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Resistance of *Eucalyptus* spp. genotypes to eucalyptus brown looper *Thyrinteina arnobia* (Lepidoptera: Geometridae)

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Abstract

Eucalyptus brown looper *Thyrinteina arnobia* (Stoll, 1782) (Lepidoptera: Geometridae) is a major lepidopteran defoliator of eucalyptus. The objective of this study was to screen various eucalyptus genotypes and identify sources of resistance to *T. arnobia*. Leaves of the *Corymbia citriodora*, *Eucalyptus alba*, *E. brassiana*, *E. camaldulensis*, *E. cloeziana*, *E. dunnii*, *E. grandis*, *E. pellita* x *E. tereticornis*, *E. resinifera*, *E. robusta*, *E. saligna*, *E. tereticornis*, *E. torelliana* x *C. citriodora* and *E. urophylla* genotypes were provided to *T. arnobia* to assess antibiosis. The duration of the larval and pupal stages (days), pupal weight (mg), pupal and larval viability (%), adult longevity (days), sex ratio and total life cycle (days) were recorded. *E. grandis*, *E. robusta*, *E. brassiana*, *E. tereticornis* and *E. saligna* were found to be highly susceptible to *T. arnobia*, whereas *E. torelliana* x *C. citriodora* and *E. dunnii* showed antibiosis and/or antixenosis. These findings can assist growers in the management of *T. arnobia* and may be useful in the breeding of eucalyptus resistant to this insect.

Keywords: Defoliator caterpillar, Eucalyptus, Host-plant resistance, Pest management.

Introduction

Eucalyptus was introduced to Brazil in 1824 and has been planted in this country for commercial purposes since 1904 (Holtz et al., 2003). The cultivation of eucalyptus over large areas has increased since 1970 due to increased demands of the charcoal industry (Batista-Pereira et al., 2006; Chen et al., 2010). Eucalyptus monocultures, similar to those of other plants, encounter several problems with chewing insect-pests, such as ants and defoliator caterpillars (Santos et al., 2000; Zanetti et al., 2003; Zanuncio et al., 2006). *Thyrinteina arnobia* (Stoll, 1782) (Lepidoptera: Geometridae) is the main lepidopteran defoliator of eucalyptus in Brazil, causing significant defoliation and reduction in wood production (Oliveira et al., 2005; Oliveira et al., 2008; Oliveira et al., 2011). Caterpillars in eucalyptus crops can be controlled by a variety of methods, such as chemical and biological insecticides (Guedes et al., 2000), predators (Soares et al., 2009), parasitoids (Oliveira et al., 2008; Pereira et al., 2008) and silvicultural methods, including understory management (Guedes et al., 2000). The proper identification of host-plant resistance (HPR) categories is essential for the successful use of HPR in breeding programs. Antibiosis is a category of resistance that impacts insect biology, either by physical or chemical plant defenses (Smith 2005). Common observations of antibiosis are the reduction of adult fecundity and the premature death of early instars. Individuals that survive the initial impact of antibiosis exhibit a reduction in size and weight at younger stages, which results in an extended immature period and longer life cycles overall. Antixenosis, also referred to as non-preference, is a type of resistance that interferes with insect behavior. Physical and chemical factors present in plants exhibiting antixenosis impact the insect's recognition of the plant as a suitable host, oviposition site, mating site, and/or shelter (Panda and Khush 1995). The resistance or susceptibility of eucalyptus to *T. arnobia* may be due to an interaction of several factors, including food quality (Mauricio and Raushers, 1997; Seifi et al., 2013). Insect resistance in eucalyptus genotypes has been reported for several species, including ants, beetles and the red gum lerp psyllid *Glycaspis brimblecombei* (Hemiptera: Psyllidae) (Hanks et al., 1991; Oliveira et al., 2004; Pereira et al., 2013). Eucalyptus hybrids are important for forestry in tropical regions but may be more susceptible to lepidopteran defoliators than pure species (Potts et al., 2004). For example, a study conducted by Oliveira et al. (1984) found that *Eucalyptus paniculata* was more susceptible to defoliator damage than *E. alba*, *E. robusta* and *E. tereticornis* (Oliveira et al., 1984). Host-plant resistance (HPR) is widely used as a control method within the precepts of integrated pest management. HPR aims to reduce the population density of pests below the economic injury level with no or minimal impact on the environment and is compatible with other control methods. Moreover, HPR incurs lower costs than chemical control and has persistent effects during the phenological stages of the crop (Smith, 2005; Seifi et al., 2013). The objective of this research was to screen and identify potential sources of resistance to *T. arnobia* in eucalyptus.

