

FINAL REPORT

Reference: COST-ONLINESTSM-FP1203-21557

Period: 2015-02-16 to 2015-02-27

STSM type: regular (from Portugal to France)

STSM Applicant: Ana Cristina Oliveira Farinha

STSM Topic: The western Conifer Seed Bug, *Leptoglossus occidentalis*: important methodologies and techniques

Host: Alain Roques, Institute for Agricultural research, Orléans (FR), alain.roques@orleans.inra.fr

I. Background and Purpose

Leptoglossus occidentalis, a serious pest of conifer seeds, native from North America, was introduced in Europe, via Italy, in 1999. Both, nymphs and adults of this species, feed on cones of different conifers, digesting the contents of developing seeds. *L. occidentalis* has already been considered a significant pest in Italy and France. Yet, there is still a substantial lack of knowledge on its biology, ecology and impact on *Pinus pinea*.

The exchange of knowledge within the Mediterranean countries, where the pinion industry has a great economic value, is essential and the most effective way to find a solution to control this bug.

The main objectives of this STSM were:

1. Learn all about rearing *Leptoglossus occidentalis* in laboratory conditions (all year populations) and in semi-natural conditions as well as learn and develop methodologies of behavior studies;
2. Learn the X-ray technique to evaluate *L. occidentalis* damage in seeds. Optimize the protocol to *Pinus pinaster* and *P. pinea* seeds; Assess damage in 2nd and 1st year cones.
3. Establish a strong collaboration with the Forest Zoology research group of INRA, with the scope of broadening the existing knowledge on this important pest and finding methods of control.

II. Description of the work carried out during the STSM

Rearing L. occidentalis

Researcher Annie Yart show me the room where all colonies were kept and explain all about the rearing process: temperature, humidity, type of cage, type of food, types of hosts. During my stay at INRA Orléans I was the responsible for maintaining the colonies (Figure 1 A). Consequently, I could learn how to maintain an all year colony. I was responsible to check if all the rearing conditions were ok, count the dead, change the food source, clean the cages and supply adults for experiments.

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Characterization of the damage caused by L. occidentalis in seeds of P. pinea

In order to use the X-ray technique to estimate *L. occidentalis* damage on pinions first is imperative to characterize and categorize this damage over time. This same characterization has already been made for *Pinus sylvestris*, *P. nigra*, *Larix decidua*, *Pseudotsuga menziesii*, *Picea alba* and *Abies bornmulleriana* by Alain Roques research team. The process would only have to be optimized for *P. pinea* and to *P. pinaster*. To do that a sample of 500 seeds were X-rayed and completely sound seeds were put aside (Figure 1 B).

A trial of exposition of this sound seeds to *L. occidentalis* was conducted from the day one of my stay (Figure 1 C, D,E).

Eight boxes each with ten sound seeds of *P. pinea* were set. In four of the boxes was added four adults of *L. occidentalis* and the other four only had the seeds and no bug (control).

Besides boxes with *P. pinea* seeds two boxes with *P. pinaster* seeds were also set. One had twenty sound seeds of *P. pinaster* and four bugs and the other the same number of seeds and no bugs (control).

All boxes were of the same size and to all it was added a fresh branch to maintain a good humidity level (previous studies mentioned in the begging of this task with other seeds had shown that without the branch the mortality of the bug was high). Whenever any individual died it was replaced by another.

