Article

Perspectives on the use of *Verbenone* to protect pine seed production from attack by *Conophthorus spp* (Coleoptera: Curculionidae, Scolytinae)

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Received 31 October 2014; Accepted 5 December 2014; Published online 1 March 2015

Abstract

In three study sites in the Michoacan State, Mexico, we tested five behavioral chemicals: *Pityol*, *4 Allylanisole*, *Verbenone* (3M MEC), *Conophthorin* and *Hexenol*, in several field trials mixed at six combinations or treatments as posible repelents for females of *Conophthorus conicolens* W and *Conophthorus teocotum* W. Beetle-host tree combinations included these two cone bores species on cones of Pinus pseudostrobus (Lidl), the first, and on *Pinus teocote* (Schl & Cham) the second ones, baited previously with five semiochemicals, including the only case of Verbenone (3 M Mec) which was sprayed alone on healthy green cones of second year growing free from the presence of cone borers and previously to fly period of scolitids as at all other treatments. Cones baited with the combination of semiochemicals *P+4AA+V* besides these sprayed with *Verbenone* were less attacked and obviously distinctibily. Moreover, cones baited only with *Pityol* and adding *Verbenone* as a spray periodically, the effect of treatment was inefficient to protect them from boring by *Conophthorus conicolens* W, whereas these baited with *P+4AA* synergized the presence of cone borer females; The combination of all these semiochemicals and added with *Conophthorin* (P+4AA+C) and *Hexenol* (P+4AA+H) had inconsistent results, although for this last case, *Hexenol* apparently was synergist when is mixed with *Pityol* and 4AA , to prevent from attack by *Conophthorus teocotum* W. The best results of study subject were obtained with the combined treatment *Pityol +4AA+Verbenone*, and significately better spraying as alone as *Verbenone* and repelling *C. conicolens* W and *C. teocotum* W.

Keywords semiochemicals; seed protection; cone borer beetles; *Pityol*; *4 allylanisole*; Verbenone; *Conophthorin*; *Hexenol*; synergistic effect.

Arthropods
ISSN 2224-4255
URL: http://www.iaees.org/publications/journals/arthropods/online-version.asp
RSS: http://www.iaees.org/publications/journals/arthropods/rss.xml
E-mail: arthropods@iaees.org
Editor-in-Chief: WenJun Zhang
Publisher: International Academy of Ecology and Environmental Sciences

1 Introduction

The mexican cone borer *Conophthorus conicolens* Wood (Curculionidae: Scolytinae) is a endemic pest widely distributed in the Northwest Pacific Coast and central región of Mexico. Its damages on the annual natural production of seeds are estimate in order of 15-60% (Del Rio and Mayo, 1988) and causing with this a hight lost and assesment on natural regeneration process in the conifer forest lands or sites under forest management techniques.

Pheromones are nowadays no only important in the detection, monitoring and suppresion as means for mass-trapping or mating disruption of pest populations (Cardé and Minsk, 1997), but in addition to their promisory use as interruptants or repellents focused to reducing pest damagesd without being directed at pest population levels (Munakata, 1977).

The subject of this study was evaluate the response of two cone borer beetles: *C.conicolens* W and *C.teocotum* W to the use of behavioral chemicals, *Pityol*, *4 Allylanisole*, *Verbenone* (3 M Mec), *Conophthorin* and *Hexenol*, in several field trials directed at six combinations and baited directly on healthy cones of second year growing, and then assessed the effects on female beetles response with perspectives for pine cones protection.

Cone borer beetles suppression does not always reduce damage, because compensatory mechanisms (increased immigration or reduced competition) can maintain damage levels even when large numbers of insects are trapped. The male trap-out strategy to disrupt mating and thereby reduce damage has promise, but it may reduce cone beetle populations and yet fail to protect cones due to female cone beetles initiate cone attack as the first step in courtship (Hedlin et al., 1980), so even unmated females kill eones. For these reasons, one of our primary goals is the development of female-specific cone beetle repellents (Rappaport, et al., 2000).

