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Susceptibility of *Eucalyptus* spp. (Myrtales: Myrtaceae) and clones to *Leptocybe invasa* (Hymenoptera: Eulophidae) in Paraná, Brazil

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Eucalyptus spp. (Mytales: Myrtaceae) originated in Australia and Indonesia, and they have outstanding importance in forestry for use as timber, pulp, and as a potential bioenergy feed-stock. *Eucalyptus* spp. have been cultivated and used around the world, and they bring economic, social, and environmental advantages because they reduce the deforestation pressure on native forests (Zheng et al. 2014).

The gall wasp, *Leptocybe invasa* Fisher & LaSalle (Hymenoptera: Eulophidae: Tetrastichinae), is of Australian origin and prevalent in many regions of the world (Jhala et al. 2010). *Leptocybe invasa* was recently introduced into Brazil, where it exhibits the behavior of endophytic oviposition, inducing the formation of galls on leaves and petioles of eucalyptus trees, with increased frequency of leaf distortion, drying, and defoliation of new branches (Rinaldi et al. 2013). The development of galls occurs due to a physiological disorder caused by an intrinsic and specific relationship between plant and insect by high dependency and specificity to small changes in morphological and phenological characteristics of plants, determining degrees of susceptibility and coevolution (Stone & Schönrogge 2003; Zheng et al. 2014). This high degree of coevolution can provide advantages for the management of the wasp because it allows the separation of species and hybrids resistant to gall formation and development of *L. invasa* (Dungey et al. 2000).

There are great differences in the susceptibility of eucalyptus to wasp attack (Mendel et al. 2004). The objective of the present study was to evaluate the susceptibility of *Eucalyptus* spp. and clones to *L. invasa* in the field. The research was carried out in Umuarama City, Paraná (PR), Brazil (23°47'21.3"S, 53°15'29.2"W, 402.57 m asl) at the Universidade Estadual de Maringa, Campus of Umuarama. *Leptocybe invasa* was evaluated in a 2.0 acre (0.86 ha) field of 1-year-old *Eucalyptus* spp. clones with an average height of 2 m, spaced 3.0 × 2.0 m. To study the susceptibility and preference of the plant parts attacked by *L. invasa*, we used 6 clones ('urograndis (seminal)', 'urograndis H13', 'I144', 'I224', 'Grancam COP 1277', and 'Urocam VM1') and 2 eucalyptus species (*E. grandis* W. Hill ex Maiden and *Corymbia citriodora* [Hook.] K.D. Hill & L. A. S. Johnson [Myrtales: Myrtaceae]).

The experiment was a randomized block in split plot design with 5 replications. The preference of occurrence of *L. invasa* was evaluated in the main plots, and the preferred location for the occurrence on the plants was evaluated in the subplots. Accordingly, the trees were divided into 3 strata (upper, middle, and lower). Each plot had an area of 216 m^2, where 4 biweekly evaluations were performed from 05/10/2013. In each evaluation, 9 branches per tree (3 branches/stratum) were collected from 7 trees per plot.

The numbers of galls per leaf and petiole of each branch were recorded. Subsequently, the percentages of leaves and petioles with galls were determined according to the genetic material and each part of the plant collected. The susceptibilities of *Eucalyptus* spp. and clones to *L. invasa* were determined by symptoms observed in branches with oviposition of the genetic material collected, and the data were subjected to analysis of variance (*F* test). The mean numbers of insects per sample unit in each stratum of the tree as well as their occurrence in leaves and petioles were compared using Tukey's test at a significance level of 0.05. We used the statistical software Assistat 7.6 Beta, and the data were transformed with log(x + 5).

All the samples of 'Grancam COP 1277' showed galls on both leaves and petioles, with the mean values of the percentages in the first and last assessments being 19.4 and 25.0% and 8.3 and 10.5% for leaves and petioles, respectively (Fig. 1). *Corymbia citriodora* had the fewest number of galls, and the percentages of leaves and petioles of the plants attacked remained below 1% in all evaluations. According to Bailey et al. (2006) resistance or susceptibility of plants to pests is predominantly inherited and can be expressed both in pure species and in hybrids. The susceptibility of *Eucalyptus* spp. observed can explain the results obtained for preference for *E. grandis* and the hybrids 'Grancam COP 1277', 'urograndis (seminal)' and 'Urocam VM1', which are derived from crosses between the species *E. grandis* × *E. urofila, E. grandis* × *E. camaldulensis*, and *E. camaldulensis* × *E. urofila*, respectively.