Results and Discussion

The biological parameters of male and female *T. arnobia* were affected when the insects fed on different eucalyptus genotypes (Tables 1 and 2).

Table 1. Mean (± SE) values of the duration of the larval stage (DLS) and duration of the pupal stage (DPS) in days and the pupal weight (PW) in mg for males of *T. arnobia* (Lepidoptera: Geometridae) on different eucalyptus genotypes.

Genotypes[1]	DLS	DPS	PW
Corymbia citriodora	27.2±0.48 abc	8.0±0.50	252.9±0.02
Eucalyptus alba	30.0±0.41 a	9.2±0.29	257.4±0.01
Eucalyptus brassiana	27.7±0.48 abc	9.0±0.76	195.0±0.03
Eucalyptus camaldulensis	28.5±0.29 abc	9.2±0.50	142.4±0.01
Eucalyptus cloeziana	26.2±0.75 bcd	8.7±0.58	279.4±0.02
Eucalyptus dunnii	-[2]	-[2]	-[2]
Eucalyptus grandis	25.7±0.63 cd	9.5±0.50	193.5±0.03
Eucalyptus pellita x *E. tereticornis*	28.7±0.48 abc	8.0±0.29	185.8±0.03
Eucalyptus resinifera	29.0±0.41 ab	9.0±1.00	180.0±0.01
Eucalyptus robusta	29.5±0.29 a	9.2±0.10	219.5±0.04
Eucalyptus saligna	25.7±0.48 cd	8.0±0.50	259.1±0.01
Eucalyptus tereticornis	28.5±0.65 abc	8.2±0.50	201.9±0.02
Eucalyptus toreliana x *C. citriodora*	-[2]	-[2]	-[2]
Eucalyptus urophylla	23.5±0.65 d	9.5±0.50	246.2±0.01
F (treatment)	1.06[*]	1.16[ns]	1.85[ns]
CV (%)	3.75	12.50	27.73

[1]Means followed by the same letter in each column are not significantly different based on the Tukey test (P≤0.05). Data were transformed [(x + 0.5)$^{1/2}$] prior to analysis. [2]All larvae fed on this treatment died (null variance). [*]Significant at 5% probability. [ns]Not significant.

