Revista de Ciências Agroambientais

Engenharia Florestal **DOI:** 10.30681/rcaa.v22i1.12009

Effect of application methods and chemical insecticides for the attack control of *Hypsipyla grandella* Zeller (Lepidoptera: Pyralidae) on the *Swietenia macrophylla* King

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Recebido: 13/12/2023; Aceito: 16/05/2024

Abstract: Chemical control of the shoot borer (*Hypsipyla grandella* Zeller) on mahogany (*Swietenia macrophylla* King) forest plantations remains a major challenge. For this reason, the objective of this study was to evaluate the effect of two application methods of three chemical insecticides plus the control on the response to insect attack, in a completely randomized design (2x4 factorial scheme). A total of 9 evaluations were carried out at intervals of 15 days between the months of May and September (0, 15, 30, 45, 60, 75, 90, 105, 120 days). The variables were standardized (*z*-function) and then transformed by the fourth root. The significant differences at error of 10% of the F test found at 30 and 45 days of the experiment could not be verified by the parametric tests because the transformations failed to bring the data closer to a standard normal distribution. Then the Kruskal-Wallis H test was performed to verify the differences between insecticides and control and the Mann-Whitney U test for the differences between application methods. The response of the relative number of attacked shoots per tree (rNAST) in the overall imidacloprid and emamectin benzoate groups differed from each other by post-hoc pairwise Dunn's correction with Holm's correction. By comparing the Wilcoxon rank sum in the fourth evaluation it was observed that the effect of emamectin benzoate microinjection on rNAST was smaller than that of the spraying method. In the same evaluation, imidacloprid and emamectin benzoate were differentiated from each other. The graphical analysis showed that in both evaluations the performance of imidacloprid was better than that of the other insecticides for the control of the mahogany shoot borer. The microinjection did not present significant effects; however, it has not impacted the growth of the trees measured by the increases in normal diameter, basal diameter, and total height.

Key-words: Mahogany shoot borer; Imidacloprid; Spinetoram; Emamectin benzoate; Trunk microinjection.

Efeito de métodos de aplicação e inseticidas químicos no controle do ataque de *Hypsipyla grandella* Zeller (Lepidoptera: Pyralidae) sobre *Swietenia macrophylla* King

Resumo: O controle químico da broca do broto (*Hypsipyla grandella* Zeller) nas plantações florestais de mogno (*Swietenia macrophylla* King) continua sendo um grande desafio. Por esse motivo, o objetivo deste estudo foi avaliar o efeito de dois métodos de aplicação de três inseticidas químicos, mais o controle, sobre a resposta ao ataque de insetos, em um delineamento inteiramente casualizado (esquema fatorial 2x4). Foram realizadas 9 avaliações em intervalos de 15 dias entre os meses de maio e setembro (0, 15, 30, 45, 60, 75, 90, 105, 120 dias). As variáveis foram padronizadas (função z) e posteriormente transformadas pela raiz quarta. As diferenças significativas do teste F com erro de 10% encontradas aos 30 e 45 dias de experimento não puderam ser verificadas pelos testes paramétricos porque as transformações não conseguiram aproximar os dados de uma distribuição normal padrão. Em seguida foi realizado o teste H de Kruskal-Wallis para verificar as diferenças entre inseticidas e controle e o teste U de Mann-Whitney para as diferenças entre os métodos de aplicação. A resposta do número relativo de brotos atacados por árvore (rNAST) nos grupos gerais de imidaclopride e benzoato de emamectina diferiu entre si pela correção post hoc de Dunn aos pares com a correção de Holm. Ao comparar a soma dos ranks de Wilcoxon na quarta avaliação observou-se que o efeito da microinjeção de benzoato de emamectina no rNAST foi menor que o do método de pulverização. Na mesma avaliação, o imidaclopride e o benzoato de emamectina foram diferentes entre si. A análise gráfica mostrou que em ambas as avaliações o desempenho do imidaclopride foi melhor que o dos demais inseticidas no controle da broca do mogno. A microinjeção não apresentou efeitos significativos; entretanto, não impactou o crescimento das árvores medido pelos aumentos no diâmetro normal, diâmetro basal e altura total.

Palavras-chave: Broca do mogno; Imidaclopride; Espinetoram; Benzoato de emamectina; Microinjeção de tronco.

1. INTRODUCTION

In Mexico and other countries with tropical areas of America, the Meliaceae shoot borer (*Hypsipyla grandella* Zeller) is a limiting factor for the success of commercial forest plantations of species of higher international value such as red cedar (*Cedrela odorata* L.) and mahogany (*Swietenia macrophylla* King) (DÍAZ-MARTÍNEZ et al., 2023; PULGARÍN DÍAZ et al., 2023). Even after almost a century of research, the control of this insect pest continues to be a great challenge because it involves simultaneous effect and response factors such as insect-plant interaction, methods of application of chemical products, active substances and formulations of insecticides and multiple environmental factors (ALLAN et al., 1976; GOULET et al., 2005; HOWARD & MERIDA, 2004; NEWTON et al., 1993; WYLIE, 2001). Particularly, the use of chemical insecticides is a matter of concern given the complexity involved in economic efficiency and environmental safety (ALLAN et al., 1976; DÍAZ-MARTÍNEZ et al., 2023; MAESTRI et al., 2020; NEWTON et al., 1993). However, many of these active substances are still used to protect plants in the nursery or as part of integrated pest management programs in forestry plantations (MAESTRI et al., 2020; NEWTON et al., 1993). Therefore, research into the bioefficacy of active substances and application methods to control of shoot borer remains relevant for the silvicultural production of Meliaceae (ALLAN et al., 1973; GOULET et al., 2005; HOWARD & MERIDA, 2004).

The presence of small populations of adults can cause significant damage as females oviposit up to 300 eggs (SOLOMON, 1995). When the eggs hatch, the larvae bore into the terminal shoots and create tunnels in the soft stem (GRIFFITHS, 2001; MAYHEW & NEWTON, 1998; MOREIRA et al., 2014), mainly from trees from one to eight years old (HOWARD & MERIDA, 2004). Inevitably, the attacked shoots die (MOREIRA et al., 2014). The attacks can affect all the plants in a stand (MAYHEW & NEWTON, 1998). In response to the attack, there is excessive branching and conversion of lateral shoots into leading shoots (bifurcation), which leads to deformation of the stem and consequent reduction in its commercial value (HOWARD & MERIDA, 2004; MAESTRI et al., 2020; NEWTON et al., 1993). In severe infestations there is a considerable reduction in growth (HOWARD & MERIDA, 2004). In Mexico, an attack period has been reported from May to September (BLANCO et al., 2001; SÁNCHEZ-SOTO et al., 2009).

There are many attempts to carry out integrated management of this pest through chemical control (ALLAN et al., 1973, 1976; GOULET et al., 2005; WYLIE, 2001), biological control (PULGARÍN DÍAZ et al., 2018), sexual pheromone (PINEDA-RÍOS et al., 2016), volatile compounds of healthy trees (DÍAZ-MARTÍNEZ et al., 2023), and silvicultural strategies (MAYHEW & NEWTON, 1998). Still, the difficulty of finding a method of controlling the shoot borer that is economically efficient and environmentally acceptable persists (WYLIE, 2001). Furthermore, there are few complete studies on the effect of new active substances and chemical insecticide formulations on the attack of shoot borer (ALLAN et al. 1973, 1976; GOULET et al., 2005; HOWARD & MERIDA, 2004; WYLIE, 2001). Though, some systemic active substances are effective for the control of other lepidopteran insects (SAMMANI et al., 2020; SIAL & BRUNNER 2010) and specifically the Pyralidae family (CHI et al., 2021; SINGH et al., 2016; YUE et al., 2003; ZHANG et al., 2014).

