave: *controle biológico, disseminação, entomopatógeno, fungos entomopatogênicos, infecção induzida.*

White grubs (Melolonthidae) with an annual or biannual life cycle occur in native grasslands and soybean crops in Brazil and their long larval stage coincides with the development of plants. Native species include *Cyclocephala modesta* Burm, 1855 and *Dyscinetus gagates* Burm, 1847 (Coleoptera: Melolonthidae) (CHERMAN et al., 2013 and 2014). In Brazil, natural epizootic diseases caused by the fungus *Cordyceps* sp. Fries, 1818 (Hypocreales: Cordycipitaceae), *Beauveria bassiana* (Balsamo) Vuillemin, 1912 (Hyphomycetes: Moniliaceae) and *Metharhizium anisopliae* (Metchnikoff) Sorokin, 1883 (Hypocreales: Clavicipitaceae) have been shown to cause the collapse of white grub population in wheat (GASSEN, 1992; SALVADORI, 2000; SALVADORI & PEREIRA, 2006). Chemical control methods using inseticides have been the main control strategy to reduce the population densities of insects. However, the selection pressure induced by excessive applications may lead to the development of resistance in these populations. To minimize this problem, different biological control agents can be used, especially specific entomopatogenic microorganisms (DUARTE et al., 2016).

Ophiocordyceps is the largest genus of the family Ophiocordycipitaceae, originally described by PETCH (1931) for species of the *Cordyceps*, which have septate ascospores (PETCH, 1933; KOBAYASI, 1941; SUNG et al., 2007). *Ophiocordyceps* spp. infect different insect orders, mainly Blattaria, Dermaptera, Diptera, Hymenoptera, Hemiptera, Isoptera, Lepidoptera, Mantodea, Orthoptera, Odonata and Coleoptera (ARAÚJO & HUGHES, 2014). *Ophiocordyceps melolonthae* (Louis René Tulasne and Charles Tulasne) G.H. Sung, J.M. Sung, Hywel-Jones and Spatafora, 2007 (Hypocreales: Ophiocordycipitaceae) is a strong entomopathogenic species infecting mainly white grub larvae (Melolonthidae) in Brazil (SALGADO-NETO et al., 2015).

As there is no record of infection induced by entomopathogenic fungi in white grubs (Melolonthidae) in Brazil, the objective of this study is to evaluate the capacity of *O. melolonthae*

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to induce mortality of *C. modesta* and *D. gagates* larvae, infected in the laboratory.

The study was developed under laboratory conditions (26±2ºC temperature, 50±10% relative humidity and 12h photophase). Larvae of *C. modesta* and *D. gagates* were collected from soil trenches opened in soybean crops, in the district of Arroio Grande, municipality of Santa Maria, RS (29º40'S and 53º44'W), in January/February 2014 (rainfall 75mm and temperature of 25ºC, averages for the period). Samples were separated, quantified and identified in the laboratory, using taxonomic methods, as reported by COSTA (2006), PEREIRA & SALVADORI (2006) and CHERMAN et al. (2013 and 2014).

The 2nd and 3rd instars of *C. modesta* (40 individuals) and *D. gagates* (40 individuals) larvae were separated and maintained individually in plastic recipients (20x30x12cm), containing autoclaved soil and carrot pieces. Containers were inspected daily for rearing conditions (26ºC, 12h photoperiod and 50% relative humidity) and to replace the feed, as described by PARDO-LOCARNO (2002) and PARDO-LOCARNO et al. (2005).

Extract of *O. melolonthae* was obtained from infected larvae of *Diloboderus abderus* Sturm, 1826 (Coleoptera: Melolonthidae) collected in a native grassland, in the district of Umbú, municipality of Rosário do Sul, RS (30º35'S and 54º46'W). Larvae were superficially disinfected and rinsed in sterile distilled water. Subsequently, the specimens with a whitish structure resembling a stroma of *Ophiocordyceps* were sent to the Instituto Biológico de São Paulo for molecular identification. The sequence obtained was deposited in GenBank, access code KR082313 (SALGADO-NETO et al., 2015). The stroma of *O. melolonthae* was kept in an incubation chamber (25°C, 12h photophase and 50% relative humidity) and also frozen in the laboratory.

Five larvae of infected *D. abderus* showing stroma were triturated along with ten *C. modesta* healthy larvae (3rd instars) in 250ml of sterile distilled water and the flask was placed in the incubation chamber (25°C, 12h photoperiod and 50% relative humidity) for 7 days. After this period, the contents were stirred for 10 minutes. The resulting suspension was filtered and *O. melolonthae* spores were counted with the aid of a hemacytometer and optical microscope. The resulting suspension had its concentration adjusted to 10^6 spores mL^{-1}. Subsequently, 10mL of the spore suspension in each container were inoculated with larvae and this suspension was sprayed on the surface of the soil and food, while the control treatment received only sterile distilled water.

Biological variables evaluated were the mortality rate and duration of the larval and pupal periods for both species as well as longevity in Melolonthidae larvae with induced infection from *O. melolonthae* in the laboratory. In the group without the *O. melolonthae* extract, the larval stage (second and third larval instars) of *C. modesta* lasted 99.9 days and the pupal stage lasted 10.0 days. In *D. gagates*, the larval period (second and third larval instars) lasted 53.9 days, and the pupal phase lasted 25.0 days.