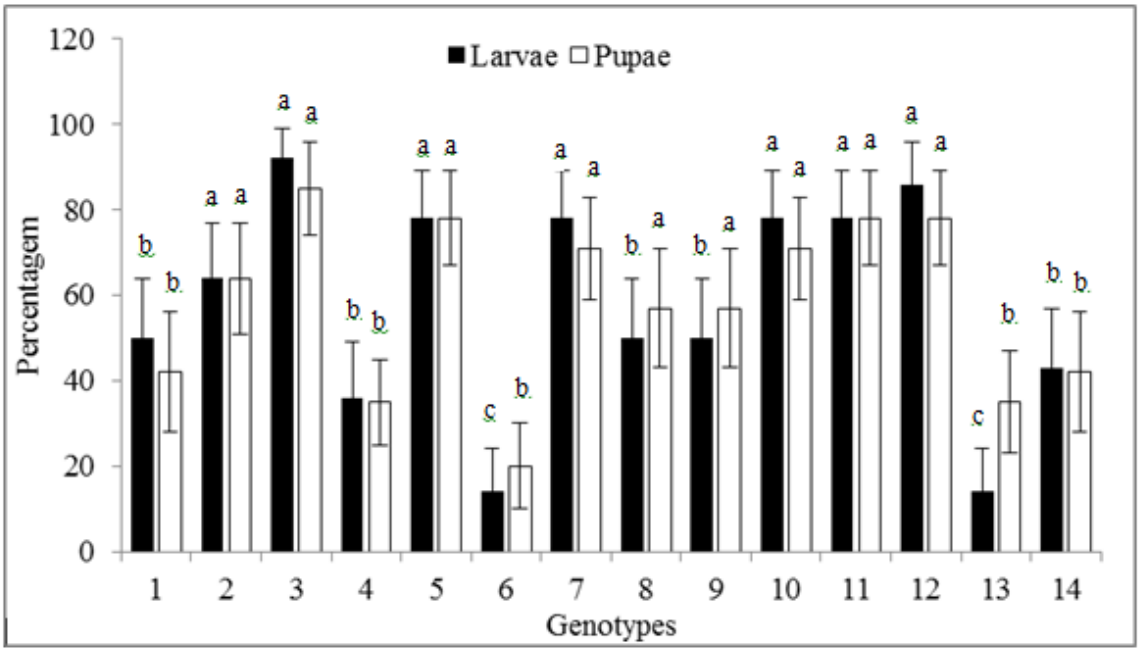

Fig 1. Mean (± SE) values of the larval and pupal viability (%) of males and females of *Thyrinteina arnobia* (Lepidoptera: Geometridae) within eucalyptus genotypes in an antibiosis bioassay. 1- *Corymbia citriodora*, 2- *Eucalyptus alba*, 3- *E. brassiana*, 4- *E. camaldulensis*, 5- *E. cloeziana*, 6- *E. dunnii*, 7- *E. grandis*, 8- *E. pellita* x *E. tereticornis*, 9- *E. resinifera*, 10- *E. robusta*, 11- *E. saligna*, 12- *E. tereticornis*, 13- *E. toreliana* x *C. citriodora* and 14- *E. urophylla*. Means followed by the same letter per column are not significantly different based on the Scott Knott test (P≤0.05%). Larvae: F (treatment) 4.62[*] and Pupa: F (treatment) 2.40[*].

The larval period of *T. arnobia* males was longer for individuals that fed on leaves of *Eucalyptus alba* and *E. robusta* when compared to individuals exposed to *E. urophylla*. The larval period was 4.8 days longer on *E. camaldulensis* than on *E. urophylla* and *E. saligna*. The pupal duration and weight of *T. arnobia* males were similar among all eucalyptus genotypes. On average, the duration of the pupal stage was 8.0 days on *Corymbia citriodora* and *E. pellita* x *E. tereticornis* and 9.5 days when insects were fed on *E. urophylla*. The pupal weight ranged from 142.4 mg in *E. camaldulensis* to 279.4 mg in *E. cloeziana* (Table 1). In females, the larval duration of *T. arnobia* that fed on *E. camaldulensis*, *E. tereticornis* and *C. citriodora* was longer than of those fed with *E. saligna* and *E. urophylla* (Table 2). Furthermore, pupal duration and weight of *T. arnobia* females were similar among all the eucalyptus genotypes tested in this study. However, the pupal duration was 8.0 days with *C. citriodora* and 9.0 days on *E. cloeziana*. The weight of pupae ranged from 367.6 mg on *E. alba* to 528.1 mg on *E. urophylla* (Table 2). The larval period of individuals that gave rise to *T. arnobia* females was longer than that of males. This may be due to the fact that females have higher nutritional needs for energy accumulation and the development of reproductive structures (Oliveira et al., 2005). *Corymbia citriodora*, *E. camaldulensis* and *E. grandis* have been found to have higher quantities of secondary compounds (Batista-Pereira et al., 2006). The presence of these compounds, especially essential oils and phenols such as tannin, can be deleterious to the biology of *T. arnobia* (Fox and Macauley, 1977; Santos et al., 2000). The duration of the larval stage was affected by the *Eucalyptus* genotypes. It was found that larvae feeding on *E. cloeziana* reached the final instar with in approximately 31 days, whereas development on its natural host guava (*Psidium guajava*) was completed within 29 days. On *E. grandis*, the duration of this larval period was of 31.4 days for males and 41.8 days for females (Holtz et al., 2003; Oliveira et al., 2005), indicating that *T. arnobia* may prefer