Spinetoram, chlorantraniliprole, and emamectin benzoate are effective for controlling *Choristoneura rosaceana* (Harris) larvae on apple (*Malus* spp.) foliage (SIAL & BRUNNER, 2010). In the brinjal (*Solanum melongena* L.) experiment, emamectin benzoate is significantly superior to other insecticides to controlling *Leucinodes orbonalis* (Guenée) (SINGH et al., 2016). According to Singh et al. (2016), the efficiency of fipronil, dimethoate and imidacloprid are far below that of emamectin benzoate. To control *Hypsipyla robusta* (Moore), Asian homologue of *H. grandella*, in *Chukrasia tabularis* A. Juss (2-year-old trees in the field), the damage caused by natural infestation can be reduced to more than 75%, 30 days after the application of carbaryl, carbosulfan, deltamethrin or fipronil (CHI et al., 2021). Damage reduction can be up to 98% with the use of alkylsulfonate or trisiloxane ethoxylate as adjuvants in the spraying mixture (CHI et al., 2021). On the other hand, imidacloprid is not effective in controlling the larvae of *Ostrinia nubilalis* (Hübner) and *Plodia interpunctella* (Hübner) (YUE et al., 2003). Imidacloprid also does not work to control mahogany shoot borer through root dipping before the spring season (HOWARD & MERIDA, 2004). The bacterial formulations, spinosad and spinetoram, act on the mortality of *Cadra cautella* (Walker) larvae and reduce the appearance of adults and the production of offspring (SAMMANI et al., 2020). Likewise, these macrolide insecticides spinetoram and spinosad, and two others abamectin and emamectin benzoate, are effective for the control of *Cnaphalocrocis medinalis* (Guenée) (ZHANG et al., 2014).

Excessive use is a reality that usually occurs to compensate for losses from the application of chemical insecticides in tropical areas mainly due to the action of rain and high temperatures, through phenomena such as leaching and evaporation (ALLAN et al., 1973, 1976). Furthermore, conventional pesticide application methods increase the risks of exposure for consumers, users, and the environment (BERGER & LAURENT, 2019; COSLOR et al., 2019). For this reason, the method of injecting insecticides into trees has emerged, with which the chemical product acts in the sap flow, which are translocated by their physiological system, making it possible to have greater protection and control against pests and diseases. and resulting in the reduction of unwanted exposures (BERGER & LAURENT, 2019; DOCCOLA & WILD, 2012). Injections of systemic insecticides into the stem with a microsyringe can be a successful technique for controlling the Meliaceae shoot borer; however, it is necessary to experimentally verify the effectiveness of this method (CIBRIÁN TOVAR, 2013).

A key rule of forest pest management is to only use one chemical pesticide to control massive damage caused by pest populations on high commercial value trees (KUMAR et al., 2020). On the other hand, if the cost of control or the potential damage to the environment caused by the active substance is greater than the estimated damage or loss, its use is not recommended (KUMAR et al., 2020). For example, imidacloprid and other highly dangerous neonicotinoids are banned in many countries for two main reasons, for the risk of groundwater contamination and, mainly, for harming domesticated honeybees and also wild pollinators (KATHURIA et al., 2023). Trunk injection of imidacloprid into tree trunks may be an alternative to reduce the potential for unwanted exposures (DOCCOLA & WILD, 2012). But there is also the residual effect of chemical insecticides applied by trunk injections in nectar or pollen, mainly depending on the seasons of the year (COSLOR et al., 2019). For example, imidacloprid is not detected in nectar or pollen when injected into apple trees (*Malus pumila* Miller) during the spring but is detected in pollen when injected in the fall. On the other hand, emamectin benzoate is not detected in nectar or pollen when injected in the fall and is detected in nectar and pollen when injected in the spring (COSLOR et al., 2019). That is, the decision to use chemical insecticides to increase the coverage and effectiveness of control involves fundamental aspects such as knowledge of the life cycle of the insect in the environment of the attack, the selection of an appropriate pesticide, the appropriate time of application and use of proper application equipment (KUMAR et al., 2020). For this reason, this study evaluates the effects of spraying and microinjection application of the insecticides imidacloprid, spinetoram and emamectin benzoate to control *H. grandella* on *S. macrophylla*.

2. MATERIAL AND METHODS

The study was carried out in a mahogany forest plantation located in the "El Tortuguero" ejido, municipality of San Juan Mazatlán, Oaxaca, Mexico (17º19'20.3" N; 95º16'15.3 W) (Figure 1). In this experimental area, it is empirically reported that there is a high concentration of infestation of *H. grandella* (Figure 2). Trees of *S. macrophylla* with the same age of 2 years (Figure 2a), distributed at 3 x 3 meters, with intervals of basal diameter between 50 to 120 mm and total height between 330 to 620 cm, ensuring homogeneity of the selected individuals and the accessibility to move application equipment (Figure 3).

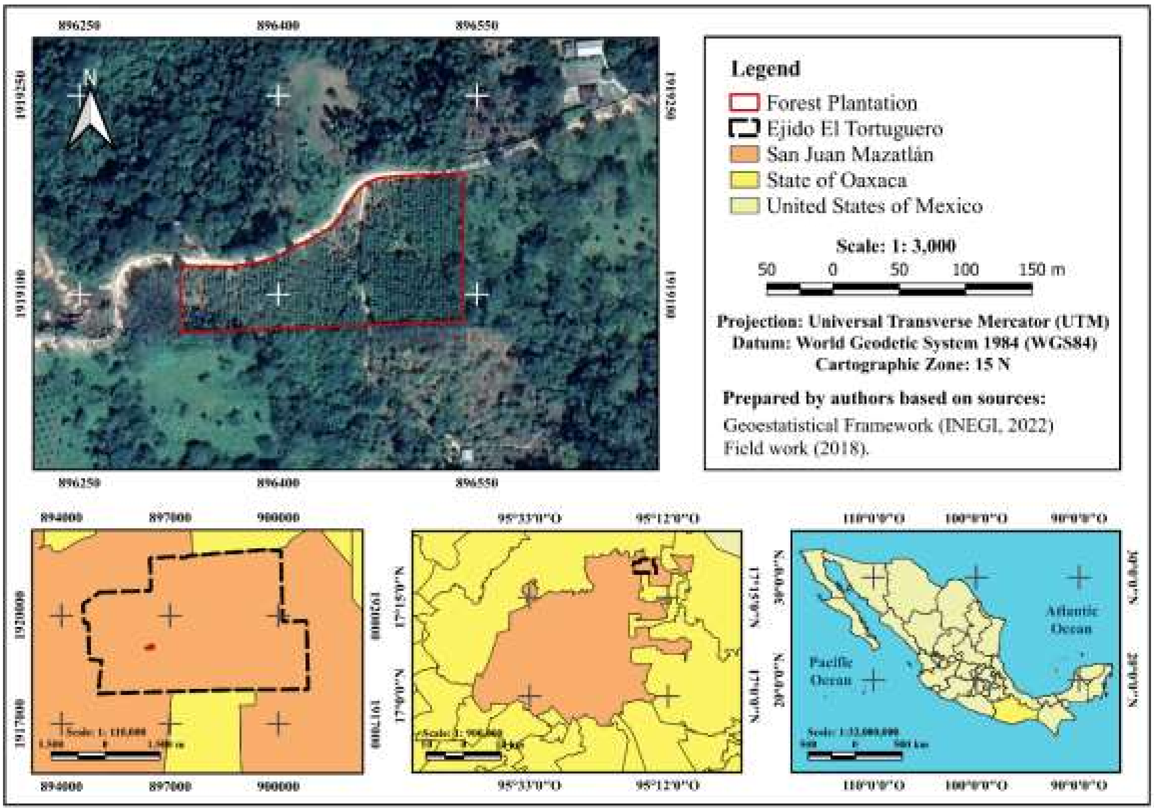

Figure 1. Location map of the study area. Forest plantation of *Swietenia macrophylla* King under attack of *Hypsipyla grandella* (Zeller).

