

## SURVIVAL OF A LEPIDOPTERAN DEFOLIATOR OF *EUCALYPTUS* IS INFLUENCED BY LOCAL HILLSIDE AND FOREST REMNANTS IN BRAZIL

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### ABSTRACT

We tested the hypothesis that *Euselasia eucerus* (Lepidoptera: Riodinidae) the intensities of mortality factors in eucalyptus plantations in xeric environments are greater near fragments of native forest and they diminish with increasing distance from the latter. Samples were collected along a transect crossing a 70-ha planting of *Eucalyptus urophylla* x *Eucalyptus grandis* hybrid extending from adjacent native vegetation to a hilltop. Sampling was completed along an elevation and distance gradient from the native forest in three environments, near the native forest (base), intermediate (mid way between the native forest and the hilltop) and distant from the native forest (hilltop) ones. Fungi, parasites and predators caused mortality of *E. eucerus* pupae. Damage to *E. urophylla* x *E. grandis* hybrid by *E. eucerus* was greater at the more distant location possibly because of plant water deficits and/or increased *E. eucerus* survival. Mortality of *E. eucerus* in all ontogenetic stages was greater in areas near the native forest. Proximity to native vegetation appeared to be a key factor that influenced survival of local *E. eucerus* populations in *E. urophylla* x *E. grandis* hybrid plantations. The results presented here reinforce the concept that native vegetation near eucalyptus plantations exerts a local effect supporting the maintenance of natural enemies of *E. eucerus*, and favoring infections by entomopathogenic fungi during the pupal stage of this insect.

Key Words: *Eucalyptus* plantation, *Euselasia eucerus*, life table, mortality, Riodinidae, *Trichogramma maxacalii*

### RESUMO

Testamos a hipótese de que os fatores de mortalidade de *E. eucerus* são mais intensos nas zonas de intercepto entre o plantio de eucalipto e fragmentos de mata nativa e mais baixos em ambiente xérico. Amostras foram coletadas em transecto, em um talhão de 70 hectares de híbridos *Eucalyptus urophylla* X *Eucalyptus grandis* próximo à vegetação nativa até o topo do morro. Essas coletas foram realizadas em gradiente de altitude e distância da mata nativa, compondo três ambientes, denominados méxico (base), intermediário (meio) e xérico (topo do morro). Oito plantas foram amostradas por ambiente e os números de ovos, larvas e pupas de *E. eucerus* por folha foram contabilizados em vinte ramos terminais de cada planta. A morte de pupas desse inseto foi causada por fungos, parasitóides e predadores. Danos por *E. eucerus* foram maiores no ambiente xérico devido ao déficit hídrico da planta e/ou ao aumento da sobrevivência de *E. eucerus*. A mortalidade em todas as fases ontogenéticas dessa borboleta foi maior em áreas próximas á mata nativa. A vegetação nativa é um componente imponente com efeito local para o controle da borboleta *E. eucerus*. Os resultados aqui apresentados reforçam o conceito de que a vegetação nativa perto de plantações de eucalipto exerce efeito local de manutenção dos inimigos naturais de *E. eucerus* e favorece a infecção por fungos durante a fase de pupa.

Palavras Chave: *Euselasia eucerus*, mortalidade, plantação de *Eucalyptus*, Riodinidae, tabela de vida, *Trichogramma maxacalii*

Lepidopteran defoliators are important pests of cultivated eucalyptus (Myrtales: Myrtaceae) due to the intensity and persistence of damage they cause (Zanuncio et al. 2003; Bernardino et al. 2007). Infestations of these species can occur throughout the year or in sporadic population explosions (Zanuncio et al. 1998, 2000). *Euselasia eucerus* (Lepidoptera: Riodinidae) is one of a few butterfly pest species of eucalyptus in Brazil (Zanuncio et al. 2000). This lepidopteran originated in South America and is a known defoliator of eucalyptus. It is found in Minas Gerais, São Paulo, Santa Catarina and Rio Grande do Sul states, Brazil (Zanuncio et al. 2000). *Euselasia eucerus* increases in density, especially during the driest months of the yr, and it has multiple generations per yr in eucalyptus plantations (Sousa et al. 2010).