2 Antecedents

Verbenone has demonstrated efficacy as an anti-aggregation pheromone for many bark beetles of the genus Dendroctonus (Coleoptera: Scolytinae), although there is not any antecedents of its use for protecting cones from attack by cone borer beetles and as alone some studs on response of some forest pests to behavioral chemicals as it is cited below:

In fact, *S-(-) VERBENONE, (1S,5S)-4,6,6-TRIMETHYLBICYCLO(3.1.1) HEPT-3-EN-2-ONE),* a beetle-produced antiaggregation pheromone also found in pines (Kainulainen and Holopainen, 2002) and a wide variety of angiosperms (Molyneux et al., 1980; Guillen and Cabo, 1996; Fournier et al., 1997; Buttery et al., 2000; Umano et al., 2000; Pintore et al., 2002; Sefidkon et al., 2002; Ghannadi and Zolfaghari, 2003; Robles et al., 2003) can be effective in limiting damage to pines by bark beetles (Payne and Billings, 1989; Payne et al 1992; Salom et al., 1995; Borden, 1997; Clarke et al., 1999; Lindgren and Miller, 2002; Kegley et al., 2003; Progar, 2005; Gibson and Kegley, 2004; Bentz et al., 2005). This behavioral semiochemical has been too previously identified in hindgut extracts of the cone borer beetle *Conophthorus coniperda* (Birgersson et al., 1995) and in Porapaq-Q collections from *C. ponderosae* (Rappaport et al., 2000).

In addition to the previous documented , as resulting from a long researches during the period 1997-2002 were realized in Western North America and Mexico which have reavealed several beahaviorally active chemicals for beetles in the genus *Conophthorus* what they have had like purpose the development and application of semiochemicals for protection of pine seed crop, studies conduced by the University of Michoacan and the USDA Forest Service, we have verified the effect antiaggregation of *Verbenone* baited in bubblecaps alone or in combinations with other semiochemicals as *Pityol*, *Conophthorin*, *4-Allynanisol* and 2 *Hexeno*l, using the model of “japan beetle traps” for the monitoring of cone borer beetles populations

(Rappaport et al., 2000; Del Rio, 2001).

The precedent studies showned that for *Conophthorus conicolens* Wood and *C.teocotum* Wood traps baited with a combination of Pityol + 4 Allynalisole(a synergizant in this case) catched more male cone bore beetles tan unbaited traps and the addition of *Verbenone* was neutralized without repellency effect, whereas the addition on the treatments either *Conophthorin* alone or *Conophthorin + Verbenone* (ratio 1:1) increased substantially the response from male cone borer beetle and moreover if these two semiochemicals go together added with *Pityol* plus *4-Allynalisone* reduce the adults trap catched. *Pityol* –baited traps did not catch significantly more tan unbaited traps (Rappaport et al., 2000).

Pityol,(+)-*TRANS*-PITYOL(2R,5S)-(+)-2-(1-HYDROXY-1-METHYLETHYL) 5METHYLTETRAHYDROFURAN, have been identified as a female produced attractant pheromone in *Conophthorus coniperda*(Schwarz) and *Conophthorus resinosae* Hopkins and as an inhibitory compound in males: *Conophthorin*((5S,7S)-(-)-7-METHYL-1,6-DIOXASPIRO-(4,5)(DECANE) (Birgersson et al., 1995; Pierce et al., 1995). Both behavioral chemicals have been isolated on other *Conophthorus* species (Miller et al., 2000).

The PHENYLPROPANOID compound or 4-ALLYLANISOL (ESTRAGOL, METHYL CHAVICOL) is an interuptant for Scolytinae(Coleoptera) in the genera *Ips* and *Dendroctonus* (Hayes and Strom, 1994).

For last, the HEXENOL("GREEN LEAF VOLATILE")= (E)-2-HEXEN-1-OL, is a chemical of short chain aliphatic known as interrupt the response to attractants in Scolytinae (Dickens et al., 1992; Wilson et al., 1996; Borden et al., 1997).

In general, it is hypothesized that a single component pheromone could be effective if deployed in a release system with the proper reléase characteristics and sustained high reléase lasting throughout the beetle flight period, as a premise for its effecttiveness. About this there are some positive experiences on this trend, anyway, Guillete et al 2006 tested with good results a sprayable wáter suspensión of microencapsulated *Verbenone*, as a repelent for the populations of *Dendroctonus valens* (Coleoptera: Scolytinae), so *Verbenone* releasing flakes for protecting individual *Pinus contorta* tres from attack by *Dendroctonus ponderosae*, although other studies did not found mean difference between the control versus treatments using a *Verbenone* pouch and *Verbenone* in bubble caps as antiaggregants for single tree protection against several bark beetles species in USA (Lister et al., 1990; Fettig, 2004), which can be due to passive release devices influenced by abiotic conditions, movement and concentration of pheromone plume on the landscape or the effect of the *photoisomerization* what degrades *Verbenone* in the environment (Kostyk et al., 1993).

