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## Biological Effectiveness of Insecticides to Control Cone Beetle in Pinyon Pine Plantations

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### ABSTRACT

Pinyon pine plantations are attacked by insects that feed on conelets and seeds, a phytosanitary problem that causes economic losses in production. The study evaluated the biological effectiveness (BE) of chemical insecticides to control *Conophthorus edulis* Hopkins in a *Pinus cembroides* Zucc. forest plantation. The treatments abamectin (18 g of active ingredient (a.i.) L<sup>-1</sup>), emamectin benzoate (19.2 g of a.i. L<sup>-1</sup>), azadirachtin (738.4 g of a.i. L<sup>-1</sup>) and an untreated check with water were applied randomly with 5 repetitions. The application was repeated 56 days later. Each repetition consisted of applying a treatment to a tree with a cohort of 20 strobili. The BE of the insecticides was determined by the percentage of healthy conelets for each treatment in 8 evaluations: 0, 19, 32, 50, 65, 83, 109 and 144 days. No significant differences were found between treatments at 5% error by the ANOVA F test. Between the months of August and September, general averages were found between 0.25 to 2.5% of attacked conelets. An increase of 21.25% was observed in the average number of dead conelets between the initial and final evaluations in September.

KEYWORDS: Forest management, Entomology, insect control, pesticides.

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## Efectividad biológica de insecticidas para controlar el escarabajo del cono en plantaciones de pino piñonero

### RESUMEN

Las plantaciones de pino piñonero son atacadas por insectos que se alimentan de piñas y semillas, un problema fitosanitario que provoca pérdidas económicas en la producción. El estudio evaluó la efectividad biológica (EB) de insecticidas químicos para controlar *Conophthorus edulis* Hopkins en una plantación forestal de *Pinus cembroides* Zucc. Los tratamientos abamectina (18 g de ingrediente activo (i.a.) L<sup>-1</sup>), benzoato de emamectina (19.2 g de i.a. L<sup>-1</sup>), azadiractina (738.4 g de i.a. L<sup>-1</sup>) y un testigo con agua se aplicaron aleatoriamente con 5 repeticiones. La aplicación se repitió 56 días después. Cada repetición consistió en aplicar un tratamiento a un árbol con una cohorte de 20 estróbilos. El BE de los insecticidas se determinó por el porcentaje de conillos sanos para cada tratamiento en 8 evaluaciones: 0, 19, 32, 50, 65, 83, 109 y 144 días. No se encontraron diferencias significativas entre tratamientos al 5% de error mediante la prueba F de la ANOVA. Entre los meses de agosto y septiembre se encontraron promedios generales entre 0.25 a 2.5% de conillos atacados. Se observó un aumento del 21.25% en el número promedio de conillos muertos entre la evaluación inicial y final en septiembre.

**PALABRAS CLAVE:** Manejo forestal, Entomología, control de insectos, plaguicidas.

### Introduction

One of the great challenges of forest management of pinyon-producing pine species is the control of insects that cause damage to harvests (Botkin and Shires, 1948; Eguiluz, 1982; Farjon and Filer, 2013; Mohedano-Caballero et al., 1999; Robert, 1977). In central-eastern Mexico, the main predator of pinyons of the species of *Pinus cembroides* Zucc. is cone beetle *Conophthorus edulis* Hopkins (Cibrián-Tovar et al., 1986; Flores Flores and Díaz E., 1991a; Flores Flores and Díaz Esquivel, 1989; Martínez Ramírez et al., 1985). The incidence of this insect in practically all seasons of the year in commercial plantations in Mexico represents important economic losses (Cibrián-Tovar et al., 1986; Flores Flores and Díaz E., 1991a; Flores Flores and Díaz Esquivel, 1989; Martínez Ramírez et al., 1985). For this reason, chemical control methods are still an alternative for managing this problem.

In Mexico, during the period from 2000 to 2021, approximately 240,000 hectares of commercial forest plantations were established with support from the federal government. Fifteen percent of this surface was planted with species of the *Pinus* genus (CONAFOR, 2022). Within this genus is the species *P. cembroides*, one of the most important of the Mexican pinyon trees (Flores Flores and Díaz E., 1991b; Flores Flores and Díaz Esquivel, 1989; Flores Flores and Díaz Ezquível., 1985; J. Flores Lara and López, 1989; Granados Victorino et al., 2015). Its importance lies in its wide natural distribution, dominance, abundance, use in establishing plantations and economic value, since it constitutes 90% of the Mexican pinyon harvest (Botkin and Shires, 1948; Eguiluz, 1982; Farjon and Filer, 2013; Mohedano-Caballero et al., 1999; Robert, 1977).

