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# Efficacy of Three Insecticides in the Control Gall Wasp *Leptocybe invasa* in *Eucalyptus urograndis* Seedlings

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**Abstract:** Forestry expertise and plantations managers are struggling to find cheaper and sustainable solutions to contain the losses caused by *Leptocybe invasa* in the last nine years on the forest stands in Mozambique. Aiming to help find a solution in the control of *L. invasa* early in the nursery, we conducted an experimental trial at the Niassa Forestry company nursery, located in the Niassa province, northern Mozambique in February 2015. Three insecticides: Acetamiprid, Thiamethoxam, Imidacloprid, with and without adherent and pH regulator were tested. The experiment had seven treatments including the control. The number of seedlings infested by the *gall wasp* were assessed 15, 30, 45 and 60 days after the seeds were sown in the nursery. Data were analysed in R package. Normality and homogeneity of variances were tested through Shapiro-Wilk and Bartlett's tests respectively. Analysis of Variance (ANOVA) and parametric means test (Tukey-HSD), were used to assess whether there was difference among the treatments. Results showed difference between treatments at 0.01% of significance after 15, 30 and 45 days and at 5% in the 60 days after sowing. With less seedlings infested by the gall wasp, Imidacloprid with and without adherent was almost superior compared to all other pesticides in all assessment. The use of adherent and pH regulators negatively affected the performance of insecticides, except in the Imidacloprid. This study findings should not however, be overall generalized, instead, more research can be conducted to verify the consistency of these results before being widely implemented.

**Keywords:** *Eucalyptus* Seedlings, Planted Forest, Insecticides and *L. Invasa*

## 1. Introduction

*Eucalyptus* plantations can provide many benefits locally and globally. These benefits include rural employment, equipping local people with new skills e.g. in managing seedlings production, planting, fertilizing, pruning, thinning, harvesting and products marketing. The plantations provide timber and non-timber products for the internal and external markets, [1,2]; The eucalyptus plantations can also maximize the use of marginal land with little or no agricultural value, contribute to local development of roads, communications, services, houses, shops, schools, health services and other infrastructure. All these goods and services are important to improve the well-being of the poor rural householders. Plantations can also enhance environmental, cultural and aesthetic ecosystem services [3], which can contribute to improve well-being and business opportunity as well as increase tax revenue for the governments [4]. In Mozambique, the National government and private organizations, started promoting commercial monoculture forest plantations since 2005. Environmental and growth conditions were suitable for the establishment of fast growing of exotic species such as pines and eucalyptus [5, 6]. Even though, most companies operating in Niassa managed to establish their fast growth *Eucalyptus* plantations, the majority of these stands were being affected by *L. invasa*. The physical and economic losses were huge after damage by *L. invasa* was discovered. Local forest companies', research institutes and private entities are struggling to find reliable and sustainable solutions, that could potentially minimize the current losses. Both *L. invasa* [7] and plantation fires are the major threats to the success of establishment of forestry projects in Mozambique [6, 8].

*L. invasa* Fisher & La Salle (Hymenoptera: Eulophidae), is not only a local problem, but also global, [9, 10]. Until 1999, *L. invasa* was unknown in the world, it was first discovered in the Middle East and the Mediterranean region in 2000 [2]; one year later was reported in India [11]. According to the Food and Agriculture Organization, (FAO, 2007[13]), the pest has currently spread through all the regions of the globe: Africa, Asia and the Pacific, Europe, Latin America, the Caribbean, Middle East and North America, displaying high natural dispersal ability throughout the areas where it has been introduced [11], which is believed to be related to its reproduction biology and intra-specific variation [10]. Fernandes and Carneiro, (2009), [14] pointed out that the *gall wasp* is capable of modifying the growth patterns of the host plant and altering the nature of plant tissue, which impairs its development and can lead to the plant death.