The differences in the susceptibility of eucalyptus materials to gall-forming insects may indicate that they possess genetic factors for both their attractiveness to *L. invasa* for oviposition and for their suitability for larval development of *L. invasa.* Another factor prevalent in most cases of susceptibility is the synchronization of the gall-forming species with the phenology of the host plant. Therefore, coevolution demonstrated between eucalyptus plants and *L. invasa* may provide greater ease in controlling this pest through the development and deployment of resistant and productive genetic materials than through chemical means (Dungey et al. 2000). In this context, the clones 'urograndis H13', 'urograndis I144' and 'urograndis I224' in all samples presented a lower percentage of leaves with galls than 'grancan COP 1277' (the most attacked), thus indicating the possibility that the 3 clones possess resistance to attack by *L. invasa*.

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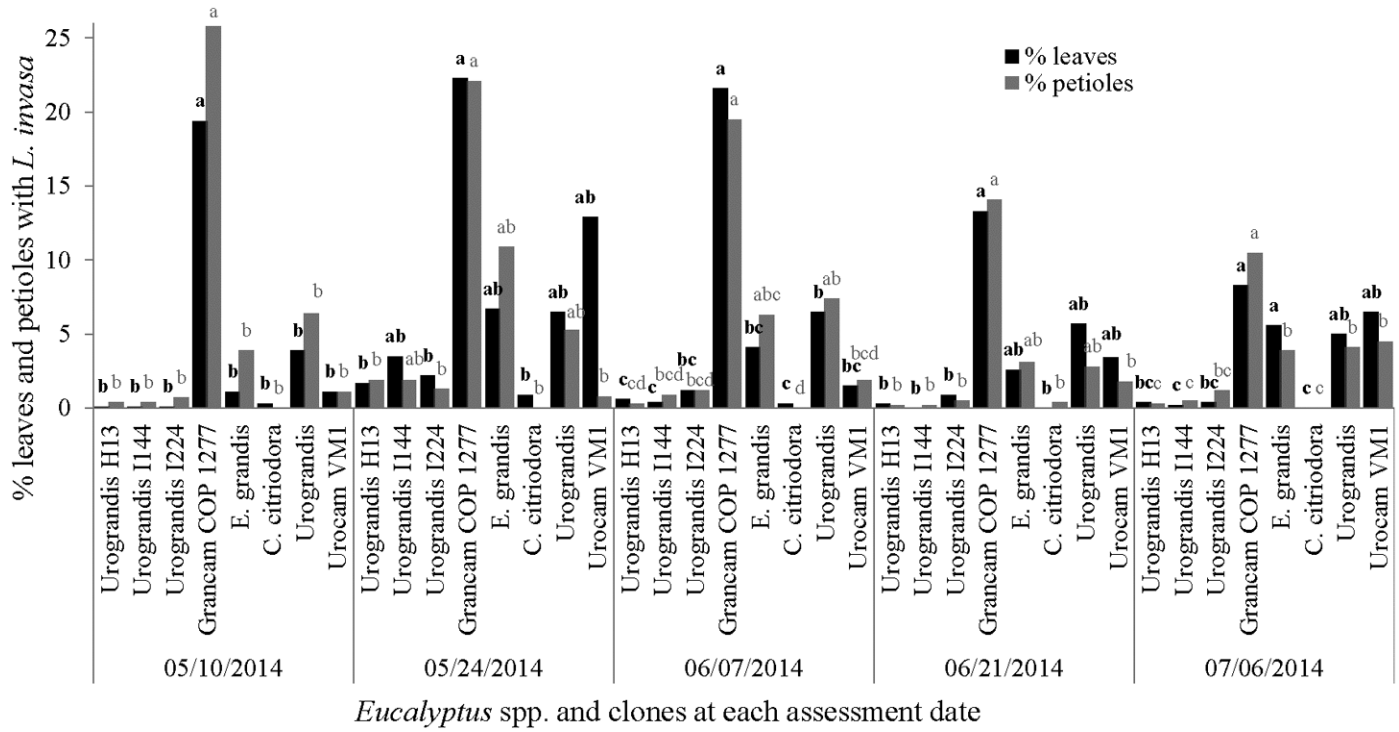


Fig. 1. Percentage of leaves and stems with galls caused by *Leptocybe invasa* in approx. 2-m-tall *Eucalyptus* spp. and clones on each sampling date at Umuarama, PR, Brazil, in 2013.

