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EARLY DETECTION AND SYMPTOMS OF *Leptocybe invasa* FISHER & LA SALLE, 2004 (HYMENOPTERA: EULOPHIDAE) IN *Eucalyptus* (MYRTACEAE) NURSERIES

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ABSTRACT

Forest production is one of the main economic activities in Uruguay, with *Eucalyptus* (Myrtaceae) being the most planted genus. *Leptocybe invasa* (Hymenoptera: Eulophidae) is one of the insect pests that affect the *Eucalyptus* plantations. The most important symptoms of the *L. invasa* attack are the galls but other symptoms have been observed such as high reduction, loss dominance on the apical region, overgrowth and some leave and branches deformations. Early detection in the nursery can reduce this pest dissemination in younger plantations.

Key words: *Eucalyptus*, *Leptocybe,* Plant protection, gall insects, Forest health

RESUMEN

Detección temprana y síntomas de *Leptocybe invasa* Fisher & La Salle, 2004 (Hymenoptera: Eulophidae) en viveros de *Eucalyptus* (Myrtaceae). La producción forestal es una de las principales actividades económicas en Uruguay, siendo el *Eucalyptus* (Myrtaceae) el género más plantado. *Leptocybe invasa* (Hymenoptera: Eulophidae) es uno de los insectos plaga que afecta a las plantaciones de *Eucalyptus*. El síntoma más importante del ataque de *L. invasa* es la formación de las agallas, pero se han observado otros síntomas tales como alta reducción, pérdida de dominancia en la región apical, crecimiento excesivo (superbrotación) y deformaciones de hojas y ramas. La detección temprana en el vivero puede reducir la diseminación de esta plaga en plantaciones más jóvenes.

Palabras clave: *Eucalyptus*, *Leptocybe*, Protección de plantas, Insectos agalladores, Sanidad forestal.

INTRODUCTION

Forest production has been the first economic activity in Uruguay since 2019 with more than U$S 2000 million in exportation (Ligrone, 2018; SPF, 2020). *Eucalyptus* spp. and *Pinus* spp. are the most planted genus in Uruguay where the former cover more than 75% of commercial plantations (Arriaga, 2018; DIEA, 2019). The main factors that cause a reduction in the productivity of forest plantations and economic losses to forest companies are insect pests and diseases (Wingfield *et al.*, 2008).

Garnas *et al.* (2016) and Hurley *et al.* (2016) proposed that the growth and frequency in the transport of goods and people (globalization) are responsible for the exponential increment in new pests (insects and diseases) reports associated with the *Eucalyptus* plantations. Plants intercontinental movement and their products (seeds, fruits, wood, and flowers) among countries is one of the main routes of the entry for exotic insect pests and diseases considered (Liebhold *et al.*, 1995, 2012; Cleary *et al.*, 2019; Meurisse *et al.*, 2019).

Gall-forming insects are the most evolved phytophagous group (Shorthouse *et al.*, 2005). This fact is probably associated with the strong interaction with their host plants to induce gall formation (Csóka *et al.,* 2017). Physiological mechanisms trough gall-insects that induce gall formation, plant tissues, and organ modifications are poorly understood. More than 50 gall insect species exist associated with eucalypt trees in Australia (Morrow *et al.*, 1994), but only ten are considered *Eucalyptus* spp. plantations pests (Paine *et al.*, 2011).

The Blue gum chalcid wasp, *Leptocybe invasa* Fisher & La Salle, 2004 (Hymenoptera: Eulophidae), is considered one of the most damaging insect pest species for *Eucalyptus* spp. plantations worldwide (Jorge *et al.*,