*Euselasia eucerus* has 5 well-defined stages, and it completes its life-cycle from egg hatch to the adult in 27 to 28 days. Its clutches contain 50 eggs on average (Murta et al. 2008; Zanuncio et al. 2009). The first few instars are social (aggregated), but the last two instars are found in smaller or isolated groups. The first few instars have weak jaws not capable of perforating the leaf cuticle, and they scrape the leaf surface, while the last few instars consume the leaves entirely. Benefits of aggregation disappear or are balanced by the costs associated with intraspecific competition of the last few instars (Reader & Hochuli 2003).

A number of factors contribute to the mortality of *E. eucerus*. Its eggs are parasitized by *Trichogramma maxacali*, Voegelé and Pointel, 1980, *T. demoraesi* Nagaraja, 1983 and *T. acacioi* Brun, Moraes & Soares, 1984 (Trichogrammatidae); its larvae and pupae are predated by the pentatomids, *Podisus nigrispinus* Dallas, 1851, *Brontocoris tabidus* (Signoret, 1852) and *Supputius cincticeps* (Stål, 1860); and its pupae are parasitized by *Itopectis* sp. (Ichneumonidae), *Galeopsomyia* sp. (Eulophidae) and the fungus *Paecilomyces fumosoroseus* (Wizea). The parasitism of *E. eucerus* eggs was reported to be the main source of mortality of populations of this lepidopteran (Zanuncio 2009). Substantial mortality of *E. eucerus* larvae and other Lepidopteran can be caused by the predation of birds, ants and many others invertebrates (Greeney et al. 2012). *Podisus nigrispinus*, a common predator, is used for the biological control of the lepidopteran defoliators (Bortoli et al. 2011), but its effectiveness depends on prey density (Oliveira et al. 2001). Overall predation induces evolutionary responses by prey to that avoid, repel or escape attackers, causing indirect changes in aggregation pattern (Soares et al. 2009; Greeney et al. 2012). The physiological regulation of temperature and water balance are likely to have the greater impact on larval mortality (Allen 2007). However, the dynamics of the effects of entomopathogenic fungi and insect

natural enemies on *E. eucerus* populations in the field are unknown.

The presence of native vegetation bordering plantations may be important on a local scale because it may lead to increased densities of natural enemies (Sperber et al. 2004) possibly because of the greater diversity of resources and niches (Tews et al. 2004). For example, *Oxydia vesulia* (Lepidoptera: Geometridae), a defoliator of eucalyptus, is more abundant in plantations without native vegetation remnants (Bragança et al. 1998; Murta et al. 2008). This pattern has also been reported for *E. eucerus* (Murta et al. 2008) and other lepidopteran defoliators with remnants of native vegetation increasing diversity of hymenopteran parasitoids in eucalyptus plantations (Bragança et al. 1998).

Remnants of native vegetation surrounding plantations increase the local availability of soil resources such as moisture, carbon and nitrogen, which are essential for the growth and development of plants (Chen et al. 2013). Therefore, the distance from a remnant forest is a compounding factor because of the decreasing predation/parasitism (Lill & Marquis 2001) and increased opportunity for herbivory through increased plant stress (White 1984) and/or quantitative reductions of chemical defenses (Ribeiro & Basset 2001).

Remnant forests may also affect the diversity and abundance of natural predators on *E. eucerus*. The effectiveness of natural enemies can be evaluated with life tables to show mortality rates caused by different factors recorded in each stage of the insect cycle (Silva et al. 2008). Knowledge of these dynamics should facilitate the improvement of strategies to control eucalyptus pests.

The objective of the present study was to test the hypothesis that distance from the remnant forest is a factor affecting the survival and population sizes of different life stages of *E. eucerus*. We posited that the greatest mortality rate of this insect would be found in the zones where eucalyptus plantations interface with native forest remnants.