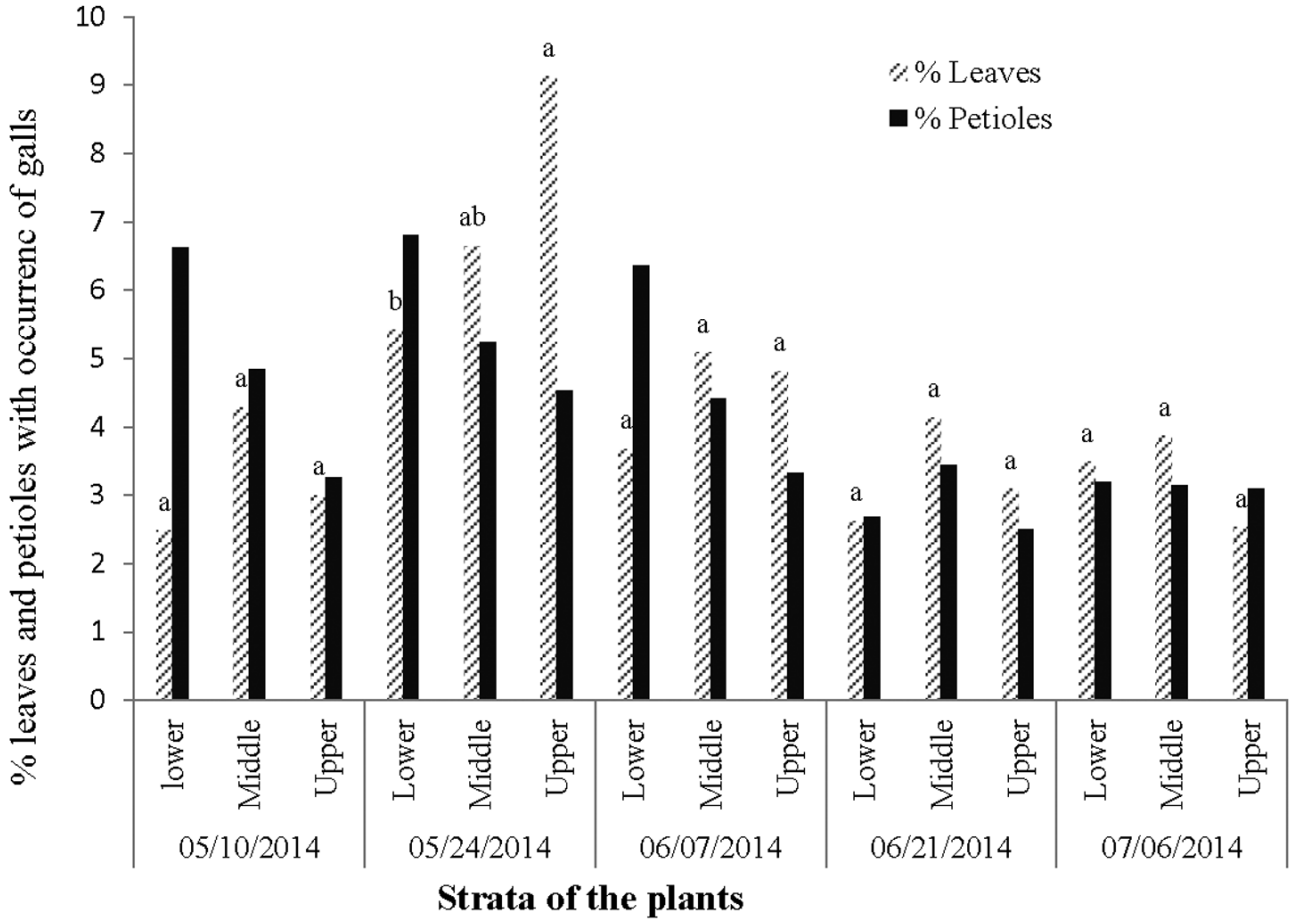


Fig. 2. Percentage of leaves and stems with galls caused by *Leptocybe invasa* in plants in each stratum (lower, middle, upper) of approx. 2-m-tall *Eucalyptus* spp. and clones at Umuarama, PR, Brazil, in 2013.