Figure 2. Forest plantation under attack of *Hypsipyla grandella* (Zeller): a) Mahogany (*Swietenia macrophylla* King) trees (*mt*) with 2 years-old; b) Shoot terminal attacked (*sta*); c) Beginning of wilting of the leaves (*wl*) of the attacked shoot; d) The terminal shoot begins to dry (*dr*); e) Dead shoot (*ds*); f) Presence of sawdust and insect excrement (*se*); g and h) Larvae of the Meliaceae borer shoot (*la*) inside the tunnel (*tu*) formed for feeding and for a pupal chamber. San Juan Mazatlán, Oaxaca, Mexico (2018).

Figure 3. Application of chemical treatments: a) Spraying of the tree canopy from the ground; b) Spraying with the use of a ladder to reach the tree canopy; c) Open a 3.6 mm diameter hole (three-point drill bit) in the north-northeast part of the tree using a cordless drill (DeWalt model DCD -771C2B3), at an approximate angle of 30°, 30 mm deep and between 5 to 10 cm above the soil surface; d) Vaccine-Tree microinjection equipment (20 ml). San Juan Mazatlán, Oaxaca, Mexico (2018).

In a completely randomized design in a 2 x 4 factorial scheme, eight treatments were tested corresponding to the combination of the application methods and insecticides factors. There were 7 repetitions per treatment. The two levels of application methods were spraying (Figure 3a and b) and microinjection (Figure 3c and d). The four levels of insecticides correspond to three chemical insecticides (imidacloprid, Confidor® 350 SC, with a formulation of 350 g of active ingredient (a.i.) L^{-1}; spinetoram, Palgus®, with a formulation of 60 g of a.i. L^{-1}; and emamectin benzoate, Denim® 19 CE, with a formulation of 19.20 g of a.i. L^{-1}) and a control (untreated check) with distilled water. The concentrations of the insecticides imidacloprid, spinetoram and emamectin benzoate used in the doses applied by microinjection were, respectively, 1.155, 1.02, 0.096 g of a.i. L^{-1}. The concentrations of imidacloprid, spinetoram and emamectin benzoate for the spraying doses were, respectively, 0.07, 0.06 and 0.01152 g of a.i. L^{-1}. Doses of 3 ml were applied by microinjection, making a 3.6 mm diameter hole (three-point drill bit) in the north-northeast part of the tree (avoiding possible damage from sunlight), using a cordless drill (DeWalt model DCD - 771C2B3), at an approximate angle of 30°, 30 mm deep, and between 5 to 10 cm above the soil surface (Figure 3c). Before each drilling, the drill bit was disinfected with a solution of 5% quaternary ammonium salts and 10% of hypochlorite L^{-1}. This formulation was applied with a spray bottle before and after the injection point. Cork buffers were placed to mainly prevent the entry of water and insects that could cause damage to the tree. The microinjections were performed once on May 25, 2018. The Vacuna-Tree microinjection equipment (Figure 3d) designed by the work team was used: Biologist Israel Aquino Morales, Dr. Víctor David Cibrián Llanderal and Dr. David Cibrián Tovar, currently in the registration process with the Mexican Institute of Industrial Property (IMPI).

The fumigations were carried out with a Jacto Sprayer Fumigator (Jacto - XP-20) 20 L, with a 600 mm gun, you can reach higher pressure 100 psi (6.8 bar) faster and with less pumping (Figure 3a and b). The spraying was repeated every fifteen days during the months of May to September. Between 7:00 and 10:00 a.m., the shoots were sprayed at drip point with an approximate expenditure. Weed control was carried out by hand every 30 days. There were a total of 9 evaluations between 15-day intervals between the months of May and September (0, 15, 30, 45, 60, 75, 90, 105, 120 days). The primary variables analysed were number of attacked shoots per tree (NAST) and number of healthy shoots (tender shoots and young leaves) per tree (NHST). Of the total number of shoots per tree (NHST + NAST), the relative number of attacked shoots per tree (rNAST, %) was calculated, the main variable of this study. Also, the normal diameter (NDia, mm), basal diameter (BDia, mm) and total height (THei, cm) of the trees in the different treatments were also analysed. The increases in NDi, BDia and THei were calculated by the differences between the last evaluation values and the first one.

Other secondary variables were calculated from the NHST: number of shoots with abiotic damage per tree (NSADT) and number of new healthy shoots per tree (NNHST). The two variables were obtained by the difference between the number of the subsequent evaluation and the number of the previous evaluation. Positive numbers indicate an emergence of new shoots in the evaluation (NNHST). Negative numbers indicate a loss of shoots due to the action of abiotic phenomena such as the incidence of wind (NSADT). All statistical procedures were performed in the R programming environment (R Core Team 2023). The main statistical packages used in the graphical analysis analyses were: "ggplot2" (WICKHAM et al., 2023) and "ggstatsplot" (PATIL, 2021).

3. RESULTS AND DISCUSSION

The rNAST variable was subjected to statistical tests of normality (LILLIEFORS, 1967) and homoscedasticity (BARTLETT, 1937; LEVENE, 1960) and to the F test of the analysis of variance (ANOVA) at 10% error for each of the 9 evaluations. The variables were transformed twice, first with a standardization function (*z*-score) and then with a fourth root function (Table 1). The variables without transformation had very high ANOVA coefficients of variation (CV), but with the transformations, these coefficients were reduced. However, even after mathematical transformations, except for the eighth evaluation, the normality of rNAST could not be detected by the Kolmogorov-Smirnov test (Lilliefors correction) for the other evaluations (Table 1). The transformations also managed to produce the desired homoscedasticity verified by the Bartlett and Levene tests (Table 1). Still, due to not meeting the requirement of normality, the significant differences found by the ANOVA F test were not verified by the parametric tests of multiple comparisons between averages. Instead, non-parametric Kruskal-Wallis (1952) (Figures 4 and 6) and Mann-Whiney (1947) (Figure 5) tests were used to verify differences between application methods and chemical insecticides.

Table 1. Summary of statistical tests for the effect of application methods and chemical insecticides for the control of *Hypsipyla grandella* (Zeller) on the relative number of attacked shoots per tree (%) of *Swietenia macrophylla* King. San Juan Mazatlán, Oaxaca, Mexico (2018) (Part I).