*L. invasa* was first reported in Mozambique by Chirinzane et al., (2014), [15] in 2011, ten years after being discovered in India. But the first outbreak was reported in the Niassa Province in October 2013, after the first cluster of *E. urograndis* trees attacked was detected in Icuve, Naconda and Mussa plantations in Chimbonila district. Later in February 2014, it was also detected in the nursery in the seedlings of E. *urograndis, E. urophylla* and *E. pellita* [7]. So far, it is estimated that the pest has affected roughly 6000 hectares of old and newly established stands in Niassa Province. Garlet et al. (2013), [16], observed that the pest can destroy up to 90% of plantations, causing huge losses.

These and other facts highlight the importance of finding fast and suitable control strategies through research and experiments. Biological control is probably the most sustainable and environmentally friendly solution to manage an alien pest over large plantation areas [17]. Biocontrol strategies have not yet been tested in Mozambique and it require a long time for relevant and conclusive results to be obtained despite reports, Chirinzane et al., (2014), [15] of the parasitoid *Megastigmus sp.* (Torymidae) occurrence. Urgent solution is highly recommended and needed to contain the devastating effects of the pest, that is why, in 2015, a research experiment was condacted in the Niassa Forestry Company nursery, to test the efficiency of three types of pesticides (Imidacloprid, Acetamiprid and Thiamethoxam), in the control of *L. invasa* in early the stages of nursery seedling growth. These three insecticides (Acetamiprid, Imidacloprid, Thiamethoxam), have been tested in several occasions elsewhere in attempt to control the gall wasp, with promising results [3]. The same insecticides were also recommended provisionally by National Services of Health and Agri-Food Quality of Argentina to combat *L. invasa* http://www.senasa.gob.ar/normativas/resolucion-180-2012, on its resolution180/2012, and are all allowed under Mozambican national legislation. See for instance the list of pesticides registered in the Ministry of Agriculture and Food Security [18].

# 2. Methodology

## *2.1. Site Location and Trial Establishment*

On 19th February 2015 an experimental trial was established and conducted in the main nursery of the Niassa Forests Company, located in Litunde at the coordinates 13º19'38.29" S 35º46'09.43" E, approximately 80km from Lichinga City, the capital of Niassa province, in Northern Mozambique.

Only one clonal species of Eucalyptus (*E. urograndis)* was used to establish the experimental trial. At the time of its establishment, the seedlings had only 40-days after being transplanted in the nursery. The trial consisted of 6 treatments and one control, namely: three types of pesticides, Acetamiprid with 222g/l of active ingredient; Imidacloprid, with 200g/l of active ingredient and Thiamethoxam with 250g/kg of active ingredient, with and without adherent (Weltall), and the control treatment with neither pesticide no adherent, (See all details in table 1).

The trial was established in a Complete Randomized Block Design (RCBD) with 5 replications, each repetition was composed of three boxes containing 98 seedlings. In total, fifteen boxes with 98 seedlings per treatment were established, summing up 1470 observations (see table 1 and figure 1). The distance between treatments was precisely one meter, to avoid contact among different insecticides, hence committing bias, as the application was made using a knapsack sprayer. Each treatment received four applications of insecticides, with 15 days interval between each application. The first application was on 19th February, soon after the establishment of the trial. In total, the trial was conducted for a period of 90 days, after observing the emergence of the first adult insects.

***Table 1.*** *Summary of Treatments Used in the Experiment.*

| Nº | Nº Treatments | Concentration (g or ml/l) | pH regulator (ml/l) | Adherent ml/l |
|---|---|---|---|---|
| T1 | Thiametoxam (Thia) | 3g | 0 | 0 |
| T2 | Imidacloprid (Imid) | 3ml | 0 | 0 |
| T3 | Acetamiprid (Acet) | 3ml | 0 | 0 |
| T4 | Thiametoxam + Adherent (ThiaAd) | 3g | 0.25 | 1 |
| T5 | Imidacloprid + Adherent (ImidAd) | 3ml | 0.25 | 1 |
| T6 | Acetamiprid + Adherent (AcetAd) | 3ml | 0.25 | 1 |
| T7 | Control | 0ml | 0 | 0 |

*Figure 1. Engineer Daniel Castilho, Manager of the nursery in the right, the company director in the middle and the nursery research assistant, far left. Photo was captured by the authors, 30 days after the trial establishment).*

### 2.2. Data Collection and Analysis

Data was collected in the same day, before the application of the pesticide. The first assessment was made 15 days after the establishment of the trial, while the second, third and fourth assessments were carried out 30, 45 and 60 days after the establishment of the trial respectively. The evaluation consisted of counting the number of plants showing gall infestation in each of the experimental units. The counts were used to estimate percentage of seedlings infestation per treatment, after the first, second, third and fourth assessment. The emergence of adult gall wasps was detected by observing the holes left on the leaves.