In the group with the extract of *O. melolonthae*, the mortality rate of *C. modesta* and *D. gagates* larvae was 85% and 75%, respectively (Figure 1), demonstrating the high rate of larval mortality (Figure 2). *C. modesta* (15%)

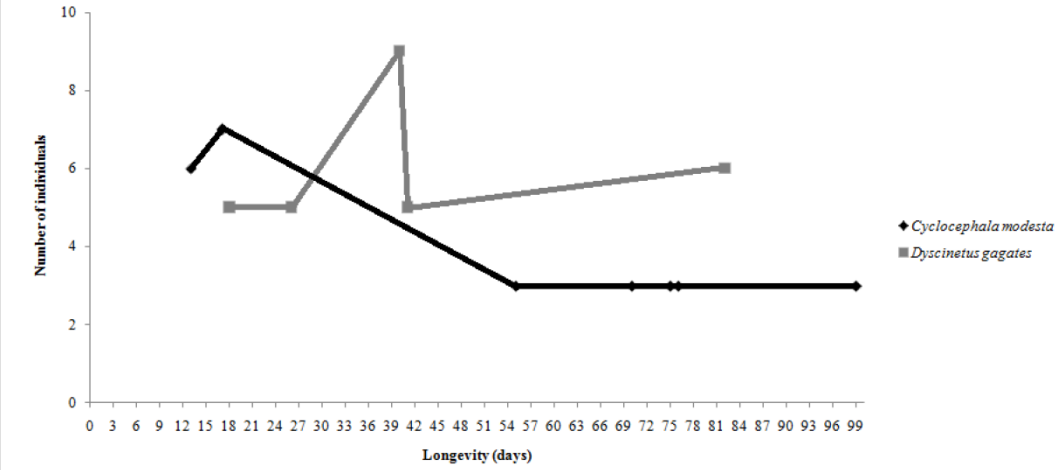

Figure 1 - Longevity distribution of *C. modesta* and *D. gagates* larvae, exposed to the entomopathogen O. melolonthae.

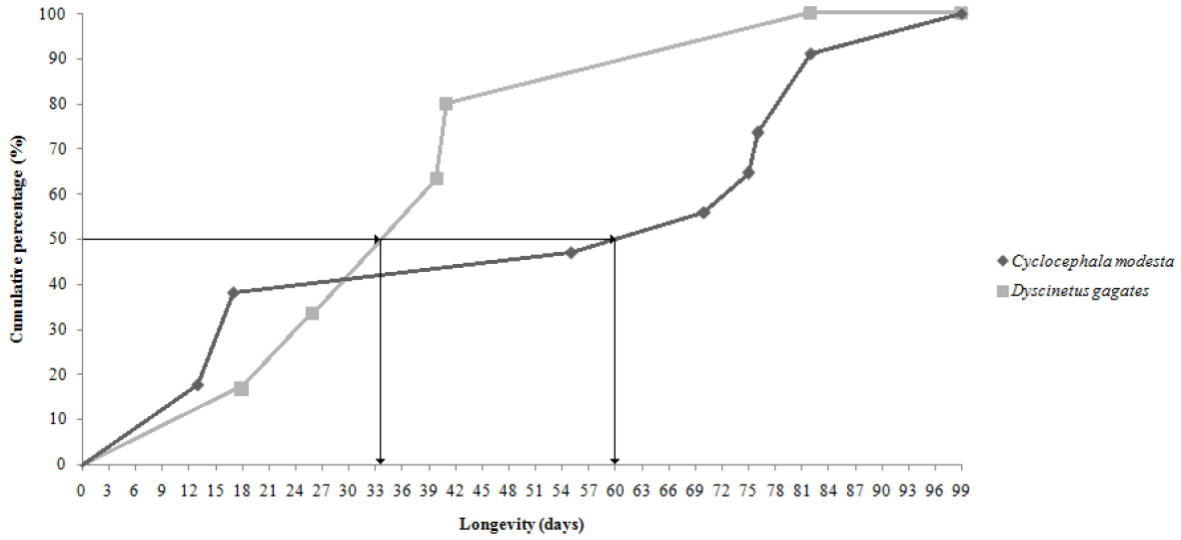

Figure 2 - Cumulative mortality of *C. modesta* and *D. gagates*, exposed to the entomopathogen O. melolonthae.

and *D. gagates* (25%) larvae had a gross survival rate of only (20%); however, they showed malformations (50%) of survivors and death during the pupa-adult metamorphosis.

Distribution of white grubs in soybean crops occurred in aggregation, with the accumulation of specimens in infestation spots, as observed by LIEBHOLD et al. (1993). The period of larval activity of two species studied was similar to other Scarabaeidae collected in Brazil, as observed by RODRIGUES et al. (2010) and NOGUEIRA et al. (2013). Larval phase of *Cyclocephala tucumana* Brethes, 1904 and *Cyclocephala melanocephala* Fabricius, 1775 (Coleoptera: Melolonthidae) last 191.9 days and 70.7 days respectively (NOGUEIRA et al. (2013) and the larval phase of *Cyclocephala verticalis* Burmeister, 1847 (Coleoptera: Melolonthidae) last 195.7 days (RODRIGUES et al., 2010). White grubs (*C. modesta* and *D. gagates*) have a rhizophagous action that attacks soybean roots and inoculates *Fusarium oxysporum* Schlechtendahl emend. Snyder and Hansen, 1940 (Hypocreales: Tuberculariaceae) dispersing root rot in soy crops (SALGADO-NETO et al, 2016). The observed differences in the mortality rate are related to the differing periods of the life cycle between the two species. Knowing the duration of the larval stage in these species will help to induce their mortality.

The extract of *O. melolonthae* caused a gross rate mortality in white grubs of 80%. This study confirmed the pathogenicity of *O. melolonthae* in larvae of *C. modesta* and *D. gagates* in the laboratory. The induced infection of white grubs by entomopathogenic fungi opens new possibilities for biological control. Duration of life cycle and behavior of white grubs are important for integrated management and precision agriculture.

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