Evaluation	Variation Source	Degree of Freedom	Mean Square	F Test	Coefficient of Variation (%)	K-S Test¹	Bartlett's Test²	Levene's Test³
				p-value		p-value		
May 25†‡	Application Methods (AMe)	1	0.03	0.69			0.60	0.55
	Chemical Insecticides (CIn)	3	0.20	0.37			0.53	0.28
	Interaction	3	0.02	0.94			0.91	0.80
	Residuals	48	0.18		25.40	<0.01		
	Total	55	0.43					
June 09†‡	AMe	1	0.05	0.55			0.70	0.34
	CIn	3	0.02	0.91			0.80	0.98
	Interaction	3	0.24	0.15			< 0.01	0.50
	Residuals	48	0.13		23.81	< 0.01		
	Total	55	0.44					
June 24†‡	AMe	1	0.21	0.40			0.67	0.60
	CIn	3	0.88	0.04			0.76	0.03
	Interaction	3	0.14	0.69			0.99	0.63
	Residuals	48	0.29		38.60	<0.01		
	Total	55	1.52					

[1]Kolmogorov-Smirnov normality test with Lilliefors correction. [2]Bartlett's test for homogeneity of variances. [3]Levene's test for homogeneity of variances. [4]Water application. Double transformation: †Variable standardized by the z-function. ‡Standardized variable transformed by the fourth root.

Table 1. Summary of statistical tests for the effect of application methods and chemical insecticides for the control of *Hypsipyla grandella* (Zeller) on the relative number of attacked shoots per tree (%) of Swietenia macrophylla King. San Juan Mazatlán, Oaxaca, Mexico (2018) (Part II).

Evaluation	Variation Source	Degree of Freedom	Mean Square	F Test	Coefficient of Variation (%)	K-S Test[1]	Bartlett's Test[2]	Levene's Test[3]
				p-value		p-value		
July 09†‡	Application Methods (AMe)	1	0.10	0.51			0.62	0.55
	Chemical Insecticides (CIn)	3	0.22	0.42			0.77	0.66
	Interaction	3	0.50	0.09			0.93	0.91
	Residuals	48	0.22		31.31	<0.01		
	Total	55	1.04					
	AMe: Control[4]	1	0.05	0.66				
	AMe: Imidacloprid	1	0.29	0.23				
	AMe: Spinetoram	1	0.03	0.77				
	AMe: Emamectin benzoate	1	1.24	0.02				
	CIn: Microinjection	3	0.04	0.93				
	CIn: Spraying	3	0.68	0.03				
July 24†‡	AMe	1	0.05	0.44			0.20	0.27
	CIn	3	0.03	0.74			0.29	0.75
	Interaction	3	0.11	0.24			<0.01	0.52
	Residuals	48	0.08		24.35	<0.01		
	Total	55	0.27					
August 08†‡	AMe	1	0.23	0.33			0.28	0.40
	CIn	3	0.25	0.38			0.28	0.33
	Interaction	3	0.17	0.55			<0.01	0.52
	Residuals	48	0.24		32.51	<0.01		
	Total	55	0.89					
August 23†‡	AMe	1	0.12	0.54			0.52	0.44
	CIn	3	0.09	0.84			0.82	0.56
	Interaction	3	0.22	0.55			0.98	0.90
	Residuals	48	0.30		33.16	<0.01		
	Total	55	0.73					
September 07†‡	AMe	1	<0.01	0.99			0.78	0.84
	CIn	3	0.31	0.45			0.65	0.09
	Interaction	3	0.72	0.11			0.97	0.94
	Residuals	48	0.34		32.46	0.06		
	Total	55	1.37					
September 22†‡	AMe	1	0.04	0.73			0.75	0.88
	CIn	3	0.06	0.91			1.00	0.99
	Interaction	3	0.37	0.34			0.99	0.95
	Residuals	48	0.33		33.44	<0.01		
	Total	55	0.80					

[1]Kolmogorov-Smirnov normality test with Lilliefors correction. [2]Bartlett's test for homogeneity of variances. [3]Levene's test for homogeneity of variances. [4]Water application. Double transformation: †Variable standardized by the z-function. ‡Standardized variable transformed by the fourth root.

By the ANOVA F test, at 30 days a significant difference in the rNAST response between insecticides was detected (Table 1). Due to the absence of normality, the rNAST variable was then subjected to the Kruskal-Wallis H test and it was verified that the evidence against the null hypothesis of equality between groups of chemical insecticides in favour of the alternative is low (*p*-value = 0.07) (Figure 4). The results of the variables without and with transformation are shown in boxplots (Figure 4), where it is possible to verify that the transformation of the rNAST variable does not alter the statistics of the non-parametric test compared to the non-transformed variable. The partial epsilon squared (0.13) with 90% confidence intervals was automatically calculated as a measure of the effect size for the Kruskal-Wallis test (Figure 4). The value of 0.13 (Figure 4) indicates a "medium" size of the effect (FIELD, 2017) of chemical insecticides on rNAST. Dunn's post hoc pairwise test (DUNN, 1964) with Holm's correction (HOLM, 1979) for multiple comparisons was applied for the Kruskal-Wallis H test, whose acceptable significance level in this study was 10% error. The graphs (Figure 4 a and b) show the pairwise comparisons and their respective significance levels. With the significance of 10% error, the alternative hypothesis of difference between groups can only be accepted for the insecticides imidacloprid and spinetoram (Figure 4 a and b).