#### MATERIALS AND METHODS

Samplings were performed at a Nipo-Brazilian Cellulose S. A. company plantation (Celulose Nipo-Brasileira S.A. CENIBRA) in the Municipality of Belo Oriente, Minas Gerais State, Brazil (S 19° 29' 36.7" W -42° 24' 62.9") during an outbreak of *E. eucerus* butterflies in May and Jun 2005. This region is characterized by sharp relief, average temperature of 24.7 °C and annual rainfall of 1,220 mm (CENIBRA S.A. Meteorological Station, Belo Oriente). The climate according to the Köppen classification is hot and humid, with rainy and hot summers and dry and cold winters. Sampled eucalyptus plantations exhibited origi-

nal Atlantic Forest biome with semi-deciduous seasonal vegetation structure. Remaining fragments of the native vegetation, mainly at the bottoms of valleys, are parts of legal reserves and permanent preservation areas which accumulate water and maintain lakes, forming the hydrographic Rio Doce basin (Perônico 2009).

Collections were located in a 70-ha plot of a 3-yr-old hybrid eucalyptus (*Eucalyptus urophylla* × *Eucalyptus grandis*) plantation. Three 150-m long transects were delimited in this plot simulating a hydric stress gradient. Each transect encompassed the following environments: mesic (close to the native vegetation and at the valley bottom with 230 m elevation; and a high moisture content), intermediate (intermediate elevation at 255 m) and xeric (hilltop at 280 m elevation, with a lower moisture content and greater distance from the native forest). Eight plants 20 m distant one from the other were selected per transect, and 3 lower basal branches were removed from each. We randomly chose 20 terminal branches from these 3 branches as the maximum number to minimize the effect of basal branch size, and transported to the laboratory in individual bags, where the numbers of *E. eucerus* eggs, larvae and pupae per leaf were counted. The ontogenetic stage of each individual was determined. Pupae infested with fungi were separated from healthy individuals based on the whitened appearance of the mass of fungal filaments, while parasitized pupae were identified based on the characteristic emergence holes left by parasitoids. Finally, pupae were considered to have been predated when the external layer of each was damaged, leaving part of the exoskeleton of the individual, without internal content.

Insect stages with aggregation behavior (L1, L2 and L3) were combined into one category for the life table calculations. Also those stages with mainly solitary individuals (L4 and L5) were combined. These two categories were treated as L1-L3, representing larvae with aggregation behavior, and L4-L5, those with solitary behavior.

The effects of the environments on density and survival of different ontogenetic categories of *E. eucerus* were tested with generalized linear models in 3 stages: (1) with the abundance of *E. eucerus* as a changeable response to transect environments (xeric, intermediate and mesic) and the ontogenetic category (eggs, L1-L3 larvae, L4-L5 larvae and pupae) as an explicative variable; (2) with the proportion of *E. eucerus* per plant as a response variable and the transect environments and ontogenetic categories as explicative variables; and (3) with the proportion of pupae as a response variable and the environments and mortality factors (parasitoids, predators and fungi) as explicative variables. Individuals collected were grouped by plant, with 8 replications per environment. Each complete model was subjected to an analysis of the residues to verify the adequate

distribution of residuals and differences between the levels were verified by an analysis of contrast (Crawley 2010).

The numbers of live pupae and adults present in the field were estimated with a static life table (Silva et al. 2008). The L1 to L5 stages were determined according to the lengths of the *E. eucerus* larvae. In this type of life table, the absolute differences in the numbers per category (from egg to adult) are attributed to the mortality in each stage.

Field data were estimated based on the *E. eucerus* developmental stages using Kobayashi method (Kobayashi 1968). This method is based on (1) a provisional estimate of the number of individuals that die per stage, assuming a uniform mortality rate in all stages during the sampling interval, and (2) a correction of these estimates, assuming that mortality differs in some stages but is constant for each one. Number of individuals per stage ( $n$ ) was used to calculate mortality factors ( $k_i$ ).

Mortality factors (Varley & Gradwell 1970) were calculated per phase or stage with the following equation:  $k_n = \log N - \log N_s$ , where  $N$  = the initial population and  $N_s$  = the surviving population at the end of the related stage. The greater the value of the factor,  $k$ , the greater the intensity of the mortality factor.