Analysis were performed in R program. Normal distribution of the standard residuals and the homogeneity of variances were tested through Shapiro-Wilk and Bartlett's test respectively. Visual inspection of the standard residuals (normal q-q plots), was also made. Since the variances of the treatments were not homogeneous (see appendix A1), Box-Cox transformation was performed, based on the R-*MASS package* (see formula 1).

$$N_{Trees'} = {N_{Trees}}^2 * \lambda \qquad (1)$$

Where: $N_{Trees}$ is the total number of trees infested by Gall Wasp. The lambda λ value of 0.5 that was selected based on the likelihood function at 95% of significance. The transformation was adequate for all four assessments (see table A1 in the appendix). After transformation, data were re-tested for normality and homogeneity, subsequent Analysis of Variance (ANOVA) and the respective test of means were used to test the difference among treatments. The parametric test, (Tukey-HSD) in the R-*package agricolae* was used to compare the differences between treatments. All analyses were made considering the four periods in which the assessments were done: 15, 30, 45, and 60 days respectively.

## 3. Results

In Table 2 are presented results of all treatments after 15, 30, 45 and 60 days. Out of the brackets are the total number of seedlings affected, while the numbers in the brackets represent the percentage of seedlings affected by *L. invasa.* It can be observed that, there was no treatment free from the gall wasp. Wherein in average all treatments performed nearly better than the control, except Thiamethoxam with adherent, which was slightly worse than the control, with exception of the last assessment: 118(8.08%), 123(8.37%), 56(4.79%) and 36(3.08%) respectively. With less than 1% of seedlings affected by the *gall wasp, except* in the first assessment (15 days), Imidacloprid (bandit), seems to display superior effect than the other treatments. Imidacloprid (bandit) with adherent, though, performed even better, except in the first assessment where the percentage of seedling affected was 2.24%. Acetamiprid with no-adherent performed second best, even though in all evaluations the percentage of seedlings affected with *gall wasp* was more than 1%: 43(2.93%), 27(1.84%), 19(1.62%) and 25(2.14%) for 15, 30, 45 and 60 days respectively. Thiamethoxam presented less performance, when compared with all treatments, except the control, but when the adherent is added, it tended to perform even worse, especially in the first two assessments, 118(8.03%) for 15 days and 123(8.37%) after the second assessment. It can be observed that, on average, the use of adherent tends to increase the number of seedlings infested, in all treatments, but with more negative effect in Imidacloprid (AcetAd) and Thiamethoxam (ThiaAd).

***Table 2.*** *Performance of the 7 treatments after 15, 30, 45 & 60 days of assessment (out of brackets are the total numbers of seedlings affected, while in the brackets is the percentage of affected seedlings).*

| No | Nº Treatments | Days | | | |
|---|---|---|---|---|---|
| | | 15 | 30 | 45 | 60 |
| T1 | Thia | 88 (5.99) | 81 (5.51) | 39 (3.33) | 36 (3.08) |
| T2 | Imid | 9 (0.61) | 13 (0.88) | 5 (0.43) | 11 (0.94) |
| T3 | Acet | 43 (2.93) | 27 (1.84) | 19 (1.62) | 25 (2.14) |
| T4 | ThiaAd | 118 (8.03) | 123 (8.37) | 56 (4.79) | 36 (3.08) |
| T5 | ImidAd | 33 (2.24) | 8 (0.54) | 2 (0.17) | 7 (0.60) |
| T6 | AcetAd | 52 (3.54) | 63 (4.29) | 39 (3.33) | 30 (2.56) |
| T7 | Control | 116 (7.89) | 116 (7.89) | 50 (4.27) | 47 (4.02) |
| Mean | 7 | 65.57 (4.46) | 61.57 (4.19) | 30.00 (2.56) | 27.43 (2.34) |