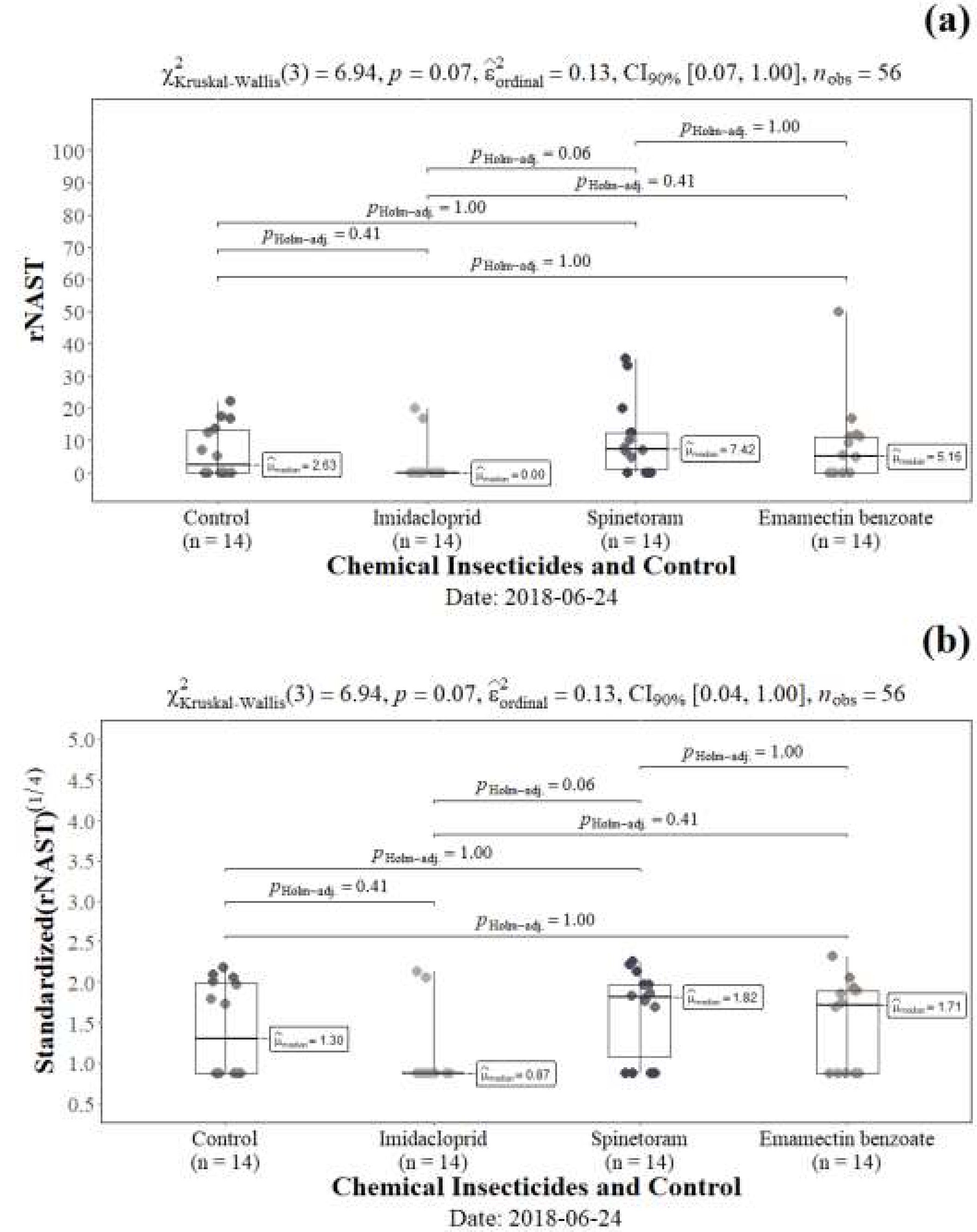

Figure 4. Boxplots for group comparisons. Response variable: relative number of attacked shoots per tree (NAST, %). Insect: *Hypsipyla grandella* (Zeller). Host: *Swietenia macrophylla* King. Not transformed (a) and transformed variable (b). Kruskal-Wallis H test to check the difference between chemical insecticides and control: Dunn's pairwise test; Holm's adjustment method. Epsilon squared interpretation: medium effect. Standardized function (*z*-scored).

Parametric tests for comparing averages or even non-parametric ranges calculated based on medians are often used without a graphical verification of the final distribution of the data. When observing the distribution of the observations for each insecticide, we see that the observations for imidacloprid are concentrated in the zero percentage of relative attack (Figure 4a), indicating its greater effectiveness compared to the other insecticides and the control. The next evaluation at 45 days, a significant interaction between the evaluated factors could be detected (Table 1), where, in turn, the differences between the application of emamectin benzoate by microinjection and by spraying were graphically analysed (Figure 5), and the differences between insecticides and control within the spraying method (Figure 6). Using the Mann-Whitney U test, it was observed that microinjection of the insecticide emamectin benzoate was more efficient for the control of shoot borer than application by spraying (Figure 4 a and b). The value of the Wilcoxon biserial rank (-0.63) (Figure 5 a and b) indicates that the effect of the application methods on emamectin benzoate was very large (FUNDER & OZER, 2019). In this fourth evaluation, differences between imidacloprid and emamectin benzoate were also detected as in the third evaluation, however, now within the spray method (Figure 6 a and b). Again, the graphic analysis provides support to verify that the spraying of imidacloprid maintained its control effect on insects until 45 days of the experiment (Figure 6 a and b). From the fifth evaluation onwards, no significant differences were detected between the effects of the factors on the rNAST response (Table 1).

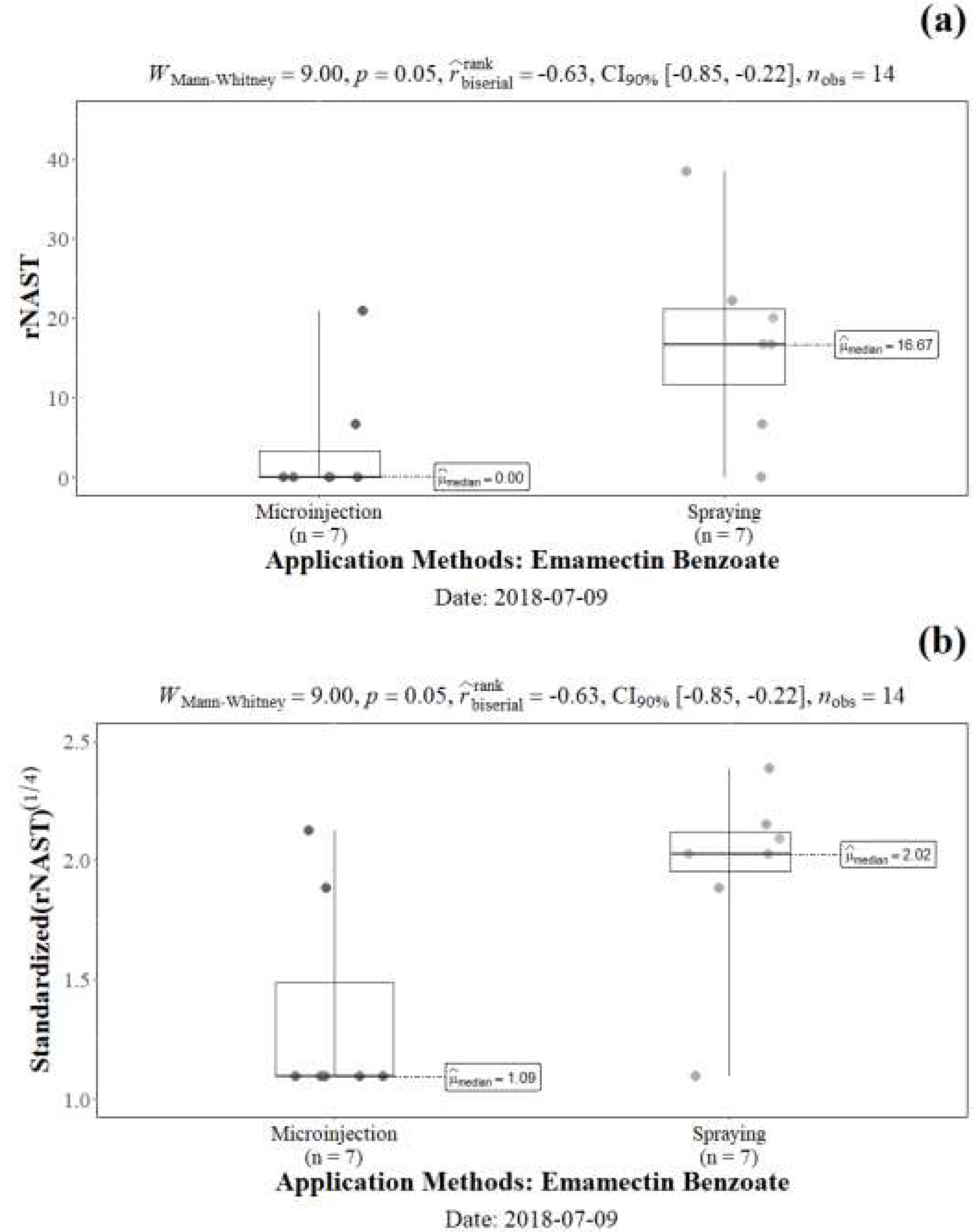

Figure 5. Boxplots for group comparisons. Response variable: relative number of attacked shoots per tree (rNAST, %). Insect: *Hypsipyla grandella* (Zeller). Host: *Swietenia macrophylla* King. Not transformed (a) and transformed variable (b). The Mann-Whitney U test to check the difference between microinjection and spraying of emamectin benzoate. Biserial-rank interpretation: very large effect. Standardized function (*z*-scored).