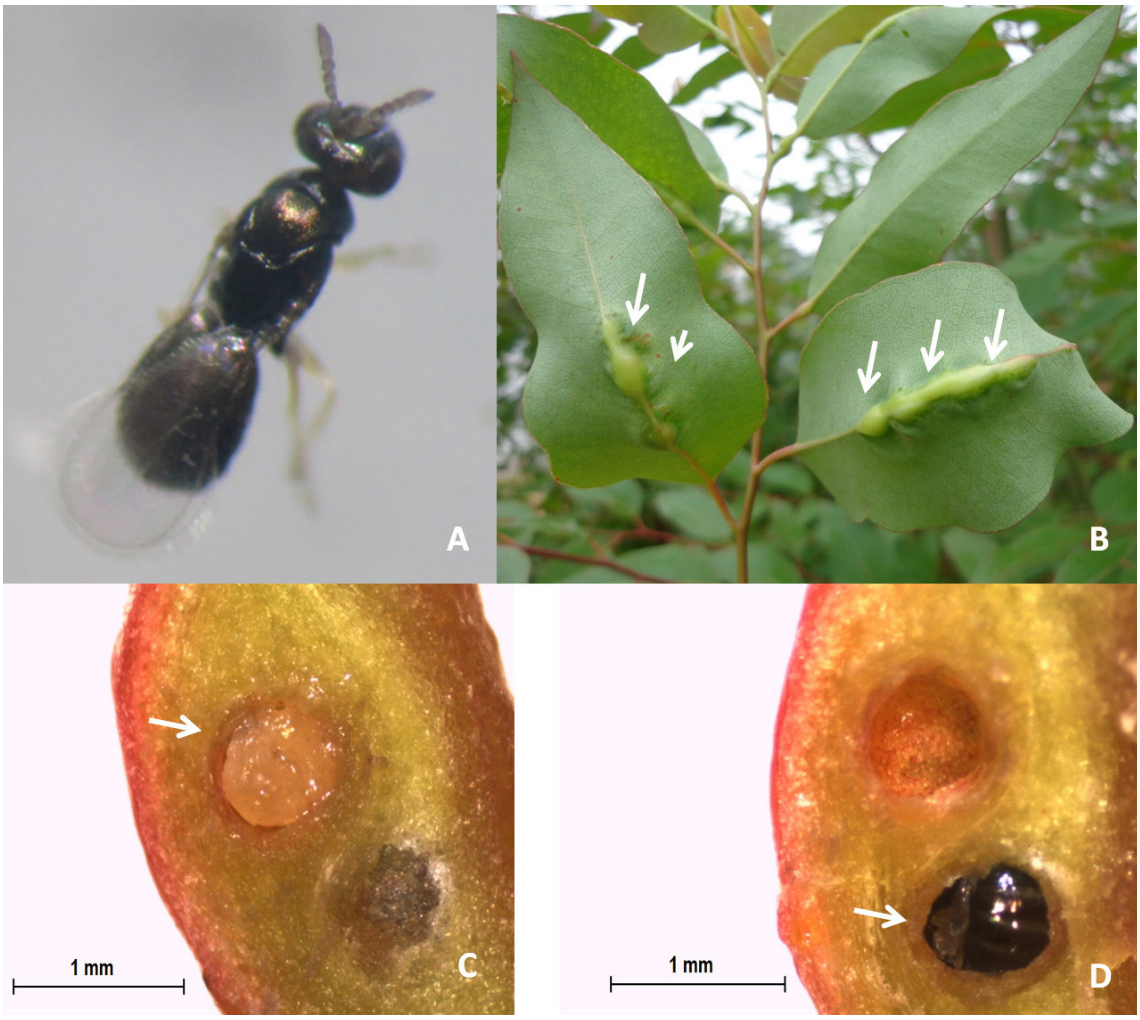

Fig. 1. *Leptocybe invasa*. A. Female. B. Mature galls in midrib and petiole in *E. grandis*. C-D. Horizontal section of *L. invasa* galls. C. Mature larva. D. Pupa.

2016; Kenis *et al.,* 2019). This species causes significant damage to eucalypt plantations and nurseries, due to the aggressiveness of their attack, its rapid dispersal and adaptation to new environments (Zheng *et al.*, 2014; Csóka *et al.*, 2017). The first detection of this species was in Israel in 2000, causing serious damage in young *Eucalyptus camaldulensis* Dehnh (Myrtaceae) plantations (Mendel *et al.,* 2004). Additionally, in the year 2007 it was noticed in the Bahia state from Brazil, and affected hybrid clones of *E. grandis x camaldulensis* (Costa *et al.*, 2008). *Leptocybe invasa* was observed in Uruguay since 2011. The first record of this pest was on red gum urban trees in the city of Montevideo spreading out to Artigas, Paysandú, Rio Negro, Salto, San José, Soriano and Tacuarembó departments (Jorge *et al.,* 2016; Martinez *et al.*, 2019).