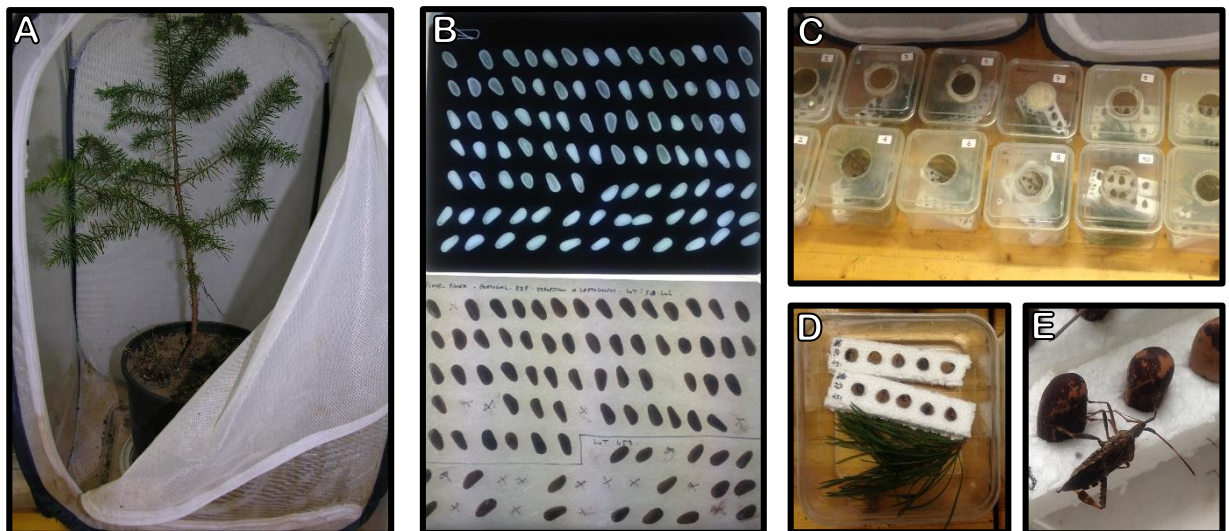


Figure 1. (A) Rearing cage, (B) selection of sound seeds of *P. pinea* for the trial of exposition of the seeds do *L. occidentalis*, (C) trial boxes, (D) trial box with *P. pinea* seeds and (E) zoom on a box with *P. pinea* seeds where is visible an adult of *L. occidentalis* feeding on a seed.

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Estimation of the damage caused by L. occidentalis in seeds of P. pinea from forest stands of Portugal

The characterization of the damage caused by *L. occidentalis* carried out in the previous task allows us to estimate this bug damage in pine nuts from samples collected in the field resorting to the same technique – the x-rays.

A random sample of 100 seeds (whenever possible) from each plot in each site was submitted to x-ray (Table 1). A total of 2093 seeds were x-rayed.

Table 1. Sample of seeds of *P. pinea* from forest stands submitted to X-ray.

District	Site	Plot	Number of seeds to x-ray
Setúbal	Maria Moita	1	87
		2	100
Setúbal	Herdade da Palma	1	100
		2	100
		3	100
Setúbal	Sta Margarida do Sado	1	60
		2	60
Santarém	Abegoaria	1	113
		2	100
Santarém	Herdade da Espirra	1	85
		2	100
		3	100
Santarém	Vale Mouro	1	145
Santarém	Seed orchard of Coruche	1	40
		2	40
Santarém	Casa de Bragança	1	163
Santarém	Machoqueira do Grou	1	100
		2	100
		3	100
		4	100
		5	100
		6	100
Total		22	2093

III. Description of the main results obtained

Characterization of the damage caused by L. occidentalis in seeds of P. pinea

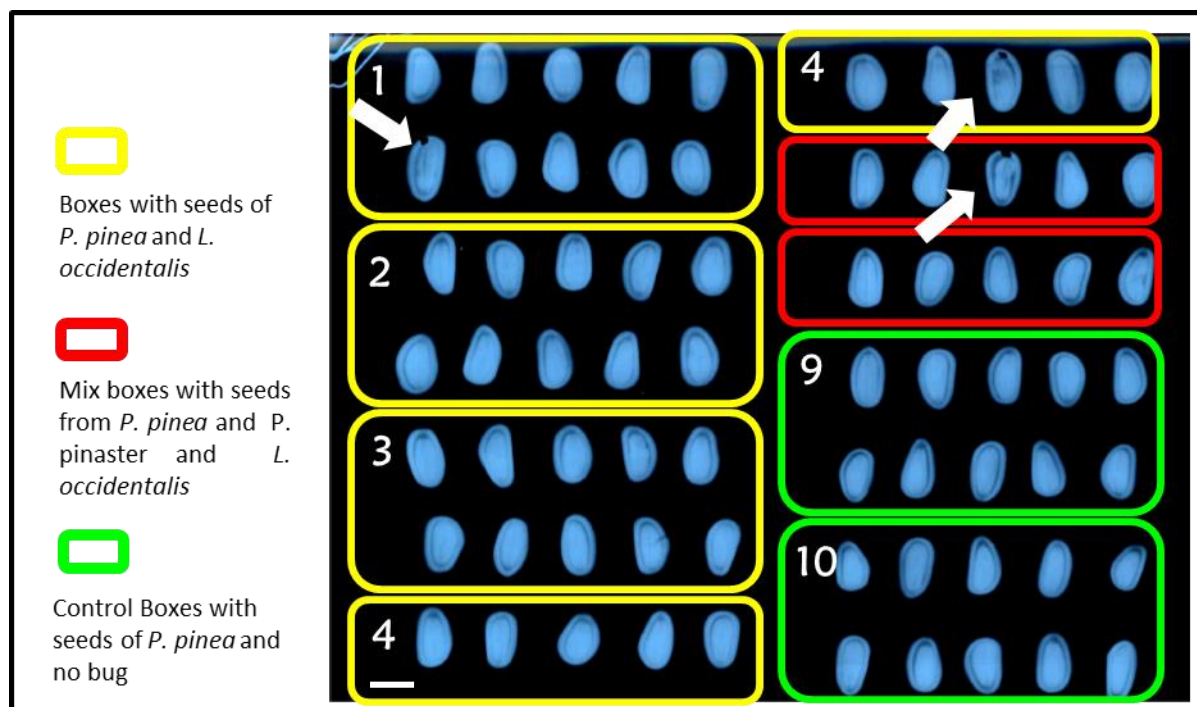
This pilot study allow us to understand better the damage made by *L. occidentalis* on stone pine seeds. Weekly X-rays on the seeds exposed to *L. occidentalis* enable us to follow the damage that can be assigned to this bug (Figure 2). Control seeds were important to track possible changes in seeds over time or related to the exposition to x-rays that would not be due to the action of the bug. These seeds remained intact throughout the experiment not showing any damage.