An Abstract of this study was early presented by Author into programme and Conferences of the IUFRO WP 7.03.05 Integrated Control of Scolytid bark beetles, in Vienna, Austria, 2007.

There is not much scientific information regarding to the use of pheromones to protecting the cone and seed crops from attack by cone borer beetles and the disponible literature generally is referent and of utlity to monitoring of them, due to which this paper has a special importance.

3 Methodology

3.1 Behavioral chemicals and release devices

Polyethylene bubblecaps were used as release devices (Phero Tech. Inc., Delta, Brithish Columbia) and the description of semiochemical-releasing devices, including Release rate (mg/day) as follows:

(+-) *trans*-Pityol (**P**)	Polyethylene bubblecap	0.14(chemical purities: >97%)
Conophthorin(10%)(**C**)	Eppendorf tube 1.5 ml	0.0.025
(E)-2-Hexen-1-ol(**H**)	Polyethylene bubblecap	3.0
4 Allylanisole(**4A**)	Polyethylene vial	75 (chemical purities: 80%)

3 MEC Verbenone (20% AI)(**V**) , a sprayable water suspension of microencapsulated verbenone, sample used for research purposes only(EPA EST. N0. 10350-CN-01, lot. # 120778-85-1).

The anterior chemicals were tested directly after top branches bearing cones were ramdomly choice, marked and monitored during all fly period of scolytids(June-September) in 4 diferent experiments carrying the next combinations of them as treatments as follow:

P+4A

P+4A+V(3 MEC Verbenone 20%AI)

P+4A+C

P+4+H

P + V(3 MEC Verbenone 20%AI)

V(3 MEC Verbenone 20% AI)

Fig. 1 Release devices used (left to right): Polyethylene bubblecaps (*Pityol*,*Hexenol*), Eppendorf tubes (*Conophthorin*), Polyethylene vials (4-AA) and Sprayable wáter suspensión (microcapsulate *Verbenone*).

3.2 Locations, host species and beetles species

Conophthorus conicolens Wood on *Pinus pseudostrobus* Lindl was studied in a seed production area managed by The Forest Community of San Juan Parangaricutiro, Michoacan, Mexico (19 25´N, 102 15´W and 19 27´N, 110 22´W), and in other site: the Forest Park Community Park in San Lorenzo, Michoacan, Mexico (19 31´37" N, 102 04´50 "W).

Conophthorus teocotum Wood on *Pinus teocote* Schl. & Cham. In a sparse stand near the town of Paracho, Michoacan., Mexico (19 39´N, 102 05´W).

The first and last one of locations mentioned were before experimental sites where Rappaport et al. (2000) tested the most behavioral chemicals here used for massive catching and effecting on beetle response.

3.3 Field tests of semiochemicals to protect pine cones from insect damage

Field experiments were carried out in 1999 and 2001, and all the combinations of semiochemicals mentioned above were applied before to the beginning of attack period for both *Conophthorus* species, by ending May of each year (Del Rio and Mayo, 1988).

On each tree was previously and randomlized choiced a variable number of branches from its middle crown bringing cones so for treatments for applying of semiochmicals as other ones more for control treatments at the same tree. For the case of these treatments including Verbenone (3 MEC), this was applied repetly on ones every month (June-August) on cones as a sprayable water suspension.

The cones baited and or sprayed with semiochemicals and their respective others unbaited or unsprayed (these last were used as Control treatments) were monitored during all the long period of flying and attacking from June till August and they were finally identified as attacked or healthies.

3.4 Evaluation of cone infestations

Initial cone counts on each individual test tres during the summers of 2 years projected for this study to determine the incidence of attacks by the cone bore beetles. Cones were examined and counted weekly to measure the effects of the different semiochemicals tested and these tallied as “infested” showed the typical signs of beetle attack (Fig. 3); These cones drop and tallied as “lost”in each treatments were registered as damages because it is characteristic they drop to soil more easy tan healthy cones, overall after strong winds. Final counts for all experiments and treatments were conducted in late August ending the adults fly period to determine cone borer infestations. A total of 52 trees were marked for 6 different tests and 536 cones monitored.

Fig. 2 Field trials: shoot with treatment P+4A.

Finally, percentaje data for cones damages were transformed to arcsin $\sqrt{X}$ (Zar, 1996); Therefore, the response variable evaluated was the percentages of cones infested by cone borer beetles for different trials and afterwords data each test were subjected to an simple oneway ANOVA to compare statistically significant differences among the cones damaged versus treatment, performed using Minitab 17 software.