## RESULTS

A total of 46,360 *E. eucerus* individuals were obtained from 3,084 leaves sampled from 408 branches of the *Eucalyptus urophylla* × *E. grandis* hybrid ( $7.5 \pm 0.32$  SE leaves per branch and  $15.6 \pm 2.6$  SE individuals per leaf), including 37,780 eggs ( $13.1 \pm 2.3$  SE eggs per leaf); 7,267 L1-L3 larvae ( $2.07 \pm 0.65$  SE larvae per leaf); 973 L4-L5 larvae ( $0.3 \pm 0.11$  SE larvae per leaf); and 340 pupae ( $0.1 \pm 0.02$  SE pupae per leaf). A total of 26,547 individuals were found on plants in the xeric environment ( $18.38 \pm 1.31$  SE eggs per leaf,  $5.49 \pm 0.84$  SE L1-L3 larvae per leaf,  $0.68 \pm 0.23$  L4-L5 larvae per leaf, and  $0.2 \pm 0.01$  pupae per leaf); 12,228 in the intermediate environment ( $13.24 \pm 1.3$  SE eggs per leaf,  $0.69 \pm 0.18$  SE L1-L3 larvae per leaf,  $0.08 \pm 0.3$  SE L4-L5 larvae per leaf and  $0.07 \pm 0.01$  pupae per leaf); and 7,624 in the mesic environment ( $5.69 \pm 0.63$  SE eggs per leaf,  $0.68 \pm 0.12$  SE L1-L3 larvae per leaf,  $0.15 \pm 0.04$  SE L4-L5 larvae per leaf and  $0.04 \pm 0.001$  pupae per leaf).

The total numbers of eggs (generalized linear model (GLM), quasi-Poisson:  $\chi^2_{(1,22)} = 5,648.5$ ,  $P < 0.001$ ), L1-L3 larvae (GLM quasi-Poisson:  $\chi^2_{(1,22)} = 6,989.5$ ,  $P < 0.001$ ), L4-L5 larvae (GLM quasi-Poisson:  $\chi^2_{(1,21)} = 710.87$ ,  $P < 0.01$ ) and pupae (GLM quasi-Poisson:  $\chi^2_{(1,22)} = 152.53$ ,  $P < 0.001$ ) were highest in the xeric environment and there was no significant difference between the intermediate and mesic ones (Fig. 1). The proportion of eggs