Seeds, both *P. pinea* and *P. pinaster*, exposed to *L. occidentalis* presented damage throughout the experiment.

This experiment is still ongoing at INRA Orléans under the responsibility of Annie Yart.

Pinions from Stone pine submitted to *L. occidentalis* and to x-ray will now be open and the seed will be characterized and photographed in order to elaborate a table of correspondences between damage assessed by x-ray and damage assessed by direct observation of the seed. This work is being conducted now.

Figure 2. X-ray to seeds of *P. pinea* and *P. pinaster* exposed to *L. occidentalis*. Results after 5 days of exposure. Arrows point to damage made by *L. occidentalis*. Scale bar - 10 mm.

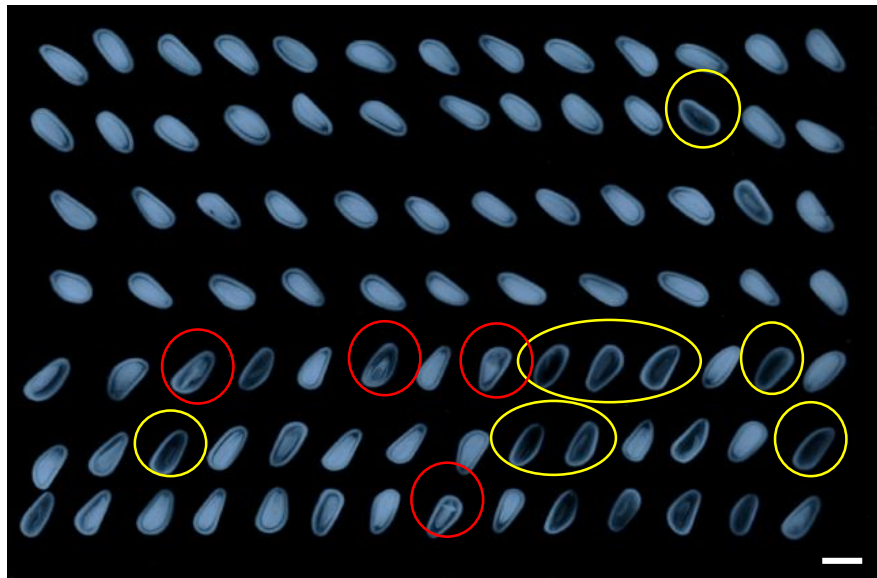


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*Estimation of the damage caused by *L. occidentalis* in seeds of *P. pinea* from forest stands of Portugal*

A sample of seeds from all plots was x-rayed and the seeds were characterized (Figure 3). Results from this task are being processed now with a photographic software which allow us to measure the percentage of the seed that is damaged and so estimate the average damage from each plot and site. The characterization of the damage caused by *L. occidentalis* obtained in the previous task was key for calibrating the software and not get overestimation of the damage related to this insect.

Figure 3. X-ray on a sample of *P. pinea* seeds from Abegoaria: Yellow circles - Damage not due to *L. occidentalis*; Red circles – damage possible due to *L. occidentalis*. Scale bar - 10 mm.



Assess damage in 2nd and 1st year cones revealed to be a challenging goal. X- Ray technique proved not to be suitable to assess damage in young cones due to its large dimensions and opacity. There was also an attempt to isolate the small seeds that are still in development in order to submit them to x-ray but these has also been unsuccessful and very hard to do. New ways to assess damage in young cones must be thought out.

IV. Future collaboration with host institution and foreseen publications

The collaboration with INRA Orléans and all its researchers was a rich and full experience. In fact it is still ongoing with the experience of the seeds exposed to *L. occidentalis*. Besides this experience new exchange of knowledge and experience was also a guarantee. An idea on a molecular approach to my PhD project

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was discussed in Orleans and will hold true this year. The objective will be to study if the populations of *L. occidentalis* present in Europe which were found to be originate from multiple introductions in Europe, also display different ecological and biological traits related with host preference, thermic preferences and thermic tolerances. A publication in this matter is one of the objectives within the PhD project.

The experience with seeds exposed to *L. occidentalis* will be repeated in order to have more replications and allow for a strong and reliable characterization of the damage thought time. A joint publication is expected at the end.

Results from the last point studied in this STSM, “Estimation of the damage caused by *L. occidentalis* in seeds of *P. pinea* from forest stands of Portugal” will contribute to better discriminate and quantify damage related only to this pest and therefore estimate its economic impact in Portugal.