Fig. 3 Left: Green cones of second year; Rigth: Cone attacked showing pitch tube on enter point bored by *Conopthorus conicolens* W female.

4 Results and Discussion

The results below are grouped on 4 tables and figures and corresponding each one by experiment (4). In general, all them show than trees protected with the combination *Pityol+4 Allylanisole+Verbenone* (3 M Verbenone 20%) and these sprayed only with same *Verbenone* had fewer beetle –killed cones that trees treated with other semiochemicals combination or without protection (Control or T), undepending from the cone beetle species (Figs 4 and 6; Fig 8: Control 3 versus P+4ª+V, and Fig 10: Control versus V), but protective effect of *Verbenone* is nullified when added *Pityol*(Fig 8: Control 2 versus P+V), while the treatment *Pityol* added with *4 Allylanisole* (P+4A) were more attacked by cone borer beetles (Figs 4 and 6; Fig 8: Control 1 versus P+4A):

Treatment	NO. Trees	NO. Cones	Mean percentage of attacked cones
Control	13	20	20.8 ± 8.1a
P+4A	13	24	30.1 ± 9b
P+4A+C	13	25	10.7 ± 5.5c
P+4A+V	13	21	0d
P+4A+H	13	15	21.1 ± 9a

* Means followed by the same letter in each column of the same experiment are not significantly different at p=0.05.

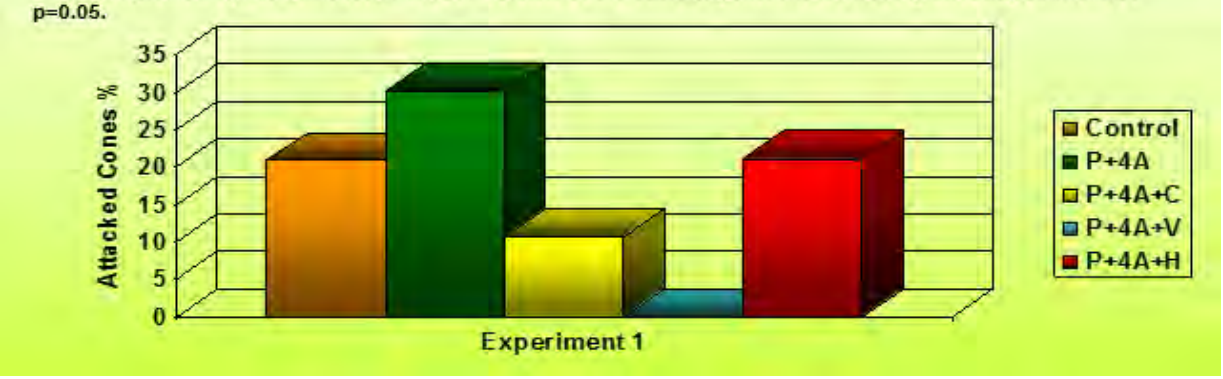


Fig. 4 Experiment 1: *Conophthorus conicolens* W on *Pinus pseudostrobus*, San Juan Nuevo Parangaricutiro, Michoacán, México (19° 25´ N, 102° 15´ W, 1999. Cone borer beetle damage (X ± SE), attacked cones.