In forest management scenarios focused on seed production in forests, plantations, seed stands, seed orchards and germplasm producing units. Insects that feed on cones, fruits, seeds, and shoots are of great importance due to the impact they have on the harvest (Miranda, 2021). Insect predation of conelets and seeds in Mexican pinyon trees can cause crop losses of more than 90% and is frequently greater than 60% (Cibrián-Tovar et al., 1986; Flores Flores and Díaz E., 1991b; Flores Flores and Díaz Esquivel, 1989; Flores Flores and Díaz Ezquível., 1985; J. Flores Lara and López, 1989; Martínez Ramírez et al., 1985). *Conophthorus edulis* Hopkins is the main insect that causes mortality in cones and conelets of *P. cembroides* in Mexico. Damage between 40 to 60% has been reported in conelets production (Cibrián-Tovar et al., 1986; Flores Flores and Díaz E., 1991a; Flores Flores and Díaz Esquivel, 1989; Martínez Ramírez et al., 1985). Looking for alternatives to reduce the economic impact of the pest attack, the objective of the research was to determine the biological effectiveness (BE) of chemical insecticides to control *C. edulis* on a *P. cembroides* forest plantation.

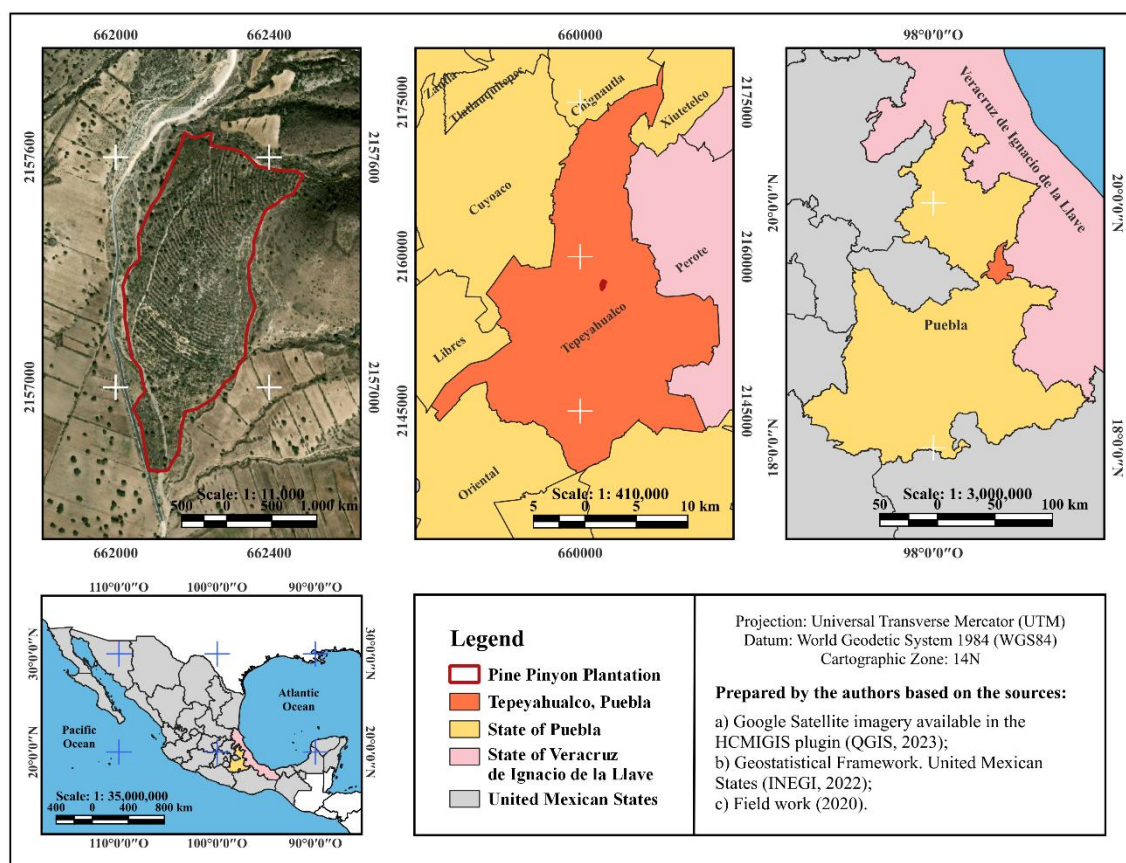