Leptocybe invasa adults are small wasps between 1.0-1.4 mm body length, with metallic blue-green color on the thorax and dark brown abdomen, (Mendel *et al.*, 2004) (Fig. 1 A). The asexual reproduction by thelytokous parthenogenesis is the main reproductive method of *L. invasa* because the sex ratio is skewed to females (Do´anlar, 2005). The role of males is unknown, but it is presumed that they would secondarily contribute to the sexual reproduction of the species (Zheng *et al.,* 2018). *Leptocybe invasa* life cycle and annual generations are unknown for the environmental conditions of Uruguay.

The females lay their eggs in the midrib and petioles of the leaves of several *Eucalyptus* spp. (Dittrich-Schröder *et al.*, 2012). After the larva hatch, it induces the gall formation on leaves and branches

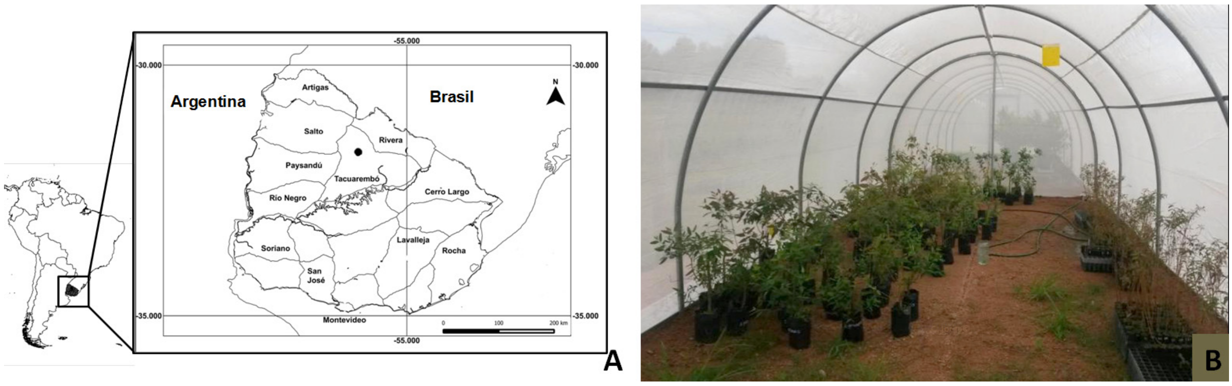

Fig. 2. Study site. A. Locality in Tacuarembó City (black dot). B. Infected plants with *L. invasa.*

in young plants (Fig. 1 B). The entire life cycle occurs inside the gall (Fig. 1 C-D). In addition, they can cause deformations, defoliation, reduction of the photosynthetic area, growth loss (less productivity) and can cause the death in highly susceptible genotypes (Wilcken *et al*., 2015; Jorge *et al*., 2016; Martinez *et al*., 2019). In cases of high infestation and susceptible materials, they can attack branches and trunks in seedlings or young plants, rendering them productively unviable (Mendel *et al*., 2004).

As well as it happens with several species of phytophagous insects the main dispersal path of *L. invasa* is through the transport of attacked seedlings (Liebhold *et al*., 2012; Roques *et al*., 2016; Meurisse *et al*., 2018). Considering that forest cycle production starts in the nursery is important sending healthy plants to the field (Jorge, 2019). For this reason, it is significant to train the nursery staff to detect early symptoms. Most of *L. invasa* signs and symptoms present small size hindering their detection without training.

