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Leptoglossus occidentalis damages on stone pine female reproductive structures

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Abstract. Stone pine (*Pinus pinea*) is an important forest species in, generating several ecological and economic benefits, particularly with fruit production (pine nuts). The detection of *Leptoglossus occidentalis* in since 2010 seems to be related to a significant decrease in pine cones production and productivity despite the difficulty to detect and quantify its damages. *L. occidentalis* is a sucking insect which feeds by inserting its stylet between the cone scales to reach the developing ovules of several conifer species. In order to understand which stone pine reproductive structures can be affected by the different instars of *L. occidentalis,* measurements were made on the insect stylet, body length and distances between the ovules and cone surfaces from flowering to cone maturation (1st, 2nd and 3rd year cones). The results show that *L. occidentalis* biological cycle overlap with all reproductive structures of stone pine. All development stages of *L. occidentalis* can damage the ovules, except the 1st instar on the 3rd year cones. In the 2nd year cones, cellular damages were also observed in the interior cone scale surface between scales. The stylet length and distance between the ovules and cone surface are determinant factors to allow insect feeding.

Keywords. *Pinus pinea* – Western conifer seed bug – Ovule damages – Stylet length.

I – Introduction

Stone Pine (*Pinus pinea*) is an important forest species in generating relevant economic benefits, particularly through pine nut production. In the latest years an increasing of pests and diseases affecting cones (Sousa *et al*., 2014) was detected demanding the study of its interactions with the stone pine reproductive phenology. Data collected from the main industries (Pimpão, 2014) revealed that in the latest years, cone production and pine nut productivity have dropped to alarming values. The cone production in the 2010-2011 campaign was about 120 million kg, whereas in 2011-2012 was reduced to more or less 25 million kg. The pine nut productivity rounded about 3.5-4% and decreased in the two consecutive campaigns in 2010/2011 (3.3%) and 2011/2012 (2.5%). At the same time, in the field, an increasing number of aborted 1st and 2nd year cones were also detected and were suspectedly related to pests, namely *Leptoglossus occidentalis* (Sousa *et al*., 2012).

L. occidentalis is a sucking insect native to which feeds on seeds of various species of the genus *Pinus* and other conifers (McPherson *et al.*, 1990; Bates *et al.*, 2000a; Strong *et al.*, 2001, Bates and Borden, 2005). The species was first reported in Italy (1999) and in Portugal in 2010 (Bernardinelli and Zandigiacomo, 2001; Sousa and Naves, 2011). Currently it seems to be distributed throughout Portugal and it is usually present in *P. pinea* stands. Several methods have been tested to control the species but an effective method has not been found yet. *L. occidentalis* feeds on developing seeds by inserting its mouthparts between cone scales and affected cones do not show any external damage symptoms (Bates *et al.*, 2000*b*; Strong *et al.*, 2001). The aim of this study is to evaluate *L. occidentalis* feeding capacity in all phenological phases of stone pine female reproductive structures.

II – Material and methods

Measurements were made on the insect stylet and body length (nymphs and adults) and distances between the ovules and cone surfaces from flowering to cone maturation (1st, 2nd and 3rd year cones). The stylet and body length of 5 insects for each 5 nymphal and adult stages were measured using a digital caliber.

For the measurements of the distances between the ovules and cone surface, a field plot with ten trees located in Santa Suzana (Sintra council) originated from natural regeneration was selected (38º 55' 23.2" N, 9º 22' 46.5" W; 90-100m a.s.l.). Cone samples of 1st, 2nd and 3rd year were weekly and randomly collected from 19th November 2012 to 21st June 2013 (3 cones per stage). Samples of reproductive structures from the different phenological stages were fixed, during at least 48 hours, in FAA 1:1:18 - formalin: acetic acid: ethanol (70%) at 4 °C (Johansen, 1940; Ruzin, 1999). Dehydration was achieved through progressive ethanol/water series and finally included in paraffin. Histological sections (healthy and damage ovules) of 10µm thickness were obtained using a rotary microtome (Leica RM2255) and stained with Heidenheim Hematoxylin. Image acquisition was performed using a Digital Microscope Leica© DMS1000 with LAS (Leica Application Suite) V4.4 software. Measurements of the distances from the cones surface to ovules (5 ovules/cone) were performed using the software scale bar and ImageJ software in irregular distances.