Effect of the nursery environment (blocks) was only detected in the first assessment (p-value = 0.033*), at 0.05 of significance. While differences among the treatments were observed in the all assessed period, at 0.001 of significance from 15 to 45 days and 0.05 in the last assessment, after 60 days, (see appendixes A2). Table 3 presents results from the Tukey-HSD test of means. The test was performed based on the transformed data, which are in the brackets, whereas the values out of the brackets are the true means. Results show no significant difference between the control and Thiamethoxam, as well as Thiamethoxam (+adherent), in all assessments, meaning that both treatments presented the worst performance. In general, Imidacloprid with adherent did well than that with no adherent, despite the slight difference in the first assessment. Acetamiprid (Acet and AcetAd), appears to be an intermediate pesticide, between Thiamethoxam and Imidacloprid, but when pH and adherent are added, the effect of the pesticide is weaker, although the difference is not so significant. The overall effect of the different treatments, either with adherent and non-adherent is converging, from the first to the last assessment. The difference between ThaiAd and Imid in the first and last assessment are 21.8 and 6, respectively.

***Table 3.*** *Comparison of means among the different groups of treatments using Tukey-HSD test at 0.05 of significance. Figures in the column followed by same lowercase letters are not statistically different.*

| **Treatments** | | **Days** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **15** | | **30** | | **45** | | **60** | |
| T4 | ThaiAd | 23.6(4.81) | a | 24.6(4.90) | a | 11.2(3.29) | a | 7.2 | a |
| T7 | Cont | 23.2(4.79) | a | 23.2(4.77) | a | 10.0(3.13) | a | 6.1 | a |
| T1 | Thai | 17.6(4.11) | ab | 16.2(3.96) | ab | 7.8.6(2.61) | a | 5.8 | a |
| T6 | AcetAd | 10.4(3.11) | bc | 12.6(3.42) | ab | 7.8(2.71) | a | 4.4 | ab |
| T3 | Acet | 8.6(2.82) | bc | 5.4(2.25) | bc | 3.8(1.88) | ab | 4.0 | ab |
| T5 | ImidAd | 6.6(2.43) | cd | 1.6(1.08) | c | 0.4(0.28) | c | 0.5 | c |
| T2 | Imid | 1.8(1.31) | d | 2.6(2.40) | c | 1.0(0.77) | bc | 1.2 | bc |
| | | 13.11(3.34) | | 12.31(3.39) | | 6.0 (2.10) | | 4.17 | |

When we zoom together the data of all time periods of assessment (Figure 2), one can observe that, there is a considerable relation between efficiency and dispersion, in the sense that, the less efficient the treatment is, the more dispersed are the results, (see for instance Thai and ThaiAd). But the correlation tends to decrease with the number of times that the pesticide is applied, with an exception for Thiamethoxam.