## 1. Materials and Methods

### 1.1. Study area

The study was carried out in a *P. cembroides* forest plantation with an altitude range between 2,361 to 2,456 masl, located in Pizarro, Tepeyahualco, Puebla. (Figure 1). The climate according to the Köppen classification modified by García (1986) is semi-arid (BS1k'w). According to the World Reference Base for Soil Resources (WRB), Leptosols

(LP) Eutric (eu) Lithic (li) and Regosols (RG) Calcaric (ca) Skeletic (sk) predominate in the study area (INEGI, 2007).

**Figure 1.** Forest plantation of *Pinus cembroides* Zucc. under attack by *Conophthorus edulis* Hopkins.



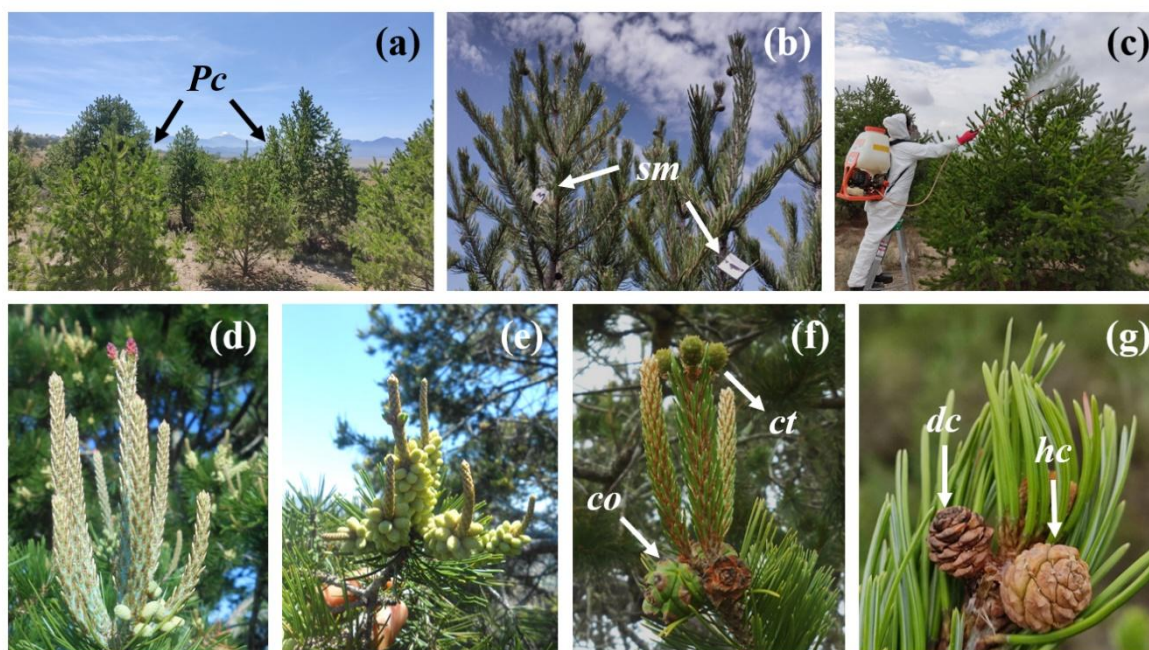
## 1.2. Experimental design and sampling

Sampling was carried out in the period from July to December 2020 (Figure 2), during the conelet growth stage and the attack period of adults of the second generation of the cone beetle life cycle. From the forest plantation (Figure 2a), 20 trees were evaluated, severely attacked by the insect, with an approximate age of 11 years, presence of conelets, diameter between 12.5 to 15.5 cm, total height between 3 to 5 m. The experiment was conducted in a completely randomized design with four treatments (three insecticides and an untreated check) with five repetitions each. Each repetition consisted of applying a



treatment to a tree with a cohort of 20 strobili (conelets), individually identified, with an aluminium tag for monitoring during the study period (Figure 2b).