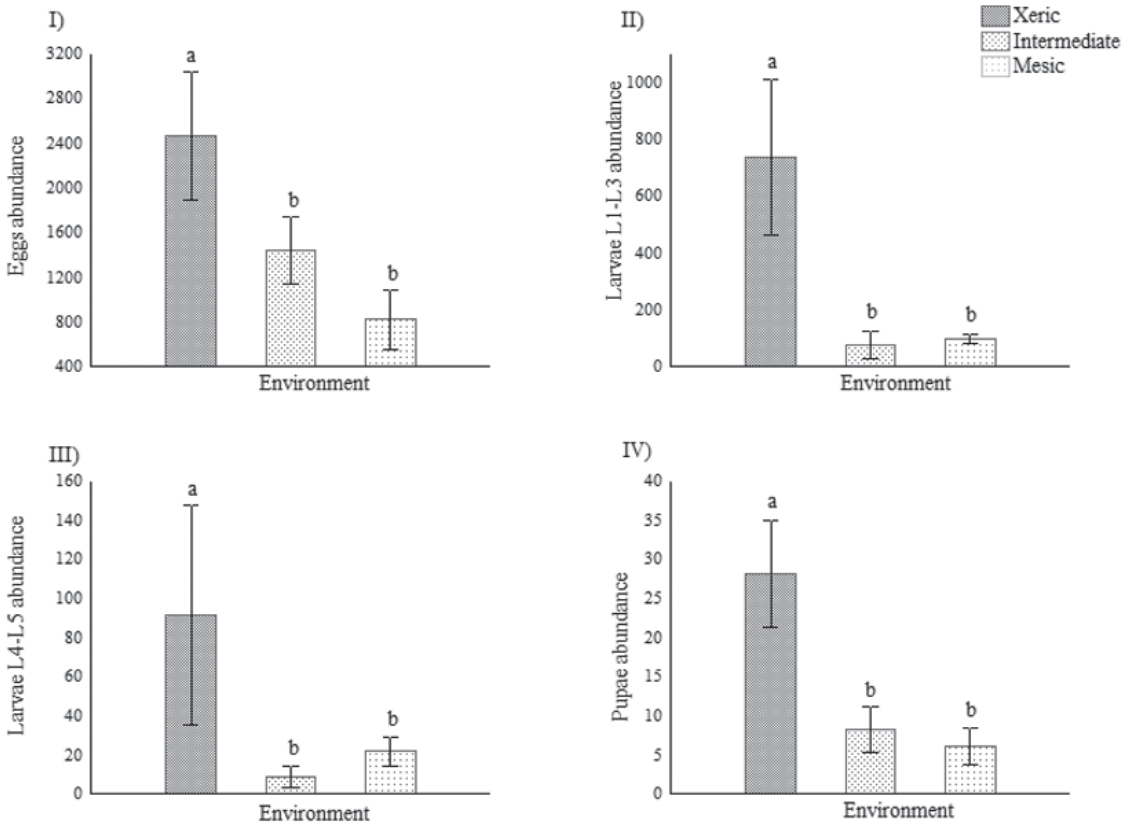


Fig. 1. Number of eggs (I), L1-L3 larvae (II), L4- L5 larvae (III), and pupae (IV) of *Euselasia eucerus* (Lepidoptera: Riodinidae) per plant of hybrid *Eucalyptus urophylla* x *Eucalyptus grandis* in the xeric, intermediate and mesic environments in Belo Oriente, Minas Gerais State, Brazil. Bars with different letters indicate statistically significant differences between means. Data represented as mean ± standard error.

was greater in the intermediate transect environment (GLM quasi-binomial:  $F_{[1,22]} = 2,060.9$ ,  $P < 0.05$ ) than in the xeric and mesic environments (Fig. 2). However, the number of live caterpillars in the L1-L3 stage was higher in the xeric environment (GLM quasi-binomial:  $F_{[1,22]} = 2,633.7$ ,  $P < 0.01$ ) than in the mesic and intermediate environments. The proportions of L4-L5 larvae (GLM quasi-binomial:  $F_{[2,21]} = 482.98$ ,  $P = 0.12$ ) and pupae (GLM quasi-binomial:  $F_{[2,21]} = 12.289$ ,  $P = 0.66$ ) were similar between the environments (Fig. 2). The estimated egg mortalities were high and similar in the mesic and intermediate environments (94% and 88%, respectively), with greater  $K_n$  values in these environments (0.925 and 1.284, respectively).

The number of viable eggs of *E. eucerus* was greatest in all environments, followed by L1-L3 larvae. However, the survival pattern changed between environments beginning from the L1-L3 stage, with greater numbers of L4-L5 larvae and pupae in the xeric (GLM quasi-binomial:  $F_{[1,$

$30] = 42,455$ ,  $P < 0.0001$ ) and intermediate environments (GLM quasi-binomial:  $F_{[1, 30]} = 42,275$ ,  $P < 0.0001$ ) (Fig. 2), which showed similar val-

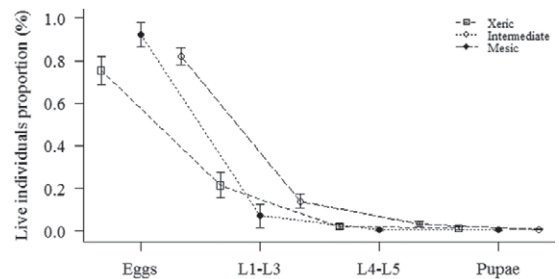


Fig. 2. Percentage of alive individuals of *Euselasia eucerus* (Lepidoptera: Riodinidae) per plant of hybrid *Eucalyptus grandis* x *Eucalyptus urophylla* in Belo Oriente, Minas Gerais. Analyzes were made by environment and by stage per environment (xeric, mesic and intermediate). Data represented as mean ± standard error.

ues between these ontogenetic phases. Survival between the L1-L3 and L4-L5 stages was similar in the mesic transect environment where a decreased proportion of pupae was found (GLM quasi-binomial:  $F_{(2,29)} = 20,471, P < 0.0001$ ) (Fig. 2).