The galls induced by *L. invasa* are spherical in shape and most cases present in the midrib and petiole of *Eucalyptus* leaves (Figure 1B) (Dittrich-Schröder *et al*., 2012; Jorge *et al*., 2016). According to Mendel *et al.* (2004), four to five stages of maturation of *L. invasa* galls could be distinguished based on their size and coloration. These externally visible gall states are associated with the developmental stage of *L. invasa* larvae within them. Although there is a general pattern of shape, differences in the size, and galls coloration have been observed in the different *Eucalyptus* species or genotypes attacked by this pest (Zheng *et al*., 2014). However, there is no graphic information about the symptoms generated by the wasp in the different species of *Eucalyptus* spp. attacked in the nursery. Therefore, the objective of this work was to qualitatively show the different symptoms caused by *L. invasa* in *Eucalyptus grandis* W. Hill ex Maiden and *Eucalyptus tereticornis* Sm. (Myrtaceae) growing in Uruguayan nurseries.

MATERIALS AND METHODS

Study site

The study was carried out in Tacuarembó city, Uruguay (31º44'26.19"S; 55º58'48.43"W) (Fig. 2 A). This locality has an annual mean temperature of 17.9 ºC and an average rainfall of 1280 mm (INUMET, 2020). During the summer of 2018-2019 some plants of *E. tereticornis* and *E. grandis* were selected and were exposed to *L. invasa.* The choice for this period was because according to Jorge (2019) is the season of the year where adults of *L. invasa* are most abundant in Tacuarembó, Uruguay, and therefore is easier to find females to submit the *Eucalyptus* plants to this pest.

METHODOLOGY

Plant infestation

For this study were selected 25 seedlings of *E. tereticornis* (Santa María nursery) and 25 cuttings of six *E. grandis* clones (LUMIN® nursery). The plants were placed in an open nursery surrounded by mature *Eucalyptus* trees and young eucalypts of 1 m of height with mature *L. invasa* galls (third and fourth stage according to Mendel *et al.* (2004) to promote their natural infestation. They were kept in these environmental conditions for about a month to guarantee the *L. invasa* oviposition. After that, plants were taken to a closed nursery to evaluate the evolution of the eucalyptus gall wasp symptoms (Fig. 2 B).

Fig. 3. Comparison between a seedling with symptoms of *L. invasa* attack (A) and a healthy seedling (B).

Symptoms evaluation

Qualitative evaluations of the morphological changes observed in the plants were carried out weekly after *L. invasa* attack. The symptoms were graphically recorded through photographs. Gall stages were classified according to Mendel *et al.* (2004).

Pictographic symptom guide

In order to register the *L. invasa* galls development, weekly photographs were taken since female oviposition until adult's emergence holds. These pictures were employed to elaborate a pictographic guide with the different symptoms observed in *E. tereticornis and E. grandis studied plants.*

RESULTS

Symptoms of *L. invasa* attack were different in relation to oviposition and development of immature larvae within leaves and trunk tissues for *E. grandis* and *E. tereticornis* (Figs. 3-6).

The first symptoms observed in both *Eucalyptus* species were the scars done by the females during oviposition (Fig. 4 A-C). The oviposition marks made by the females on the midrib were easier to observe than those generated at the petiole and trunk level. The genotypes who present tender leaves exhibited deformation and some perforations in the leaf surface (Fig. 4 C-D). This is probably made with the ovipositor during unsuccessful egg lying events. *E. tereticornis* plants were more attacked by *L. invasa* than those of *E. grandis* studied. That was observed because all red eucalyptus trees presented symptoms of attack by blue gum chalcid wasp. But only some plants of the most susceptible genotypes of *E. grandis* studied were attacked.

For *E. tereticornis* was observed a considerable development of galls, generally larger in size than those in *E. grandis* genotypes (Fig. 6). The highly susceptible genotypes in both species presented galls and deformation on branches, trunk, and leaves (Figs. 5 A, 6 B). Moreover, gall formation causes abnormalities in plants develop. These abnormalities were observed

Fig. 4. *L. invasa* symptoms in the studied plants of *Eucalyptus grandis*. A-C. Scars of oviposition. D-E. Damaged leaves with tissue perforations.

in both *Eucalyptus spp.* and were characterized by a high reduction, loss in dominance on the apical region, lower elongation in the internodes, and overgrowth (Fig. 5-6). A few materials of *E. grandis* were slightly affected with only oviposition marks present.