Table 2. Mean (± SE) values of the duration of the larval stage (DLS) and duration of the pupal stage (DPS) in days and the pupae weight (PW) in mg for females of *T. arnobia* (Lepidoptera: Geometridae) on different eucalyptus genotypes.

Genotypes[1]	DLS	DPS	PW
Corymbia citriodora	32.6±1.53 a	8.0±0.29	372.5±0.07
Eucalyptus alba	31.8±2.00 ab	8.4±0.58	367.6±0.01
Eucalyptus brassiana	31.4±0.50 ab	8.0±0.29	369.8±0.02
Eucalyptus camaldulensis	33.2±0.50 a	8.4±1.04	521.5±0.04
Eucalyptus cloeziana	31.0±1.04 ab	9.0±0.50	456.0±0.07
Eucalyptus dunnii	-[2]	-[2]	-[2]
Eucalyptus grandis	31.4±0.50ab	8.8±0.29	440.2±0.12
Eucalyptus pellita x *E. tereticornis*	30.0±0.76 ab	8.4±0.29	516.3±0.09
Eucalyptus resinifera	31.4±0.50 ab	7.4±0.29	446.6±0.02
Eucalyptus robusta	32.2±0.58 ab	8.6±0.29	409.3±0.09
Eucalyptus saligna	28.4±0.29 b	8.2±0.29	445.5±0.06
Eucalyptus tereticornis	33.0±1.53 a	8.2±0.29	456.8±0.04
Eucalyptus toreliana x *C. citriodora*	-[2]	-[2]	-[2]
Eucalyptus urophylla	28.4±0.29 b	8.6±0.29	528.1±0.02
F (treatment)	3.66[*]	1.26[ns]	1.13[ns]
CV (%)	5.94	10.03	27.12

[1]Means followed by the same letter per column are not significantly different based on the Tukey test (P≤0.05). Data were transformed [$(x + 0.5)^{1/2}$] prior to analysis. [2]All larvae fed on this treatment died (null variance). [*]Significant at 5% probability. [ns]Not significant.