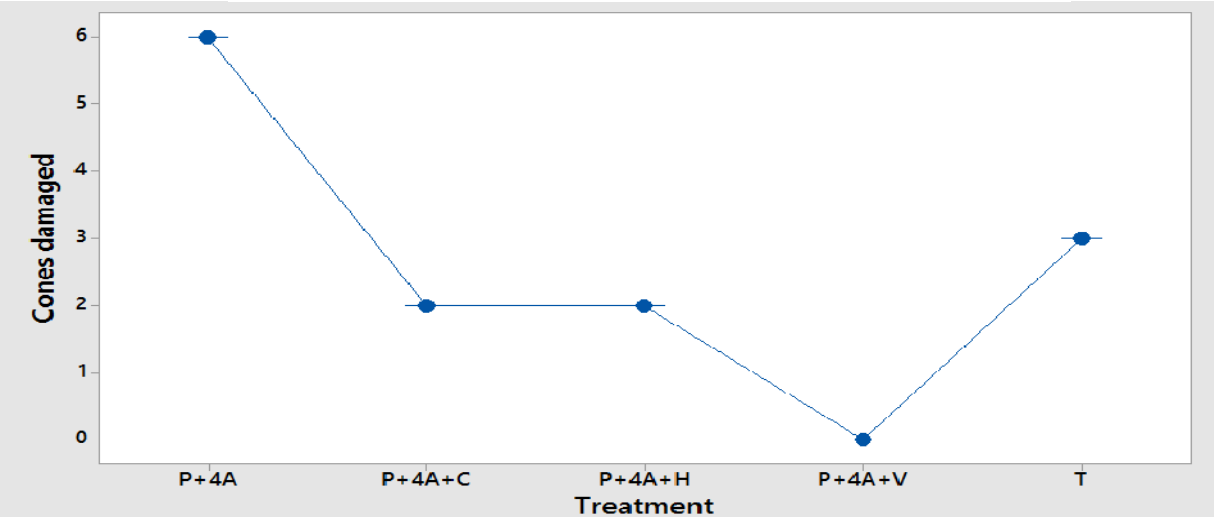


Fig. 5 Experiment 1: Interval plot of cones damaged for 4 treatments versus control (T) (95% Cl for the Mean). The pooled standard deviation was used to calculate the intervals.

Treatment	NO. Trees	NO. Cones	Mean percentage of attacked cones
Control	7	17	35,5 ± 11,3a
P+4A	7	14	36,3 ± 12,5a
P+4A+C	7	14	36,3 ± 12,5a
P+4A+V	7	18	0c
P+4A+H	7	16	18,5 ± 8,5b

* Means followed by the same letter in each column of the same experiment are not significantly different at p=0.05.

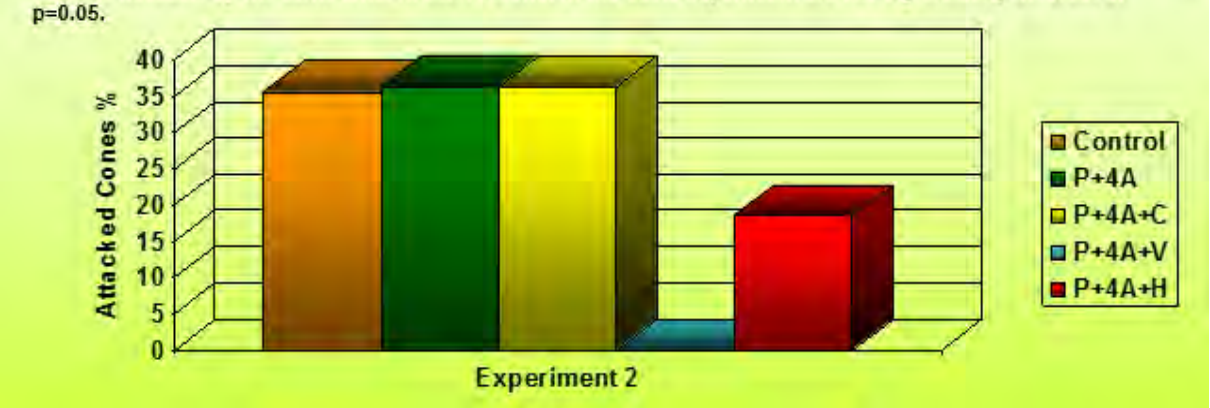


Fig. 6 Experiment 2: *Conophthorus teocotum* W on *Pinus teocote*. Paracho, Michoacán, México (19° 39´ N, 102°05´W, 1999. Cone borer beetle damage(X ± SE), attacked cones.