DISCUSSION

In the latest years, *L. invasa* has been increasing sanitary problems in commercial *Eucalyptus* plantations and nurseries and is considered one of the main pests for this crop worldwide (Csóka *et al.*, 2017; Kenis *et al.*, 2019). The lack of information about its bioecology and indirect symptoms of *L. invasa* attack difficult fast detection and adequate management.

Since the blue gum chalcid wasp develops within the tissues of eucalypts it is difficult to see in the first stages of development, facilitating its dispersal together with the affected seedlings. Trade-in plants and vegetal components are considered the mains means of dispersion and entry of exotic pest insects to the countries (Liebhold *et al.*, 2012; Meurisse *et al.*, 2019). Therefore, it is fundamental to be able to describe the initial symptoms of *L. invasa* attack, and train nursery staff to recognize them and to be able to identify the attacked plants before the field transport.

Although there are several studies worldwide that evaluate the susceptibility of different *Eucalyptus* species to *L. invasa* (Mendel *et al.*, 2004; Dittrich-Schröder *et al.*, 2012; Zhu *et al.* 2012; Luo *et al.* 2014; Petro *et al.*, 2014), there is no graphic material

Fig. 5. *L. invasa* symptoms in the studied seedlings of *Eucalyptus grandis.* A. Deformation and overgrowth, B-C. Overgrowth. D. Unviable seedling: deformation caused by galls in the trunk, defoliation and overgrowth.

Fig. 6. *L. invasa* symptoms in studied seedlings of *Eucalyptus tereticornis*. A. Mature galls. B. Serious damaged plant by the development galls in the trunk. C. Highly overgrowth.

of the initial symptoms and indirect symptoms of the attack of this pest. Red gum trees, such as *E. tereticornis* and *E. camaldulensis* and their hybrids, are considered the most susceptible species to the blue gum chalcid wasp (Jorge *et al*., 2016). In our study we verified that *E. tereticornis* is more susceptible to *L. invasa* than *E. grandis*, because all of them showed attack symptoms. Those genotypes of *E. grandis* that did not present oviposition marks and / or symptoms of the evolution of the *L. invasa* attack, will be studied in the future to establish possible defense mechanisms of *Eucalyptus* against this insect pest. These plant responses suggest we could identify genetic resistance to *L. invasa* attacks. Therefore, being able to determine the physiological effect of *L. invasa* on the growth and development of *Eucalyptus* is vital to establish management strategies based on genetic resistance.

Once formed, the galls are an easily identifiable symptom of *L. invasa a*ttack. However, this pest causes other symptoms that are usually unnoticed during the seedling's classification. Therefore, determining them and producing a graphic record will allow to train people who work in nurseries. Early detection can facilitate the management of this pest.

One of the main factors that affect life cycle length and insect survival is temperature (Bentancourt *et al*., 2009; Gullan & Cranston, 2017). In other countries, it has been reported a greater abundance of *L. invasa* during the warmer months of the year, and consequently high level of attack (Zhu *et al*., 2012). This makes necessary to increase frequency of inspections in the nurseries and field during the hottest months.

CONCLUSIONS

The present work develops basic knowledge about the first symptoms caused by *L. invasa*, such as the oviposition marks and plant deformation. Genotypes with tender leaves exhibited deformation and some perforations in the leaf surface made by the females during the oviposition. This guide will help the personnel who work in the nurseries to identify other symptoms of this pest attack apart from gall formation. At the same time, it is the first step to carry out future studies to generate precise evaluations of the damage caused by this important forest pest and adequate management strategies.

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REFERENCES

Arriaga M.E. 2018. Resultados de la cartografía forestal nacional. DGF-MGAP, 21 pp.

Bentancourt C.M., Scatoni I.B. & E. Morelli. 2009. Insectos del Uruguay. Universidad de la República. Facultad de Agronomía -Facultad de Ciencias. Montevideo, Uruguay. 658 pp.