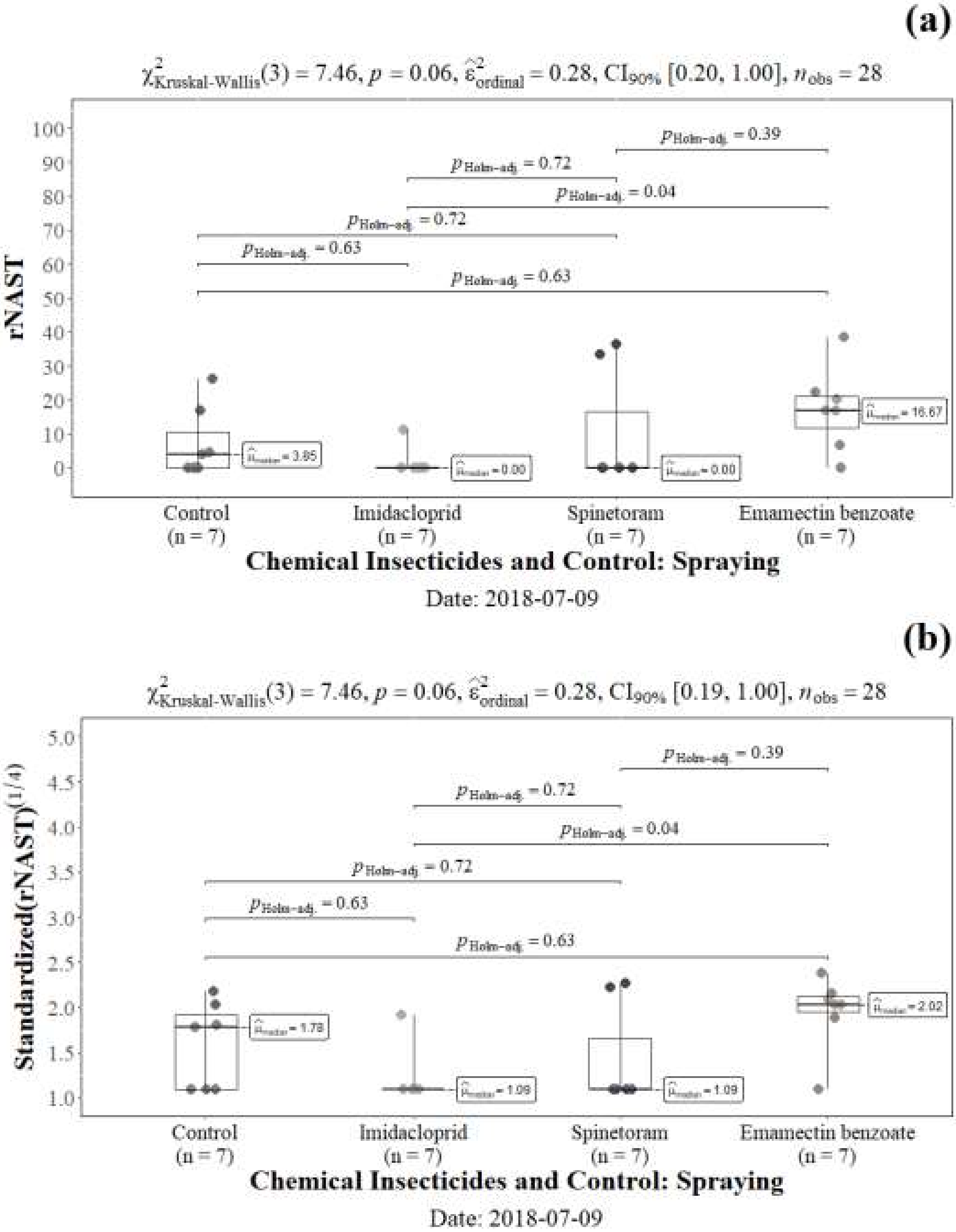

Figure 6. Boxplots for group comparisons. Response variable: relative number of attacked shoots per tree (rNAST, %). Insect: *Hypsipyla grandella* (Zeller). Host: *Swietenia macrophylla* King. Not transformed (a) and transformed variable (b). Kruskal-Wallis H test to check the difference between chemical insecticides and control in the spraying method: Dunn's pairwise test; Holm's adjustment method. Epsilon squared Interpretation: large effect. Standardized function (*z*-scored).

The NDia, BDia and THei variables were also subjected to the ANOVA F test and no significant differences were detected between averages of treatments (Table 2). Table 2 shows the overall averages and standard deviations of each evaluation and also of the increases of both variables. The distribution of the observations of the variables NHST, NAST, NSADT and NNHST for each evaluation are shown in Figure 7. From the beginning of the experiment until the third evaluation (30 days later), there was an increase of approximately 3 times in the average of NHST (Figure 7a). This fact is confirmed with the highest average of NNHST verified in the same evaluation (Figure 7d). The peaks of NAST related to sample observations occurred in the third and last evaluation, respectively (Figure 7b). The lowest average NAST was observed in the fifth evaluation (Figure 7b). The highest average NSADT was also observed in the fifth evaluation (Figure 7c).

Table 2. Summary of averages for the effect of application methods and insecticide types for the control of *Hypsipyla grandella* (Zeller) on the biometric variables of *Swietenia macrophylla* King. San Juan Mazatlán, Oaxaca, Mexico (2018).

Evaluation[1,2]	**Normal Diameter (mm)**	**Basal Diameter (mm)**	**Total Height (cm)**
		Average ± Standard Deviation	
May 25	53.50 ± 10.05	84.02 ± 12.47	454.64 ± 64.02
June 09	54.04 ± 10.12	85.14 ± 13.02	467.95 ± 64.83
June 24	54.75 ± 10.32	86.64 ± 13.39	483.39 ± 67.68
July 09	55.98 ± 10.16	88.27 ± 13.94	489.55 ± 68.40
July 24	57.14 ± 10.29	90.02 ± 14.38	501.07 ± 70.85
August 08	59.05 ± 10.65	92.11 ± 14.62	504.11 ± 70.38
August 23	61.25 ± 10.77	94.14 ± 14.76	507.68 ± 75.16
September 07	62.89 ± 10.87	96.21 ± 15.06	515.89 ± 77.10
September 22	63.86 ± 11.08	97.36 ± 15.09	529.46 ± 75.41

Overall[1,2]	Increases		
	Normal Diameter (mm)	Basal Diameter (mm)	Total Height (cm)
		Average ± Standard Deviation	
	13.34 ± 4.56	10.36 ± 2.51	74.82 ± 25.28

[1]No significant differences were detected due to the ANOVA F test at 10% error. [2]All ANOVA requirements were met without the need for mathematical transformation of variables.