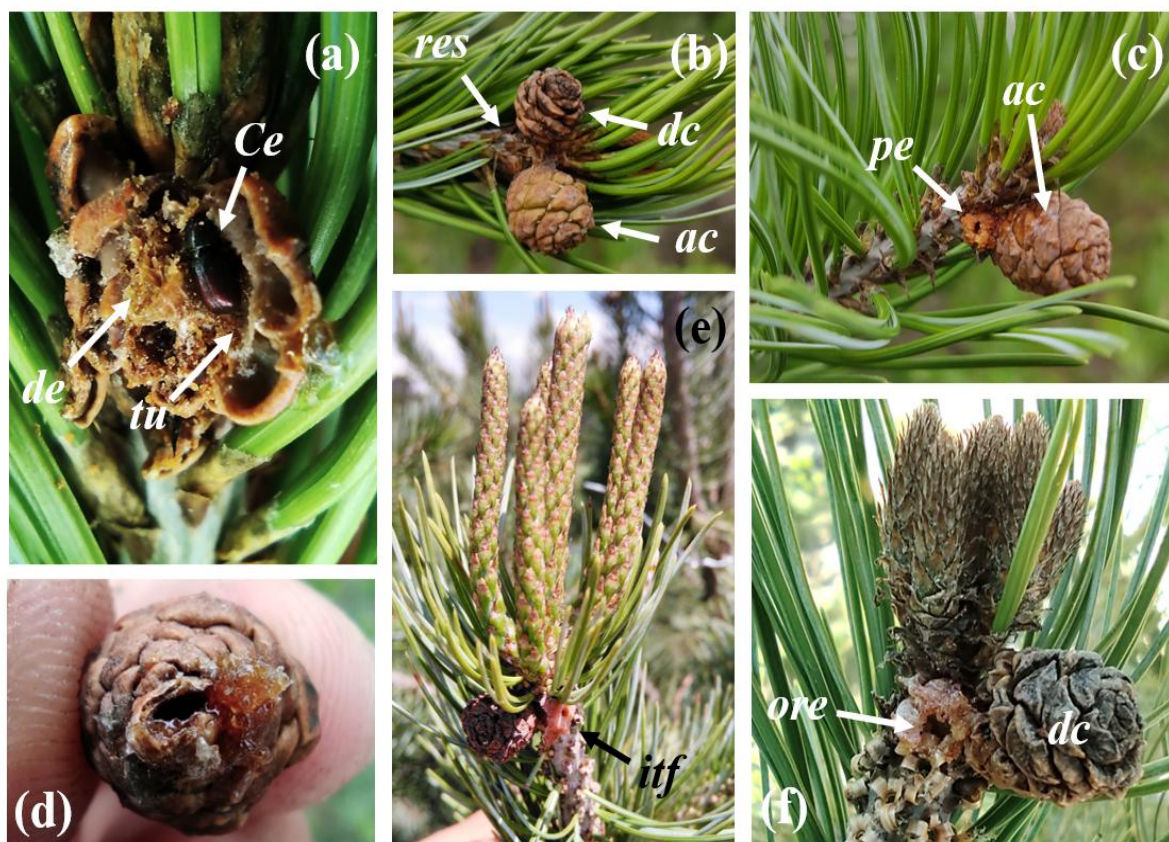
Figure 2. General information of the experiment: a) Forest plantation of *Pinus cembroides* Zucc.; b) Sampling marking (*sm*); c) Spraying chemical treatments; d) Female strobile; e) Male strobile; f) Conelet (*ct*) and cone (*co*); g) dead conelet (*dc*) and healthy conelet (*hc*). Pizarro, Tepeyahualco, Puebla (July to December 2020).



The insecticides doses that corresponded to each chemical treatment were: abamectin 1 ml L<sup>-1</sup> (18 g of active ingredient (a.i.) L<sup>-1</sup>, Agrimec® 1.8 CE), emamectin benzoate 0.12 ml L<sup>-1</sup> (19.2 g of a.i., Denim® 19 CE), and azadirachtin 0.4 ml L<sup>-1</sup> (738.4 g of a.i., Progranico® Nimicida 80 CE). The untreated check was the application of water. Applications were made on July 31, 2020 and September 25, 2020 (56 days later). Drip point fumigations were carried out in the tree crowns (Figure 2c), completely covering the strobili. To apply the treatments, a Forza 25® motorized backpack sprayer was used (model 818025, capacity 25 L, Swissmex S.A. de C.V.), with a double outlet NN-D-6 flat fan nozzle at 20 bar. The application dose was 5500 L ha<sup>-1</sup>. In both applications the expenditure was approximately 5 L tree<sup>-1</sup>.