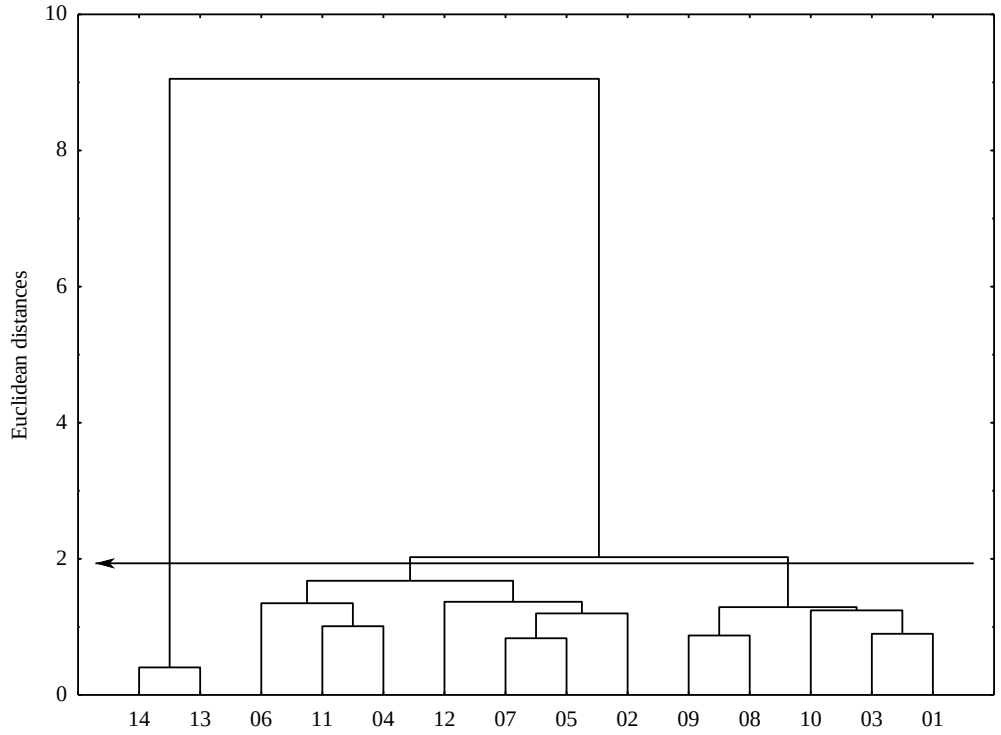

Fig 2. Dendrogram based on the biological parameters of *T. arnobia* (Lepidoptera: Geometridae) fed on various eucalyptus genotypes. 1- *Eucalyptus grandis*, 2- *E. camaldulensis*, 3- *E. robusta*, 4- *E. cloeziana*, 5- *E. pellita* x *E. tereticornis*, 6- *C. citriodora*, 7- *E. resinifera*, 8- *E. brassiana*, 9- *E. tereticornis*, 10- *E. saligna*, 11- *E. alba*, 12- *E. urophylla*, 13- *E. dunnii* and 14- *E. torelliana* x *Corymbia citriodora*. Genotypes were clustered using the UPGMA method with dissimilarity measures of Euclidean distance.

eucalyptus species over its natural host. This study demonstrated that *E. dunnii* and *E. torelliana* x *C. citriodora* negatively impacted the biological parameters and increased the mortality of *T. arnobia*, suggesting the presence of toxic compounds or feeding deterrents in these plants. The larval viability and pupal duration of *T. arnobia* ranged from 14.29 to 92.86% and from 14.29 to 78.57%, respectively. Larval viability was greatest on *E. brassiana* and was significantly lower in *E. dunnii* and *E. torelliana* x *C. citriodora*. In addition, pupal viability was also significantly lower in *E. dunnii* compared to *E. brassiana* (Fig. 1). Holtz et al. (2003) previously found that the larval viability of *T. arnobia* was 78.0% when fed on *E. cloeziana* and 29.3% on *P. guajava*, where the life cycle was 30.3 and 27.9 days, respectively. The characteristics of *T. arnobia* presented here are comparable to those determined for this insect when feeding on the leaves of *E. cloeziana*. The longevity of *T. arnobia* females was 2.4, 2.2 and 2.0 days longer on leaves of *E. cloeziana*, *E. grandis* and *E. urophylla*, respectively, compared to those on *C. citriodora* (Table 3). Furthermore, the life cycle was also influenced by the different genotypes evaluated in this study. Males that fed on *E. camaldulensis* and *E. alba* had a life cycle that was approximately 5.7 days longer than individuals that developed on *E. urophylla*. Females that developed on *E. alba* and *E. camaldulensis* had an average life cycle that was 6.3 days longer than on *E. saligna*. Interestingly, the eucalyptus

Table 3. Mean (± SE) values of the longevity of males and females (days), total cycle (days) and sex ratio of *T. arnobia* (Lepidoptera: Geometridae) within eucalyptus genotypes.