III – Results and discussion

1. Stylet and body length of *L. occidentalis* development stages

Data from body and stylet length of the five nymphal and adult stages show that stylet length increases by 2.5x from 1st to 2nd instar (2.9 mm to 7.2 mm), then increasing slowly to adult stage (12.3 mm). Body length grows at low rate until 2nd instar, doubling in the 3rd instar and rising slowly until 5th instar, increasing again at adult stage to 16.9 mm (Fig. 1).

The ratio stylet/body length at 2nd instar is 1.74 (the stylet is longer than the body), decreasing until 0.73 at adult stage (stylet shorter than body) (Figs. 1 and 2).

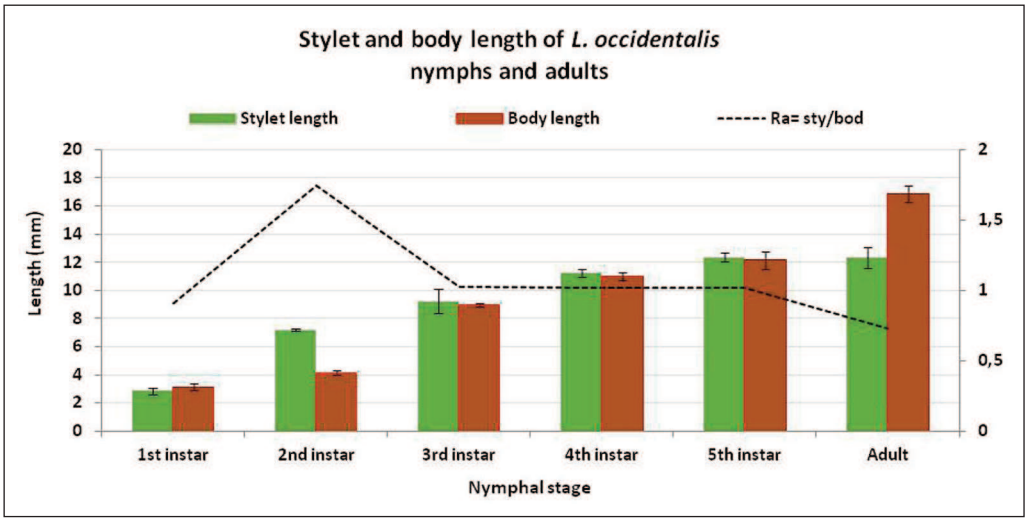

Fig. 1. Stylet and body length (mm), ratio (Ra) (stylet/body) and corresponding Standard Deviation in all *L. occidentalis* development stages.

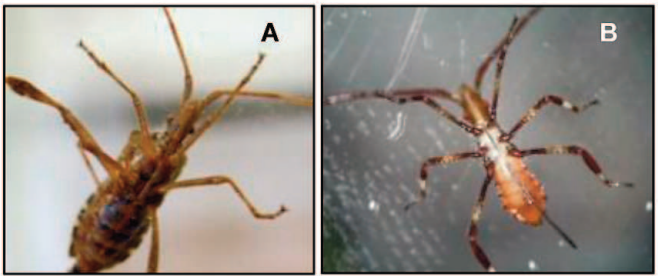

Fig. 2. ***Leptoglossus occidentalis*****. A - 2nd instar nymph where stylet length (7.2 mm) (curved in the image) exceeds body length (3.9 mm). B - Adult of** ***L. occidentalis*** **where stylet (12.0 mm) is shorter than body (16.1 mm).**

2. Distance from cones surface to ovules

Distance between the cone surface and ovule had usually irregular shape and varied according to cone stage. In the 1st year cones, distance varied from 0.3 to 2 mm, in 2nd year cones from 2 to 2.75 mm. The 3rd year cones remained quiescent (2.75-2.9 mm) until the begin of growth in March, when distance between ovules and cone surface increased from 2,9 to 6,9 mm (Fig. 3).