Cohorts of treated conelets were evaluated at 0, 19, 32, 50, 65, 83, 109, and 144 days. The phytosanitary status of each strobile was considered by the following damages (Figure 3): 1) perforation or mass of resin at the base of the peduncle and in the structure; 2) galleries inside; 3) adults, preimagos, pupae, larvae. A visual inspection was carried out to identify attacked, dead and healthy conelets (Figures 2 and 3).

Figure 3. Evidence of damage caused by *cone beetle* in pinyon conelets (*Pinus cembroides* Zucc.): a) Attacked conelet with the presence of the *Conophthorus edulis* Hopkins (Ce), adult insect and tunnels (tu) and debris (de); b) Evidences of the recent attacked conelet (ac) with the presence of secreted resin (res), and, dead conelet (dc); c) Recent attacked conelet (ac) with perforation at the base of the peduncle (pe) with a secreted resin; d) Tunnel formation of the with perforation at the base of the conelet with a secreted resin; e) Attacked conelet with initial tunnel formation (itf) in the base of the peduncle; f) dead conelet (dc) with the presence secreted old resin (ore) at the base of the peduncle. Pizarro, Tepeyahualco, Puebla (July to December 2020).



### 1.3. Statistical analysis

The BE of insecticides was calculated using the percentage of healthy conelets (Figure 2e) with no evidence of conelet beetle damage, for each treatment, replicate and date from the 19th day onwards. The data were subjected to the F test of the analysis of variance (ANOVA) with a probability level of error of 5% to determine the differences in averages between treatments (Table 1). All statistical procedures were performed in the R environment (R Core Team, 2023).

## 2. Results

The evaluation of the BE began on the 19<sup>th</sup> day of the experiment in the *P. cembroides* forest plantation (Table 1). The requirements of normality and homoscedasticity of the healthy conelets variable were met for the ANOVA, except for the evaluation on August 19, where the variable was transformed by a standardized function (Table 1). According to the F test in the ANOVA at 5% significance, there were no differences between averages of healthy conelets by treatments on each of the different days (Table 1). As of October 4, the coefficient of variation exceeds 30% and reaches 48.58% on the last day of the evaluation (Table 1). In the last evaluation (144 days after the application of the insecticides) a general relative average of 37.75% healthy conelets was found (Table 1; Figure 4). The identification of attacked conelets has a relative average of 2.5% in the month of September (Figure 4). Also, between the 1st and 19th of September there was an increase of 21.25% in the relative average of dead conelets (Figure 4). From mid-September to October the relative increase in dead conelets remained between 5 to 8% (Figure 4). Between the evaluations from November to December, the average relative increase was 2.75% (Figure 4).

## 3. Discussion

There was no BE of the insecticides evaluated compared to what was reported for *C. ponderosae* in *P. monticolae*, testing permethrin in different doses with 1 and 2 applications (Shea and McGregor, 1987). It is possible that the BE of the insecticides in the second application could have been negatively affected due to precipitation that occurred in the area after the spraying of the treatments. Only between the months of August and September it was possible to identify signs of *C. edulis* attack in the conelets evaluated



(Figure 4). On the other hand, in this study area, conelet mortality increased in the month of September (Figure 4). Strobili cohorts were marked months before the start of the experiment (Figure 2b). The evaluation dates of the experiment between the months of July and August correspond to the initial stages of conelet formation (fertilized strobili). Conelets in the developing phase were more susceptible to attack by the cone beetle (Figure 4a), resulting in an increase in conelet mortality in September (Figure 4b). In addition to this, the increase in the variability of the results found from the month of October (Table 1) may indicate the need to increase the sample size for this type of field evaluations. A decrease in conelet mortality was observed between the months of November and December (Figure 4), a phenomenon that may be associated with low temperatures of these months.

Figure 4. Time evolution of the phytosanitary condition of *Pinus cembroides* Zucc. conelets (a, b, c) under attack by *Conophthorus edulis* Hopkins in a forest plantation in Tepeyahualco, Puebla, Mexico.