Genotypes[1]	Longevity		Total cycle		Sex Ratio
	Male	Female	Male	Female	
Corymbia citriodora	6.5±0.50	6.6±0.50 b	35.0±1.50 ab	47.8±0.87 abc	0.33±0.14 b
Eucalyptus alba	6.5±0.50	8.4±0.58 ab	38.7±1.15 a	50.6±2.65 a	0.33±0.14 b
Eucalyptus brassiana	5.2±1.15	8.0±0.29 ab	35.5±1.44 ab	49.0±1.04 abc	0.50±0.15 ab
Eucalyptus camaldulensis	8.0±0.20	8.4±1.04 ab	38.7±0.50 a	51.2±0.76 a	0.75±0.13 ab
Eucalyptus cloeziana	7.0±0.29	9.0±0.50 a	35.2±1.15 ab	48.0±0.87 abc	0.33±0.14 b
Eucalyptus dunnii	-[2]	-[2]	-[2]	-[2]	-[2]
Eucalyptus grandis	8.0±0.50	8.8±0.29 a	36.2±1.26 ab	48.6±0.58 abc	0.42±0.15 ab
Eucalyptus pellita x *E. tereticornis*	7.0±0.29	8.4±0.29 ab	36.2±0.50 ab	47.6±0.50 abc	0.67±0.14 ab
Eucalyptus resinifera	7.20±1.04	7.4±0.50 ab	38.0±1.04 ab	48.8±76 abc	0.25±0.23 b
Eucalyptus robusta	6.5±0.58	8.6±0.29 ab	37.5±0.29 ab	49.8±0.55 ab	0.42±0.15 ab
Eucalyptus saligna	6.0±1.44	7.2±0.50 ab	33.0±2.47 b	44.6±0.58 c	0.50±0.15 ab
Eucalyptus tereticornis	6.2±0.37	8.2±0.29 ab	36.0±1.89 ab	49.6±1.53 abc	0.67±0.14 ab
Eucalyptus toreliana x *C. citriodora*	-[2]	-[2]	-[2]	-[2]	-[2]
Eucalyptus urophylla	6.7±0.29	8.6±0.29 a	33.0±0.58 b	45.4±0.76 bc	0.83±0.11 a
F (treatment)	1.19^{ns}	3.33^{*}	2.67^{*}	3.48^{*}	1.65^{*}
CV (%)	21.24	10.76	6.55	4.79	26.02

[1]Means followed by the same letter are not significantly different based on the Tukey test (P≤0.05). [2]All larvae fed on the indicated treatment died (null variance). Data were transformed $[(x + 0.5)^{1/2}]$ prior to analysis. *Significant at 5% probability. nsNot significant.

genotypes also influenced the sex ratio. In our study, the measured sex ratio ranged from 0.83 on *E. urophylla* to 0.25 on *E. resinifera* (Table 3). Food quality affects the biology of herbivores due to the chemical defense mechanisms of plants that act as repellents, thereby reducing the insect's ability to properly digest food (Holtz et al., 2003). The presence of tannins in the leaves, combined with proteins and digestive enzymes in the herbivore gut, may hinder digestion and reduce the developmental rate of current and future generations (Mauricio and Raushers 1997). This could potentially explain how the genotypes tested in our study influenced the biology of *T. arnobia*. *Eucalyptus grandis*, *E. urophylla* and *E. saligna* were considered susceptible to *T. arnobia*, favoring the development of the insect. Conversely, *E. robusta*, *E. alba*, *E. dunnii* and the clone *E. torelliana* x *C. citriodora* had negative effects on the biology of *T. arnobia*. A hierarchical cluster analysis based on the similarity of the biological parameters of *T. arnobia* on fourteen eucalyptus genotypes revealed differences among the groups (Fig. 2). The first cluster, including *E. torelliana* x *C. citriodora* and *E. dunnii*, was formed at a Euclidean distance of 0.2. The second cluster comprised *C. citriodora*, *E. alba*, *E. cloeziana*, *E. urophylla*, *E. resinifera*, *E. pellita* x *E. tereticornis* and *E. camaldulensis*, and the final group consisted of *E. tereticornis*, *E. brassiana*, *E. saligna*, *E. robusta* and *E. grandis*. The phenon line (Fig. 2), which represents the average similarity between genotype pairs, indicates a reference point for their division into groups (Pitta et al., 2010). Using a Euclidean distance of 2.0 to separate the genotypes into three groups, different resistance levels were established: *E. torelliana* x *C. citriodora* and *E. dunnii* were classified as moderately resistant; *C. citriodora*, *E. alba*, *E. cloeziana*, *E. urophylla*, *E. resinifera*, *E. pellita* x *E. tereticornis* and *E. camaldulensis* were found to be susceptible; and *E. tereticornis*, *E. brassiana*, *E. saligna*, *E. robusta* and *E. grandis* were found to be highly susceptible to *T. arnobia*.