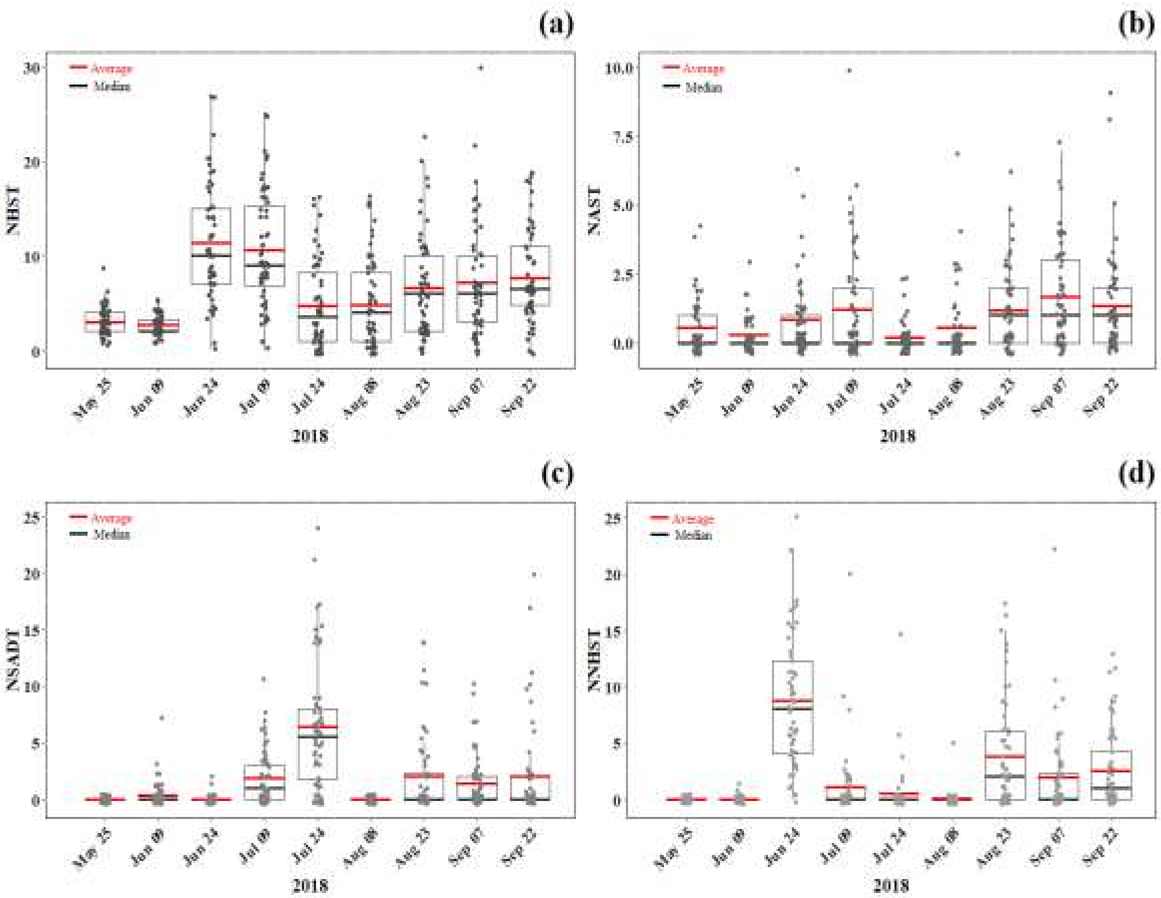

Figure 7. Boxplots analysis. Primary variables: number of healthy shoots per tree (a) and number of attacked shoots per tree (b). Secondary variables: number of shoots with abiotic damage per tree (c) and number of new healthy shoots per tree (d). Insect: *Hypsipyla grandella* (Zeller). Host: *Swietenia macrophylla* King. San Juan Mazatlán, Oaxaca, Mexico (2018).

Twenty-one trees that had been drilled for microinjection were evaluated (Table 3). At 180 days, 100% of these trees were completely healed and had expelled the cork stoppers (Table 3). The cost of the microinjection of spinetoram and emamectin benzoate were, respectively, 59.4% and 68.9% lower than the cost of spray for 1111 trees/hectare (Table 4). As soon as imidacloprid microinjection increases the cost to 9.2% when compared to the spraying method (Table 4).

Table 3. Evaluation of healing of *Swietenia macrophylla* King trees after microinjections with chemical insecticides (imidacloprid, spinetoram, emamectin benzoate and untreated check) to control *Hypsipylla grandella* (Zeller). San Juan Mazatlán, Oaxaca, Mexico (2018)

Days after microinjection	Trees completely healed	Trees <50% of the cork stoppers expelled	Trees >50% of the cork stoppers expelled	Total
75	9 (43.0%)	8 (38.0%)	4 (19.0%)	21 (100%)
90	12 (57.0%)	5 (24.0%)	4 (19.0%)	21 (100%)
105	15 (71.4%)	3 (14.3%)	3 (14.3%)	21 (100%)
120	17 (81.0%)	2 (9.5%)	2 (9.5%)	21 (100%)
180	21 (100.0%)	0 (0.0%)	0 (0.0%)	21 (100%)

Table 4. Approximate cost for each chemical treatment applied to a mahogany plantation (*Swietenia macrophylla* King) with two years-old, during a six-month period. San Juan Mazatlán, Oaxaca, Mexico (2018)

Chemical insecticide	Application Method	
	Microinjection	Spraying
	Cost of 1111 trees/hectare ($USD)	
Imidacloprid	635.59	582.30
Spinetoram	622.48	1047.75
Emamectin benzoate	626.31	909.07

The results of the statistical tests presented in this study (Table 1; Figures 4-6) exemplify the complexity that is involved in experimental designs to evaluate the control of *H. grandella* on *S. macrophylla* and others Meliaceae (ALLAN et al., 1976; DÍAZ-MARTÍNEZ et al., 2023; GOULET et al., 2005; HOWARD & MERIDA, 2004; NEWTON et al., 1993; PULGARÍN DÍAZ et al., 2023; WYLIE, 2001). Furthermore, this type of variable is very susceptible to bias due to associated phenotypic phenomena. For example, since the third evaluation the number of healthy (tender) shoots has practically tripled (Figure 7a). Figure 7d shows the increase in new shoots in the trees after 30 days of the experiment. This time interval corresponds to the period in which significant differences in the effect of the treatments on the relative number of *H. grandella* attacks on tree shoots were found (Table 1; Figures 4-6). Still, the emission of shoots in mahogany is quite accelerated due to the rainy season in tropical regions. The mature shoots (branches) were not considered in the study, because the attack is concentrated on the tender shoots (NEWTON et al., 1993). Also, it is important to highlight that this relative number of attacked shoots is altered depending on the total number of tender shoots of the tree. The absolute attack variable is not repeated in each evaluation, because new attacks were computed in each period (Figure 7b); the attacked shoot dies (Figure 2).