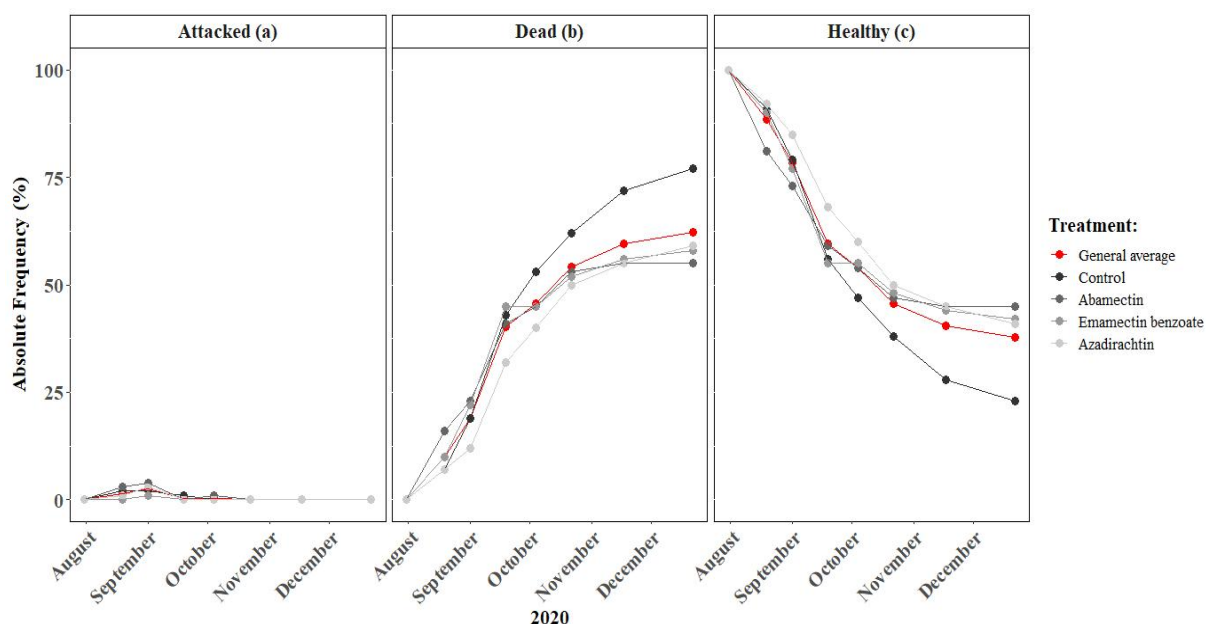


Table 1. Summary of the analysis of variance and descriptive statistics of the biological effectiveness of insecticides to control *Conophthorus edulis* Hopkins in a *Pinus cembroides* Zucc. plantation, Tepeyahualco, Puebla, Mexico