Cleary M., Oskay F., Do´mus H.T.¸ Lehtijärvi A., Woodward S. & A.M. Vettrain. 2019. Cryptic Risks to Forest Biosecurity Associated with the Global Movement of Commercial Seed. Forests, 10: 459-475.

Costa V.A., Filho E.B., Wilcken C.F., Stape J.L., La Sale J. & L. Teixeira. 2008. *Eucalyptus* gall wasp, *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae) in Brazil. New forest pest reaches the new world. Revista Agricultura, 83: 136-139.

Csóka G., Stone G.N. & G. Melika. 2017. Non-native gall-inducing insects on forest trees: a global review. Biological Invasions, 19(11): 3161-3181.

DIEA (Dirección General de Estadísticas Agropecuarias). 2019. Anuario Estadístico Agropecuario Access in: 15/8/2020. *In:* https://descargas.mgap.gub.uy/DIEA/Anuarios/Anuario2019/Anuario2019.pdf.

Dittrich-Schröder G., Wingfield M.J., Hurley B.P. & B. Slippers. 2012. Diversity in *Eucalyptu*s susceptibility to the gall-forming wasp *Leptocybe invasa*. Agricultural and Forest Entomology, 14 (1): 419-427.

Do´anlar O. 2005. Occurrence of Leptocybe invasa Fisher & La Salle, 2004 (Hymenoptera: Chalcidoidea) on *Eucalyptus camaldulensi*s in Turkey, with a description of the male sex. Zoology in the Middle East, 35: 112-114.

Garnas R.J., Auger-Rozenberg M., Roques A., Bertelsmeier C., Wingfield M.J., Saccaggi D.L., Roy H.E. & B. Slippers. 2016. Complex patterns of global spread in invasive insects: eco-evolutionary and management consequences. Biological Invasions, 18: 935-952.

Gullan P.J. & P.S. Cranston. 2017. Insetos Fundamentos da Entomologia. Editorial ROCA, 5ª Ed., Barcelona. 460pp.

Hurley B.P., Garnas J., Wingfield M.J., Branco M., Richardson D.M. & B. Slippers. 2016. Increasing numbers and intercontinental spread of invasive insects on eucalypts. Biological Invasions, 18: 921-933.

INUMET (Instituto Nacional de Metereología). 2020. Clima. Access on line: https://www.inumet.gub.uy/clima. *In:* 28/08/2020.

Jorge C. 2019. "Ecología de *Leptocybe invasa* (Hymenoptera: Eulophidae) e seus parasitoides e simbiontes em *Eucalyptus*". Tesis de Doctorado en Ciencias Forestales. Faculdade de Ciências Agronômicas - Câmpus de Botucatu, Universidade Estadual Paulista "Júlio de Mesquita Filho".

Jorge C., Martínez G., Gómez D. & M. Bollazzi. 2016. First record of the eucalypt gall-wasp *Leptocybe invasa* (Hymenoptera: Eulophidae) from Uruguay. Bosque (Valdivia), 37(3): 631-636.

Kenis M., Hurley B.P., Colombari F., Lawson S., Sun J., Wilcken C., Weeks R. & S. Sathyapala. 2019. Guide to the classical biological control of insect pests in planted and natural forests, Forestry Paper No. 182. Rome, FAO. 96 pp.

Liebhold A.M., Macdonald W.L., Bergdahl D. & Mastro V.C. 1995. Invasion by Exotic Forest Pests: A Threat to Forest Ecosystems. Forest Science Monographs, 30: 49 p.

Liebhold A.M., Brockerhoff E.G., Garrett L.J., Parke J.L. & K.O. Britton. 2012. Live plant imports: the major pathway for forest insect and pathogen invasions of the US. Frontiers in Ecology and Environment, 10:135-143.

Ligrone A. 2018. La ley primera: A 50 años de la primera Ley Forestal y en el cierre de un año particular para el sector, es tiempo de balances. Pasado, presente y futuro de la forestación en Uruguay. Revista Forestal, 8: 2-3.