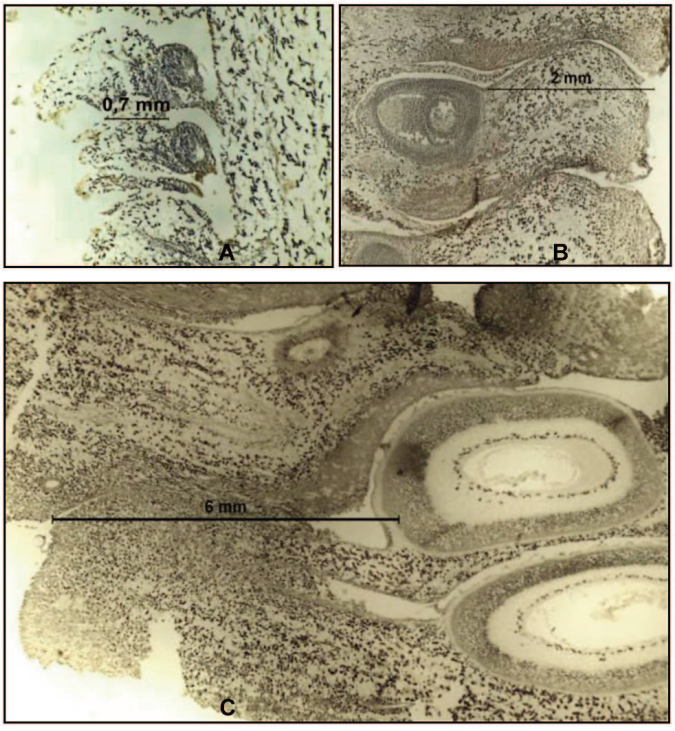

Fig. 3. Distance between the cone surface and the ovules for the 1st, 2nd and 3rd year cones collected in 11th May (2013). Measurements of real distances were performed through ImageJ software. A - 1st year 0.85 mm; B - 2nd year 2.5 mm; C - 3rd year 6 mm.

3. Relation between insect feeding and host damages

The relation between *L. occidentalis* stylet length and the distance from the ovules to the cone surface show that all nymphal stages are able to insert its stylet and suck the content of all cone stages (1st, 2nd and 3th year cones), except the 1st instar nymphs that cannot reach the ovules of the 3rd year cones (Fig. 4).

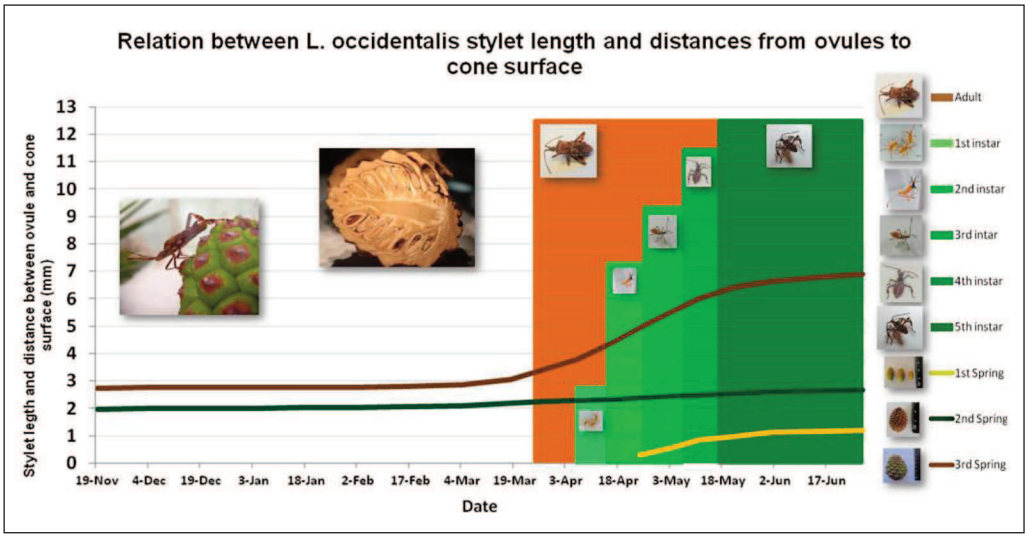

Fig. 4. Stylet length of all *L. occidentalis* stages and distance from ovules to cone surface for the 1st, 2nd and 3rd year cones.

4. Damages in reproductive structures

Internal damages were detected on 2nd year cones (11th May and 1st June 2013) (Fig. 5). Comparison of ovules with and without damage showed ovule cell degradation (Fig. 5B) and cellular damages in the gap between cone scales (Fig. 5C), compatible with *L. occidentalis* that inserted its mouthpart between cone scales to reach the ovules. However, there is uncertainty about the causing agent of ovule and cone scale damages, since cones were collected from the field. These damages are not externally detectable and makes difficult to recognize affected and unaffected cones.