Materials and Methods

The experiments were conducted at the Entomology Laboratory of the Goiano Federal Institute (IF Goiano), Campus Urutaí, Goias State, Brazil. The following species, hybrids and clones were used for this study: *Corymbia citriodora*, *Eucalyptus alba*, *E. brassiana*, *E. camaldulensis*, *E. cloeziana*, *E. dunnii*, *E. grandis*, *E. pellita* x *E. tereticornis*, *E. resinifera*, *E. robusta*, *E. saligna*, *E. tereticornis*, *E. torelliana* x *C. citriodora* and *E. urophylla*.

Caterpillar stock rearing

Thyrinteina arnobia egg masses were obtained from the Laboratory of Plant Resistance to Insects from the University of São Paulo (UNESP) in Jaboticabal, São Paulo State, Brazil. Newly hatched *T. arnobia* larvae were reared on *E. grandis* prior to experimentation. Leaf petioles of *E. grandis* available were harvested from the areas surrounding IF Goiano and were placed into 250 mL plastic bottles with water to maintain leaf turgor. The plant tissue was replaced daily until the termination of stock rearing (Marinho-Prado et al., 2011). Prior to feeding, the leaves were disinfected with a 5% solution of sodium hypochlorite for 15 minutes to reduce pathogenic microorganism infection, rinsed in water, air dried and placed in the rearing cages. Larvae were placed in glass cages (50 cm high by 30 cm in diameter) and covered with organdy fabric to prevent their escape. All cages were lined with paper towels to reduce moisture from insect frass.

Antibiosis bioassays

One newly hatched (1 day old) *T. arnobia* larva obtained from the mass rearing glass cages was transferred into a plastic bottle (28 cm high by 10 cm diameter). The base of the bottle was fixed to a plastic container (8 cm high by 13 cm in diameter) with toothpicks in sand. Water was added to the container to reduce drying of the leaves. *T. arnobia* larvae were kept in these containers until pupation, after which the individuals were sexed.

Larval, pupal and adult parameters were evaluated based on the sex of *T. arnobia*. The pupae were removed from the rearing cages and individually weighed and, 24 hours later, were placed in emergence pots (9 cm high and 7 cm in diameter). The adults that emerged were transferred to mating PVC cages (20 cm high by 10 cm in diameter) lined with paper affixed with adhesive tape and with the top was closed with organdy fabric. Adult longevity and total life cycle were calculated based on the larval and pupal durations.

The experimental design was completely randomized with 14 treatments (genotypes) and 30 replications (plastic bottle and one larva).

Statistical analysis

Data normality and variance were verified using the Kolmogorov-Smirnov and Bartlett tests, respectively (Lilliefors, 1967). When appropriate, data were transformed using the equation $(x + 0.5)^{1/2}$. An analysis of variance (ANOVA) and F test were conducted, and the means were separated at a 5% level of significance using the Tukey and Scott-Knott tests. The above statistical analyses were performed in Assistat version 7.6 (Silva and Azevedo, 2002). The hierarchical cluster analysis and Euclidean distances used for dissimilarity measures were conducted using the software Statistica version 7.0 (Sneath and Sokal, 1962).

Conclusions

Eucalyptus grandis, *E. robusta*, *E. brassiana*, *E. tereticornis* and *E. saligna* were found to be highly susceptible to *T. arnobia*. The clone *E. torelliana* x *C. citriodora* and the species *E. dunnii* exhibited antibiosis and/or antixenosis to *T. arnobia*.

Cluster analysis was effective in selecting resistant varieties and may be used as a method complementary to univariate analysis.

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