Compared with the mesic environment, mortality of *E. eucerus* larvae was lower in the xeric and intermediate environments, with 87.5% mortality for L1-L3 larvae and 69% for L4-L5 larvae in the xeric environment and 88% mortality for L1-L3 larvae and 4% for L4-L5 larvae in the intermediate environment. The  $K_n$  was higher for the L1-L3 larvae and lower for the L4-L5 larvae in the xeric and intermediate environments (Table 1). In contrast, larval mortality was higher in the mesic environment, varying from 72% in the L1-L3 stage to 77% in the L4-L5 stage, and with the highest mortality  $K_n$  found for the L4-L5 larvae (Table 1).

The L4-L5 larvae to pupae ratio differed in the mesic environment, which had the highest  $K_n$  (0.54) and 72% mortality of pupae compared with 48.5% in the intermediate environment and 55.5% in the xeric one (Table 1). Pupal mortality was attributed to parasitoids, predators and fungi, and this last factor represented the principal mortality factor in all environments, i.e., xeric (GLM quasi-binomial:  $F_{(2,21)} = 24.273, P < 0.04$ ), intermediate (GLM quasi-binomial:  $F_{(2,15)} = 30.44, P < 0.001$ ) and mesic (GLM quasi-binomial:  $F_{(2,18)} = 40.247, P < 0.0004$ ) and with a greater impact in the mesic environment (GLM quasi-binomial:  $F_{(2,18)} = 15.133, P < 0.03$ ) (Fig. 3). Parasitism was

similar between the 3 environments (GLM quasi-binomial:  $F_{(2,18)} = 4.9, P = 0.38$ ), but predation was lower in mesic and in the intermediate environments without any predated pupae (Fig. 3).

DISCUSSION

The greater number of *E. eucerus* individuals in the xeric environment may be associated with the availability of nutrients and water stress in these plants, making them more vulnerable to pests (White 1984; Thies et al. 2003). Moreover, females of this butterfly may prefer to lay eggs in xeric environments because of the lower mortality by parasitoids and fungi compared to other environments.

Egg parasitism is one of the major control factors of *E. eucerus* in the field, independent of its population density (Matsumoto et al. 2004; Murta et al. 2008). Egg mortality of *E. eucerus* decreased (70%) with a decreased  $K_n$  effect (0.525) in the xeric environment. Mortality at this stage was principally due to parasitism by *Trichogramma maxacalii*, independent of habitat. This shows the importance of *Trichogramma* species in the natural biological control of agricultural and forest pests (Zanuncio et al. 2009) and corroborates the reported severe mortality of *E.eucerus* by *T. maxacalli* (Oliveira et al. 2000; Murta et al. 2008). *Trichogramma*, a natural enemy, is broadly distributed, specialized, efficient and crucial for integrated management of this pest (Pratissoli et al. 2005).

TABLE 1. LIFE TABLE OF *EUSELASIA EUCERUS* (LEPIDOPTERA: RIODINIDAE) INDICATING THE MORTALITY FACTORS (K) IN 3 DIFFERENT ENVIRONMENTS A TRANSECT DELIMITED IN A PLANTATION OF *EUCALYPTUS UROPHYLLA* × *EUCALYPTUS GRANDIS* IN BELO ORIENTE, MINAS GERAIS STATE, BRAZIL DURING MAY AND JUN OF 2005.

Environment	Stage	Mortality source	Number of Individuals (Nx)	Mortality rate(qx)	Mortality factor (Kn)
Xeric	Eggs	Parasitism	19704	0.7012	0.525
Xeric	L1 - L3	Unknown	5887	0.8758	0.906
Xeric	L4 - L5	Unknown	731	0.6922	0.512
Xeric	Pupae	Parasitism Predation Fungus	225	0.5556	0.352
Xeric	Adults	—	100	—	—
Intermediate	Eggs	Parasitism	11495	0.9480	1.284
Intermediate	L1 - L3	Unknown	598	0.8846	0.938
Intermediate	L4 - L5	Unknown	69	0.0435	0.019
Intermediate	Pupae	Parasitism Predation Fungus	66	0.4848	0.288
Intermediate	Adults	—	34	—	—
Mesic	Eggs	Parasitism	6581	0.8812	0.922
Mesic	L1 - L3	Unknown	782	0.7788	0.655
Mesic	L4 - L5	Unknown	173	0.7168	0.548
Mesic	Pupae	Parasitism Predation Fungus	49	0.7143	0.544
Mesic	Adults	—	14	—	—