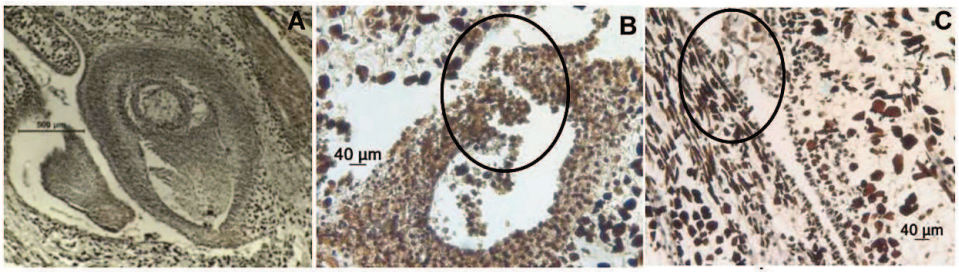

Fig. 5. Cone longitudinal sections where damages were detected in 2nd year cones. A - Developing ovule without damage (11th May 2013); B - Developing ovule with damage (11th May 2013); C - Cone scale surfaces with cellular damage (1st June 2013).

IV – Conclusions

This study reveals that *L. occidentalis* biological cycle overlaps with the development of all stone pine reproductive structures, having the capacity to feed on developing seeds (except the 1st instar on the 3rd year cones). Damages in developing ovules compatible with *L. occidentalis* feeding activity were also detected, suggesting that *L. occidentalis* can cause a significant decrease in cone production and productivity. Further studies are required in order to gather more information about the interaction between the host and this agent.

References

Bates S.L. and Borden J.H., 2005. Life table for *Leptoglossus occidentalis* Heidemann (Heteroptera: Coreidae) and prediction of damage in lodgepole pine seed orchards. In: *Agric For Entomol.,* 7, pp. 145-151.

Bates S.L., Borden J.H., Kermode A.R. and Bennett R.G., 2000a. Impact of *Leptoglossus occidentalis* (Hemiptera: Coreidae) on Douglas-fir seed production. In: *Journal of Economic Entomology,* 93, pp. 1444-51.

Bates S.L., Lait C.G., Borden J.H. and Kermode A.R., 2000b. Effect of feeding by the western conifer seed bug, *Leptoglossus occidentalis* (Heteroptera: Coreidae), on the major storage reserves of developing seeds and seedling vigor in Douglas-fir. In: *Tree Physiology,* 21, pp. 481-487.

Bernardinelli I., Zandigiacomo P., 2001. *Leptoglossus occidentalis* Heidemann (Heteroptera, Coreidae): a conifer seed bug recently found in northern Italy. In: *Journal of Forest Science,* 47(2), pp. 56-58.

Johansen D., 1940. Plant microtechnique. New York: McGraw Hill Book. New York; London: McGraw-Hill Book Company, Inc., 523 p.

McPherson J.E., Packauskas R.J., Taylor S.J. and O'Brien M.F., 1990. Eastern range extension of *Leptoglossus occidentalis* with a key to *Leptoglossus* species of America North of Mexico (Heteroptera: Coreidae). In: *The Great Lakes Entomologist,* 23, pp. 99-104.

Pimpão M., 2014. *Leptoglossus occidentalis*: Bioecologia e previsão de impacte económico em Portugal. Master thesis. ISA, Lisbon.

Ruzin S., 1999. Plant Microtechnique and Microscopy. Oxford, New York: Oxford University Press. May 20th 1999, 322 p.

Sousa E. and Naves P., 2011. The western conifer seed bug *Leptoglossus occidentalis* Heidemann, 1910 (Heteroptera: Coreidae) in Portugal. In: *Boletin de Sanidad Vegetal – Plagas,* 37(1), pp. 65-67.

Sousa E., Ferreira C., Pimpão M., Naves P., Valdivieso T. and Varela C., 2012. Sanidade dos povoamentos de pinheiro manso em Portugal: Valorização da Fileira da Pinha/Pinhão. [online] [Consulted in June 2016]. National Institute for Agricultural and Veterinary Research I.P. Alcácer do Sal Municipal Auditorium, September 18th 2012. http://www.unac.pt/attachments/162_03_Edmundo%20Sousa%20-%20INIAV.pdf.

Sousa E., Pimpão M., Valdivieso T., Naves, P., Santos L., Godinho-Ferreira P., Varela C., 2014. New data about biotic factors that affect stone pine production in Portugal. Medinsect 4: Entomological research in Mediterranean ecosystems. IUFRO-Working Group 7-03-14. Antalya (Turkey), 9-14 April, 2014.

Strong W.B., Bates S.L. and Stoehr M.U., 2001. Feeding by *Leptoglossus occidentalis* (Hemiptera: Coreidae) reduces seed set in lodgepole pine (Pinaceae). In: *Canadian Entomologist,* 133, pp. 857-865.