| Variation Source                                       | Degree of Freedom | Healthy Conelets (2020)                                           |               |               |               |               |               |               |               |
|--------------------------------------------------------|-------------------|-------------------------------------------------------------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
|                                                        |                   | July 31                                                           | Aug 19        | Sep 1         | Sep 19        | Oct 4         | Oct 22        | Nov 17        | Dec 22        |
|                                                        |                   | Mean Square                                                       |               |               |               |               |               |               |               |
| Treatments                                             | 3                 | NA                                                                | 63.30†        | 125           | 175           | 143.33        | 141.25        | 348.33        | 497.92        |
| Residuals                                              | 16                | NA                                                                | 146.39†       | 339.37        | 307.5         | 340.63        | 349.38        | 350           | 336.25        |
| Total                                                  | 19                |                                                                   |               |               |               |               |               |               |               |
| Statistical tests                                      |                   | p-value                                                           |               |               |               |               |               |               |               |
| F-test                                                 |                   | NA                                                                | 0.73†         | 0.78          | 0.64          | 0.74          | 0.75          | 0.42          | 0.26          |
| Shapiro-Wilk test                                      |                   | NA                                                                | 0.45†         | 0.63          | 0.48          | 0.80          | 0.44          | 0.73          | 0.82          |
| Bartlett's test                                        |                   | NA                                                                | 0.93†         | 0.65          | 0.22          | 0.48          | 0.51          | 0.50          | 0.78          |
|                                                        |                   | Coefficient of Variation (%)                                      |               |               |               |               |               |               |               |
|                                                        |                   | NA                                                                | 13.7†         | 23.47         | 29.47         | 34.18         | 40.86         | 46.19         | 48.58         |
| Treatments                                             |                   | Absolute Frequency (Average ± Standard Deviation, %) <sup>3</sup> |               |               |               |               |               |               |               |
| Untreated Check <sup>1</sup>                           |                   | 100.00 ± 0.00                                                     | 91.00 ± 12.45 | 79.00 ± 16.36 | 56.00 ± 9.62  | 47.00 ± 16.43 | 38.00 ± 12.04 | 28.00 ± 10.95 | 23.00 ± 13.51 |
| Agrimec® 1.8 CE (Abamectin) <sup>2</sup>               |                   | 100.00 ± 0.00                                                     | 81.00 ± 21.33 | 73.00 ± 23.87 | 59.00 ± 24.34 | 54.00 ± 24.85 | 47.00 ± 22.25 | 45.00 ± 23.18 | 45.00 ± 23.18 |
| Denim® 19 CE (Emamectin benzoate) <sup>2</sup>         |                   | 100.00 ± 0.00                                                     | 90.00 ± 11.73 | 77.00 ± 19.24 | 55.00 ± 10.61 | 55.00 ± 10.61 | 48.00 ± 13.96 | 44.00 ± 15.57 | 42.00 ± 16.81 |
| Progranico® Nimicide 80 CE (Azadirachtin) <sup>2</sup> |                   | 100.00 ± 0.00                                                     | 92.00 ± 6.71  | 85.00 ± 12.25 | 68.00 ± 20.80 | 60.00 ± 19.04 | 50.00 ± 23.72 | 45.00 ± 22.36 | 41.00 ± 18.51 |
| General average                                        |                   | 100.00 ± 0.00                                                     | 88.50 ± 13.68 | 78.50 ± 17.48 | 59.50 ± 16.93 | 54.00 ± 17.59 | 45.75 ± 17.79 | 40.50 ± 18.70 | 37.75 ± 19.02 |

†Variable transformed by the standardized function (z-score). <sup>1</sup>Water application. <sup>2</sup>Application rate of 5500 L ha<sup>-1</sup>. <sup>3</sup>Five repetitions per treatment.

It was reported for *C. coniperta* in a *P. strobus* seed stand, using the mating interruption control method, an average reduction in conelet damage of 64% was obtained in areas treated with the pheromone pityol (Trudel et al., 2004). In use of conventional control methods such as chemical insecticides, it is being restricted worldwide. Research has been carried out with semiochemicals for several species of *Conophthorus* from North America, with the objective of understanding the behaviour of the insect and exploring its use as control methods mainly in seed orchards (Miller et al., 2000; Rappaport et al., 2000; Trudel et al., 2004).

A control method that can be implemented in plantation conditions is prescribed burning. The application of low intensity burning in seed stands of *P. resinosa* increased the number of conelets without *C. resinosae* infestation by between 11% and 45% (W. E. Miller, 1978). The use of fire as a control method is limited to stands with mature trees, species with fire-tolerant bark, and sites with sufficient fuel load, to ensure that this method is effective in controlling insect populations (Trudel et al. al., 2004).

## Conclusions

The application of evaluated insecticides did not increase the survival of *P. cembroides* conelets. However, in addition to the relative percentage of healthy conelets, to better understand the response of these chemical controls on visual inspection variables such as attacked and dead conelets, it is suggested to expand the number of samples and increase the number of applications in future experimental trials in the pinyon pine plantations. In addition to chemical control methods for *C. edulis*, future biological effectiveness studies should test other methods, with the aim of increasing options in a context of integrated pest management and reducing the use of insecticides.

The general average of conelets attacked between the months of August and September ranges between 0.25 to 2.5%. In the last evaluation, 144 days after the application of insecticides and untreated check, the general averages of attacked, dead and healthy conelets were 0, 62.25 and 37.75%, respectively. In the month of September there was an increase of 21.25% in the average number of dead conelets.

The methodology used to monitor the phytosanitary status of *P. cembroides* conelets can be adapted and expanded to evaluate the biological effectiveness of several control

methods and studies evaluating the impact of insects on the production of cones and conelets, and seeds.

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