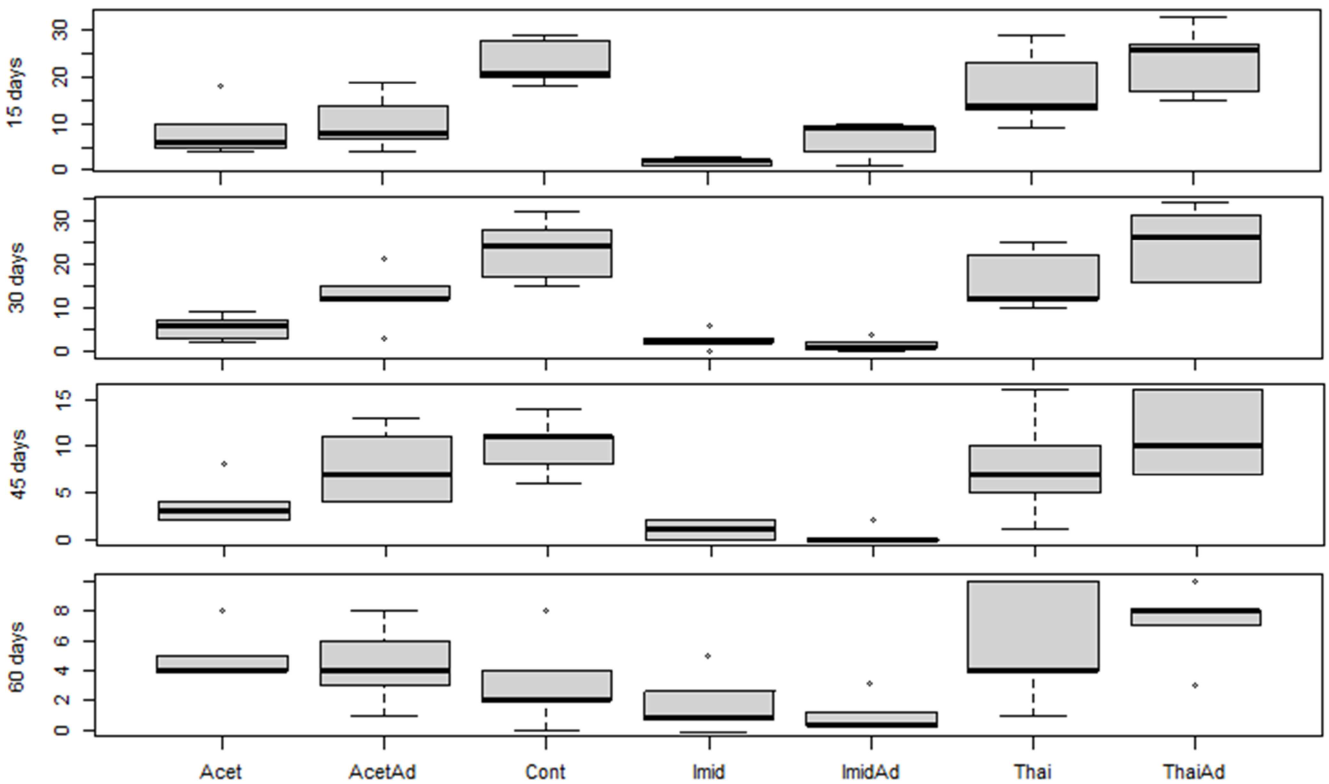


***Figure 2.*** *Comparison of 7 different treatments after 15, 30 and 45 days of experiment.*

It is important to note that in relation to the biological development of the wasp, from oviposition to emergence, only in the last assessment was observed the emergence of adult insect in the control treatment. From the (6.1%) of infested seedlings in the control treatment, 4% emerges the adult gall wasp in the last assessment.

# 4. Discussion

The effect of blocks was only slightly significant in the first assessment (p-value = 0.033*), indicating that the environment where the trial was conducted was controlled. This was not a surprise, as the trial was established in the best uniform conditions as possible. But the trial was established in RCBD because random effects like watering, spray application, light etc. are some factors that are difficult to control with a certain precision in the nursery. Novel results of this research include the fact that less than 25% of seedlings were infested by *L. invasa,* even in the control treatment, since there are authors who found more than 90% of infestation in the nursery trials experiments [9]. Thiamethoxam, either with or without adherent both presented the highest number of seedlings with *gall wasp* and performed poorly as the control treatment in all four time periods of assessment. One can relate that fact with the composition and concentration of the active ingredient in the formulation. In almost all treatments (except Imidacloprid), the use of adherent and pH regulator appeared to have an opposite effect of what was expected, hence increasing the number of seedlings infested by the *gall wasp* in all times where the assessment was conducted. Although the results are not statistically different, it is true that, the use of pH regulator and adherents tend to slightly increase the current cost of raising Eucalyptus *sp.* seedlings in the Niassa nurseries. Based on this research findings, we would suggest that, all the insecticides tested should be used with no adherent and pH regulator, as the adherent seems to cover the pores and stomata of the leaves and making it difficult for the penetration of the active ingredient, hence also reducing its systemic effect in the control of the *gall wasp*. The use of adherent decreases runoff and causes droplets to remain on the surface of the leaves and is not easily washed by rainwater [19].