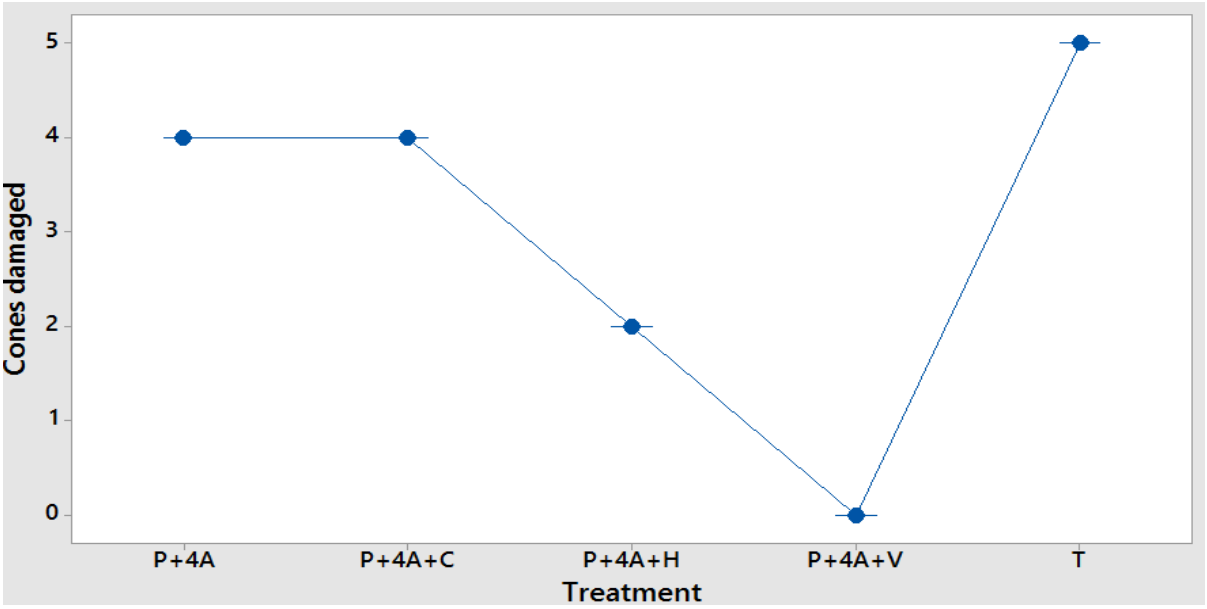


Fig. 7 Experiment 2: Interval plot of cones damaged versus control (T) (95% Cl for the Mean).The pooled standard deviation was used to calculate the intervals.

Treatment	NO. Trees	NO. Cones	Mean percentage of attacked cones
Control 1	4	22	0a
P+4A	4	17	44,3 ± 12,3b
Control 2	3	19	44,4 ± 11,7a
P+V	3	19	51,3 ± 11,7a
Control 3	5	37	57,6 ± 8,1a
P+4A+V	5	39	30,5 ± 7,3b

* Means followed by the same letter in each column of the same experiment are not significantly different at p=0.05.