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With regard to the occurrence of galls on leaves and stems in the various tree strata of the *Eucalyptus* spp. and clones, the averages were statistically different only for leaves in the second evaluation. Furthermore, the highest percentage was obtained for the upper stratum, with 9.3% of leaves with galls (Fig. 2). In the evaluation of the preferred sites of attack by *L. invasion* on the tree (leaves and petioles), we noticed that regardless of the species or clone evaluated or from which stratum the samples were collected, wasp attack occurred in the same proportion of leaves and petioles (Table 1). In most studies on oviposition preference of *L. invasion* in eucalyptus, this pest species occurred on leaves, shoots, and stems of the eucalyptus (Mendel et al. 2004; Garlet et al. 2013; Rinaldi et al. 2013).

Regardless of the sampling period and the tree stratum evaluated, the average numbers of galls per leaf and per petiole were significantly higher for 'Grancam COP 1277' than for any other eucalyptus materials. 'Grancam COP 1277' averaged 14.46 and 14.25 galls on the leaves and petioles, respectively (Table 1). The clones 'urograndis H13', 'urograndis I144', and 'urograndis I224' as well as *C. citriodora* had the lowest occurrence of galls, demonstrating low susceptibility to *L. invasa.*

The results obtained in the present study with eucalyptus confirm previously observed percentages of leaves attacked by *L. invasa*. The occurrence of galls observed are within the range of those reported previously by Mendel et al. (2004), who found that numbers of galls caused by *L. invasa* ranged from 1 to 65 per leaf. According to Rinaldi et al. (2013), the highest occurrence of galls per leaf and petiole had been observed for plants undergoing rapid growth, which was confirmed in the present study for the most-attacked hybrids.

Table 1. Average values of galls transformed by log(x+5) (original averages in parentheses), caused by *Leptocybe invasa* in leaves and petioles of approx. 2-m-tall *Eucalyptus* spp. and clones, regardless of when collected and from which stratum. The data were collected at Umuarama, Paraná (PR), Brazil, in 2013.

	No. of galls		
Eucalyptus material	Leaves	Petioles	Average
Urograndis H13	0.722 cdA (0.28)	0.717 dA (0.22)	0.719 c (0.25)
Urograndis I144	0.724 cdA (0.32)	0.721 dA (0.26)	0.722 c (0.29)
Urograndis I224	0.725 cdA (0.33)	0.721 dA (0.32)	0.725 c (0.32)
Grancam COP 1277	1.236 aA (14.46)	1.212 aA (14.25)	1.224 a (14.35)
E. grandis	0.845 bB (2.11)	0.896 bA (2.97)	0.871 b (2.54)
C. citriodora	0.709 dA (0.12)	0.7 dA (0.01)	0.704 c (0.07)
Urograndis	0.866 bA (2.60)	0.839 bcA (1.98)	0.852 b (2.29)
Urocam VM1	0.829 bcA (1.92)	0.765 cdB (0.85)	0.797 bc (1.39)
CV% (eucalyptus material)	15.63		
CV% (plants parts)	6.77		

Means followed by the same lower case letter in the columns and capital letters in the rows do not differ by Tukey test at 5% probability. CV% is Coefficient of variation.

We express heartfelt thanks to "Instituto Agronômico do Paraná (IAPAR)" and to Dr. Alex Carneiro Leal for the eucalyptus species and clones.

Summary

'Grancam COP 1277' had the highest percentage of leaves and petioles attacked by *L. invasa*, whereas 'Urograndis (seminal)', 'Urocam VM1' and *E. grandis* had lower and statistically similar values. The attack by the wasp on *C. citriodora* was relatively minor compared with the above susceptible lines. Statistical differences were observed among similar proportions of attack between the oviposition preference on the 3 strata, leaves, and petioles of the assessed trees.

Key Words: *Corymbia citriodora*; coevolution; defoliation; endophytic oviposition; gall; gall wasp resistance; susceptibility; 'Urograndis (seminal)'

Sumário

'Grancam COP 1277' teve a maior porcentagem de folhas e pecíolos atacados por *L. invasa*, enquanto 'Urograndis (seminal)', 'Urocam VM1' e *E. grandis* apresentaram valores inferiores estatisticamente semelhantes. O ataque da vespa em *C. citriodora* foi relativamente menor em comparação com as linhagens suscetíveis citadas anteriormente. Foram observadas diferenças estatísticas em proporções semelhantes entre o local de ataque e preferência para oviposição dos três estratos em folhas e pecíolos das árvores avaliadas.

Palavras Chave: *Corymbia citriodora*; coevolução; desfolha; oviposição endofítica; galhas; resistência à vespa-das-galhas; suscetibilidade; 'Urograndis (seminal)'

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