— Not applicable

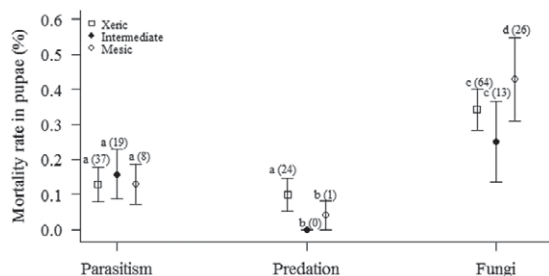


Fig. 3. Mortality rate of *Euselasia eucerus* pupae, per plant of *Eucalyptus urophylla* × *Eucalyptus grandis* in the xeric, intermediate and mesic environments in Belo Oriente, Minas Gerais. Bars with different letters indicate statistically significant differences between means. Data represented as mean ± standard error.

The greater structural complexity and variety of food resources of native vegetation increases the diversity (Thies et al. 2003), longevity and fertility of parasitoids in adjacent plantations (Oliveira et al. 2000). The mesic environment is in contact with a fragment of Atlantic Forest, which may contribute to the natural control of *E. eucerus* through this forest's positive effect on the parasitism rates of this butterfly in eucalyptus (Lill & Marquis 2001; Murta et al. 2008).

Differences in numbers of *E. eucerus* individuals per stage can be attributed to predation or parasitism or to mortality due to unknown causes, such as pathogens or climatic factors (Hajek & Leger 1994). Mortality factors of *E. eucerus* larvae are not well known (Zanuncio et al. 2009), but predation by Pentatomidae was observed during field sampling. These predators attack mainly third and fourth instar larvae (Lemos et al. 2003, Medeiros et al. 2003). Aggregation behavior of *E. eucerus* caterpillars at early stages can impede predation (Klok & Chown 1999), compared with the increased susceptibility to natural enemies of solitary individuals (Reader & Hochuli 2003). The greater size of *E. eucerus* caterpillars at third and fourth instars can facilitate their selection by predators as they yield a greater energetic return (Burger & Gochfeld 2001). Finally, the greater voracity of the final instars can increase consumption rates and the release of volatile chemical compounds that indicate the presence of eucalyptus herbivores, and thereby increase the predation risk (Radhika et al. 2003).

The greater number of *E. eucerus* individuals on eucalyptus plants in xeric areas may be caused by the higher concentration of nitrogen in the tissues of these plants and/or lower levels of chemical defenses (Thies et al. 2003). These factors would allow *E. eucerus* larvae to reach a larger size and be more susceptible to predation. In contrast, feeding on lower quality plants in mesic areas would render larvae more attractive to predators in later instar life-stages.

The mortality pattern of *E. eucerus* pupae can also be explained by their distance from the Atlantic Forest remnants, as the efficiency of predators and parasitoids depends on encounters with their hosts (Borer et al. 2007). *Euselasia eucerus* pupate on eucalyptus leaves and trunks, on other plants in the forest understory (Sousa et al. 2010), and on the ground. This behavior can make predator/parasitoid encounters unpredictable during active searching, and the predation was higher at the higher resource abundance in the xeric environment, but parasitism showed no differences between environments (Fig. 3). The same factors do not affect fungal infections, because fungi exhibit a high capacity to be spread horizontally by wind. Moreover, the greater humidity in mesic environments can favor the proliferation of fungi on *E. eucerus* pupae (Palmer et al. 1987), and humidity can favor their efficiency against pupae of this butterfly on eucalyptus plants (Zanuncio et al. 2009).

The results presented here reinforce the concept that native vegetation near eucalyptus plantations exerts a local effect supporting the maintenance of natural enemies of *E. eucerus*, and favoring fungal infection during pupal stage of this insect.

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