Luo J., Arnold R., Lu W. & Y. Lin. 2014. Genetic variation in *Eucalyptus camaldulensis* and *E. tereticornis* for early growth and susceptibility to the gall wasp *Leptocybe invasa* in China. Euphytica, 196: 397-411.

Martínez G., Jorge C., Escudero P., Martínez-Haedo J., de los Santos M. & R. Scoz. 2019. Hacia un programa de control biológico de la avispa agalladora del eucalipto. Revista INIA, 56 (1): 75-78.

Mendel Z., Protasov A., Fisher N. & J. Lasalle. 2004. Taxonomy and biology of *Leptocybe invasa* gen. & sp. n. (Hymenoptera: Eulophidae), an invasive gall inducer on *Eucalyptu*s. Australian Journal of Entomology, 43(2): 101-113.

Meurisse N., Rassati D., Hurley B.P., Brockerhoff E.G. & R.A. Haack. 2019. Common pathways by which non-native forest insects move internationally and domestically. Journal of Pest Science, 92:13-27.

Morrow P.A., Whitham T.G., Potts B.M., Ladiges P., Ashton D.H. & J.B. Williams. 1994. Gall-forming insects concentrate on hybrid phenotypes of *Eucalyptus*. *In:* Price PW, Mattson W.J., Baranchikov Y.N. (Ed). The ecology and evolution of gall-forming insects. pp. 121-134, St. Paul, MN.

Paine T.D., Steinbauer M.J. & S.A. Lawson. 2011. Native and Exotic Pests of *Eucalyptus*: A Worldwide Perspective. Annual Review of

Entomology, 56: 181-201.

Petro R., Madoffe S.S. & S. Iddi. 2014. Infestation density of eucalyptus gall wasp, *Leptocybe invasa* Fisher and La Salle (Hymenoptera: Eulophidae) on five commercially grown Eucalyptus species in Tanzania. Journal of Sustainable Forest, 33 (3): 276-297.

Roques A., Copeland R.S., Soldati L., Denux O. & M.A. Auger-Rozenberg. 2016. *Megastigmus* seed chalcids (Hymenoptera, Torymidae) radiated much more on Angiosperms than previously considered. I- Description of 8 new species from Kenya, with a key to the females of Eastern and Southern África. ZooKeys, 585: 51-124.

Shorthouse J.D., Wool D. & R. Anantanarayanan. 2005. Gall-inducing insect Nature´s most sophisticated herbivores. Basic and Applied Ecology, 6: 407-411.

SPF (Sociedad de Productores Forestales). 2020. Uruguay Forestal: Principales cifras del sector. Access: 15/08/2020, in: http://www.spf.com.uy/uruguay-forestal/

Wilcken C.F., Zaché B., Masson M.V., Pereira R.A., Barbosa L.R.& J.C. Zanuncio. 2015. A vespa-da-galha-do-eucalipto, *Leptocybe invasa* Fisher & La Salle. *En:* Vilela EF, Zucchi RA (eds) Pragas Introduzidas no Brasil. Insetos e ácaros. Pp 835-844. Fealq, Piracicaba.

Wingfield M.J., Slippers B., Hurley B.P., Coutinho T.A., Wingfield B.D. & J. Roux. 2008. Eucalypt pests and diseases: Growing threats to plantation productivity. Southern Forests, 70: 139-144.

Zheng X.L., Huang Z.Y., Li J., Yang Z.D., Yang X.H. & W. Lu. 2018. Reproductive Biology of *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae). Neotropical Entomology, 47: 19–25.

Zheng X.L., Li J., Yang Z.D., Xian Z.H., Wei J.G., Lei C.L., Wang X.P. & W. Lu. 2014. A Review of Invasive Biology, Prevalence and Management of *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae: Tetrastichinae). African Entomology, 22(1):68-79.

Zhu F., Ren S., Qiu B., Huang Z. & Z. Peng. 2012. The abundance and population dynamics of *Leptocybe invasa* (Hymenoptera: Eulophidae) galls on Eucalyptus spp. in China. Journal of Integrative Agriculture, 11: 2116-2123.

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