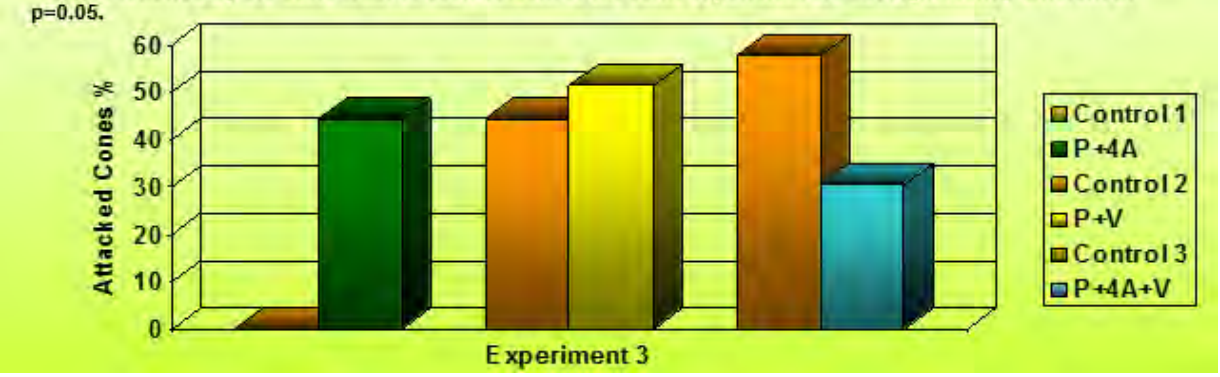
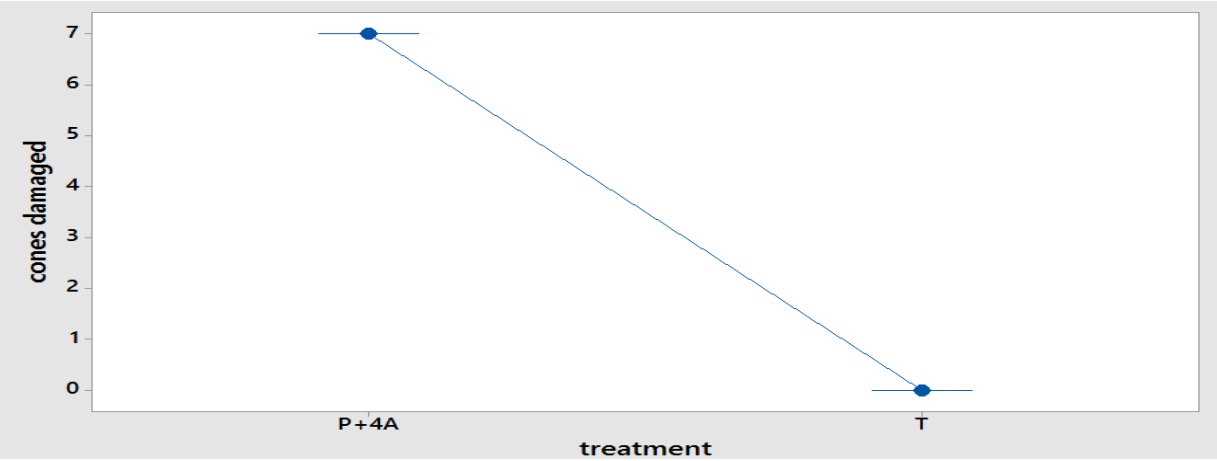
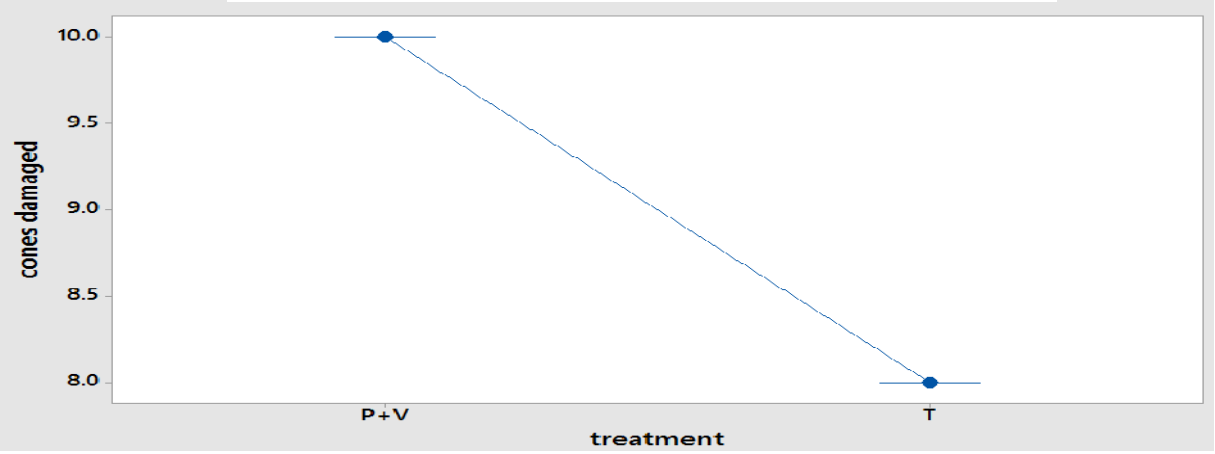


Fig. 8 Experiment 3: *Conophthorus conicolens* W on *Pinus pseudostrobus*, San Juan Nuevo Parangaricutiro, Michoacán, México (19°27´N, 110° 22´ W. Cone borer beetle damage (X ± SE), attacked cones.





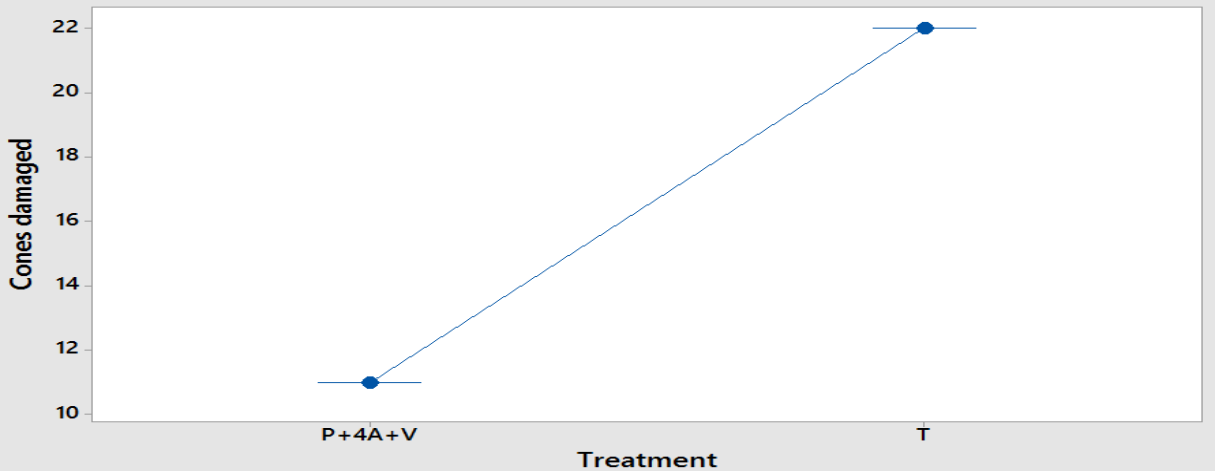


Fig. 9 Experiment 3: Interval plot of cones damaged for one treatment each versus control (T) in 3 separate trials (95% Cl for the Mean). The pooled standard deviation was used to calculate the intervals.