Imidacloprid was more effective in controlling *L. invasa* when compared to other pesticides. Javare, Prabhu, & Roopa (2010), [20], evaluating the efficacy of botanicals and synthetic insecticides in the control of *Leptocybe invasa* (Eulophidae: Hymenoptera), in *Eucalyptus sp*, observed that Imidacloprid exhibited maximum insecticidal action 15 days after application, even with concentration of 0.25ml/l, less than that used in our test. Chakrabarti (2015), [21], also found Imidacloprid as one of insecticides with better effects in the combat of gall wasp, but Jhala, Patel, & Vaghela (2010), [22] used a much lower concentration of Imidacloprid (0,008%) and found the lowest efficacy of the pesticide.

It would be interesting to follow up this test after the establishment of the seedlings in the field, since it seems that, as time passing by and the plants grow old, the number of seedlings infested by the gall wasp tends to stabilize, probably due to the strengthening of plant tissues or even adaptability to the pest. The mean development time of the ovipositor was only about 90 days, after the seeds were release in the nursery and it occurred only in the control treatment. This period was slightly reduced than the average reported in other studies that range from 3.5 to 5 months [2].

# 5. Conclusions

The results, shows that Imidacloprid with non-adherent performed better than the other 6 treatments, including the control. The addition of adherent and pH regulator didn't change the treatments, instead it even worsened the results in all three times of assessment for all tested insecticides. If someone decides to use extensively any of these insecticides under test, no adherent or pH regulator should be added, unless other studies would have been done and provided different evidences.

Concerning these findings, it can be inferred that, the maximum efficiency of Imidacloprid in the control of *L. invasa*, depends amongst other factors, on the optimal dosage and concentration. While the superiority of Imidacloprid in the control of *gall wasp* in the nursery, appears to be "good news" for the Niassa context, the insecticide is already widely used for forestry and agriculture propose, due to its availability and being cheaper in the local market.

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# Conflict of Interest

The authors declare that they have no conflict of interest.

# Appendix

***Table A1.*** *Results from the homogeneity and normality test, before and after data.*

*Transformation (square root transformation)*

| | Non-Transformed | | Transformation (Box-Cox) | |
|---|---|---|---|---|
| | homogeneity | Normality | homogeneity | Normality |
| 15 days | 0.039 | 0.067 | 0.465 | 0.082 |
| 30 days | 0.023 | 0.604 | 0.984 | 0.199 |
| 45 days | 0.012 | 0.426 | 0.798 | 0.282 |
| 60 days | 0.821 | 0.275 | | |

***Table A2.*** *Analysis of Variance for the four periods of assessment.*

| | Df | Sum.Sq | Mean.Sq | F-Value | P-Value |
|---|---|---|---|---|---|
| | 15 Days | | | | |
| Treatment | 6 | 50.571 | 8.429 | 17.308 | 0.000*** |
| Blocks | 4 | 6.121 | 1.530 | 3.143 | 0.033* |
| Residuals | 24 | 11.687 | 0.487 | | |
| | 30 Days | | | | |
| Treatment | 6 | 72.685 | 12.114 | 16.259 | 0.000*** |
| Blocks | 4 | 1.577 | 0.394 | 0.529 | 0.715 |
| Residuals | 24 | 17.881 | 0.745 | | |
| | 45 Days | | | | |
| Treatment | 6 | 41.235 | 6.873 | 13.323 | 0.000*** |
| Blocks | 4 | 2.663 | 0.666 | 1.290 | 0.3017 |
| Residuals | 24 | 12.381 | 0.516 | | |
| | 60 Days | | | | |
| Treatment | 6 | 99.371 | 16.562 | 1.656 | 0.041* |
| Blocks | 4 | 17.429 | 4.357 | 0.419 | 0.794 |
| Residuals | 24 | 249.77 | 10.407 | | |

Df = Degrees of freedom, Sum.Sq = sum square; Mean.sq = Mean Square; Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' '

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