The NHST count is also a peculiar measurement (Figure 7a). When there is no attack, the same outbreak that was computed in a previous evaluation usually emits a new prolongation, which in turn is computed again in a subsequent evaluation. For this reason, the NHST can be repeated between 15-day evaluations, as in the 30- and 45-day evaluations, which have practically the same distribution (Figure 7a). On the other hand, the NNHST variable represents the increase in new outbreaks compared to the previous evaluation (Figure 7d). Therefore, the analysis of the primary and secondary variables with their absolute values (Figure 7) is essential to verify the phenomena associated with the insect attack on the forest plantation, allowing us to correctly interpret the incidence of the pest. The peak of the number of attacked shoots is observed after 45 days of the experiment, however, this peak resembles the distribution of the attack in the last three evaluations (Figure 7b), corroborating with the information on the incidence of the pest in these months (SÁNCHEZ-SOTO et al., 2009). Furthermore, between the 45 and 60 days of the experiment, heavy rains occurred in the experimental area, and many shoots were lost due to the strong winds (Figure 7c). The phenomenon of shoots with abiotic damage was important in this area and continued from the end of August to September (Figure 7c). Apparently, mahogany trees have a growth response mechanism to the combined phenomena of biotic and abiotic attack, emitting a large number of new shoots in a relatively small-time interval (120 days of experiment) (Figure 7b, c and d).

The experiment was installed between the months of highest incidence of *H. grandella* in the tropical areas of Mexico. However, as insect attack is not uniformly distributed across the stand, the effects tend to be dispersed. In the present study, the relative number of shoots attacked per tree was used, but, most likely, another experimental design option would be to work with composite experimental units instead of individual trees. With a more robust design, the relative attack response would provide us with data that is easier to analyse, even to be transformed appropriately to meet the parametric testing criteria. Furthermore, the use of composite experimental units could reduce the measurement error depending on the distribution of the attack in the stand. It is also important to mention that the induction of attack on plants is undoubtedly one of the best options to verify the effect of application methods and insecticide types (CHI et al., 2021; SIAL & BRUNNER, 2010; SINGH et al., 2016; YUE et al., 2003). However, this type of experiments is only viable with plants in nurseries (CHI et al., 2021); It is not viable at the field level, both due to the difficulty of raising *H. grandella* larvae and the difficulty of guaranteeing that the attack occurs in a poorly controlled environment.

The active substances used in this study (imidacloprid, spinetoram and emamectin benzoate) have been reported with different levels of effectiveness for the control of Lepidoptera (SAMMANI et al., 2020; SIAL & BRUNNER 2010) and more specifically the Pyralidae family (CHI et al., 2021; SINGH et al., 2016; YUE et al., 2003; ZHANG et al., 2014). In this study it was possible to verify that no differences were observed between the effects of the microinjection and spray methods of these substances on the rNAST response (Table 1). When the insecticides were compared as independent groups, only imidacloprid and emamectin benzoate differed from each other (Figure 4). Graphically, it is possible to verify that the mode of rNAST in treatment with imidacloprid is zero (Figure 4). Unlike the results found in other studies (HOWARD & MERIDA, 2004; SIAL & BRUNNER, 2010; SINGH et al., 2016; YUE et al., 2003), in the 30-day evaluation, imidacloprid has reduced the number of attacked shoots (Figure 4). After 45 days of the experiment, it was verified that the application of emamectin benzoate by microinjection has reduced the attack on the trees by up to two times compared to the spraying method (Figure 5). However, in the overall comparisons of this fourth evaluation, there were differences between imidacloprid and emamectin benzoate (Figure 6). By graphical analysis, the maximum attack values on mahogany shoots occurred respectively in the emamectin benzoate, spinetoram and control groups (Figure 6). The mortality effect of larvae of other insects of the Pyralidae family associated with spinetoram and emamectin benzoate (SAMMANI et al., 2020; ZHANG et al., 2014) was not observed in the current study with the microinjection and spraying methods. Particularly, it was observed that the lower effect of emamectin benzoate applied by spraying may be related to losses due to rain and temperature, a common problem with the application of pesticides in tropical areas (ALLAN et al., 1973, 1976).

According to Doccola and Wild (2012), injection into trees creates wounds; however, the benefit of the active substances introduced to protect the trees outweighs the possible physical damage from the drilling wound. In the 120 days that the experiment lasted, the impact of the microinjection on the growth of the trees could not be verified (Table 2). There were no significant differences between the averages of NDia, BDia, and THei for the treatments in each of the evaluations (Table 2). Nor could differences be observed between the average increases for these same variables (Table 2). The morphological damage of *H. grandella* to mahoganies is irreversible (Figure 2), therefore, chemical control methods remain alternatives to combat the insect (ALLAN et al., 1973, 1976; CIBRIÁN TOVAR, 2013; GOULET et al., 2005; HOWARD & MERIDA, 2004; WYLIE, 2001). A negative effect of the microinjection method (Figure 3c and d) was also not observed, because after 120 days of the experiment, the sampled trees had already expelled the cork plugs and were completely healed (Table 3). The economic viability that is involved with the application cost per method was also verified (Table 4). The cost of the imidacloprid application method is practically the same between spraying and microinjection (Table 4). The difference in cost is in relation to spinetoram and emamectin benzoate, where the microinjection method could represent up to 70% reduction in expenses (Table 4). The efficacy of imidacloprid was low, because there was no response to the shoot borer control after 45 days of the experiment (Table 1). For this reason, recommending the use of this substance implies increasing its concentration or the frequency of application of the doses, consequently increasing the risk of exposure (KATHURIA et al., 2023). To reduce the risks of exposure to humans and the environment, it is essential to use microinjection as a method of applying highly hazardous substances (BERGER & LAURENT, 2019; CIBRIÁN TOVAR, 2013; DOCCOLA & WILD, 2012; KUMAR et al., 2020). Repeating the experimental trials with the other substances that did not show efficacy in the control of *H. grandella* on *S. macrophylla* (Table 1; Figures 4-6), would also imply recalculating concentrations and dose frequencies. Future evaluations should investigate the seasonal dynamics of systemic insecticide residues applied in commercial forest plantations and how these substances may affect bees (COSLOR et al., 2019) and other biotic and abiotic components of local ecosystems.

4. CONCLUSIONS

The two evaluations at 30 and 45 days of the experiment were the only ones that presented differences between treatments that combine microinjection and spraying methods and the insecticides imidacloprid, spinetoram and emamectin benzoate for the control of *H. grandella* in the forest plantation of *S. macrophylla* (ANOVA F test at 10% error).

In the third evaluation, the significant difference (Kruskal-Wallis at 10% error) of the overall rNAST response between insecticides was dismembered by Dunn's pairwise comparison test with Holm correction and showed difference between the independent groups of imidacloprid and emamectin benzoate.

Fifteen days later, in the next evaluation, a significant difference was detected (Mann-Whitney U test at 10%) in the rNAST response to emamectin benzoate, with greater effectiveness of the microinjection method. However, there was a difference between the individual insecticide groups in the spraying method (Kruskal-Wallis at 10% error). During this period, imidacloprid was more effective against the Meliaceae shoot borer compared to the other treatments. However, from 45 days to the end of the experiment (120 days) there was no effect of the applied imidacloprid concentrations of 1.155 g a.i. L^{-1} by microinjection and 0.07 g a.i. L^{-1} by spraying on the insect attack.

There was no effect of the treatments on the normal diameter, basal diameter and total height variables and their respective increments. The mahogany trees managed to heal completely after 120 days of the experiment.

Between the months of May and September, a period favoured by the rainy season, mahogany trees showed a natural capacity for continuous emission of new shoots and also in response to biotic losses due to pest attack and abiotic losses due to the action of strong winds.

5. ACKNOWLEDGMENTS

We thank the National Council of Humanities, Sciences and Technologies (Conahcyt) for the master's scholarship awarded to the first author. We thank the Ejido El Tortuguero for the permission granted to install the experiment within their property.

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