Treatment	NO. Trees	NO. Cones	Mean percentage of attacked cones
Control	20	84	41,5 ± 5,3a
V	20	115	13,7 ± 3,2b

*** Means followed by the same letter in each column of the same experiment are not significantly different at p=0.05.**

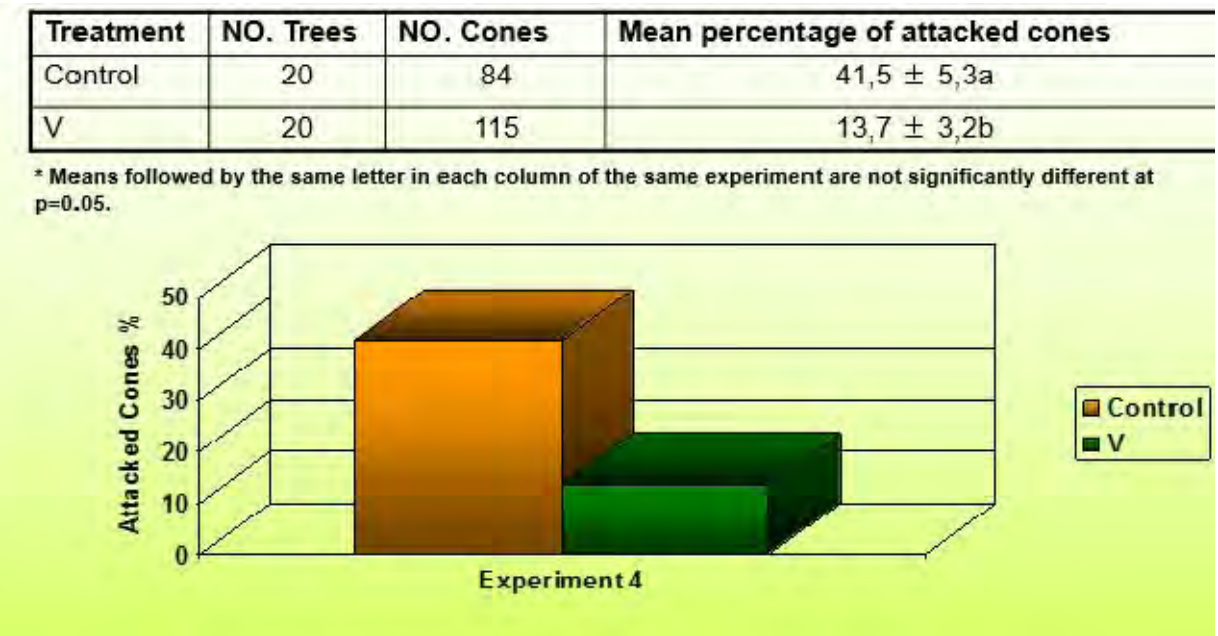


Fig. 10 Experiment 4: *Conophthorus conicolens* W on *Pinus pseudostrobus*, San Lorenzo, Michoacán, México (19°31´ 37”N, 102° 04´ 50” W. Cone borer beetle damage(X ± SE), attacked cones.

The precedent results shown that cones baited with the combination *P+4A+V* besides these sprayed with as alone *Verbenone* were less attacked and obviously and distinctively and protected from attack by *Conophthorus conicolens* W and *C .teocotum* W, too, to diference of the results obtained for all trials made it is showed that cones baited only with *Pityol* and adding *Verbenone* as a spray periodically, its effect was none in the cones protection from cone borer *Conophthorus conicolens* Whereas these baited with the combination *Pityol* and *4-AA* synergized the cones number attacked by both scolytids species, when the combinations of all semiochemicals and added with *Conophtorin* and *Hexenol* as other ones treatment where the effect was not consistent and *Hexenol* for the first case the results were negatives, but this apparently was synergist for *Pityol* and 4-AA in the cones protection from attack by *Conophthorus teocotum* W.

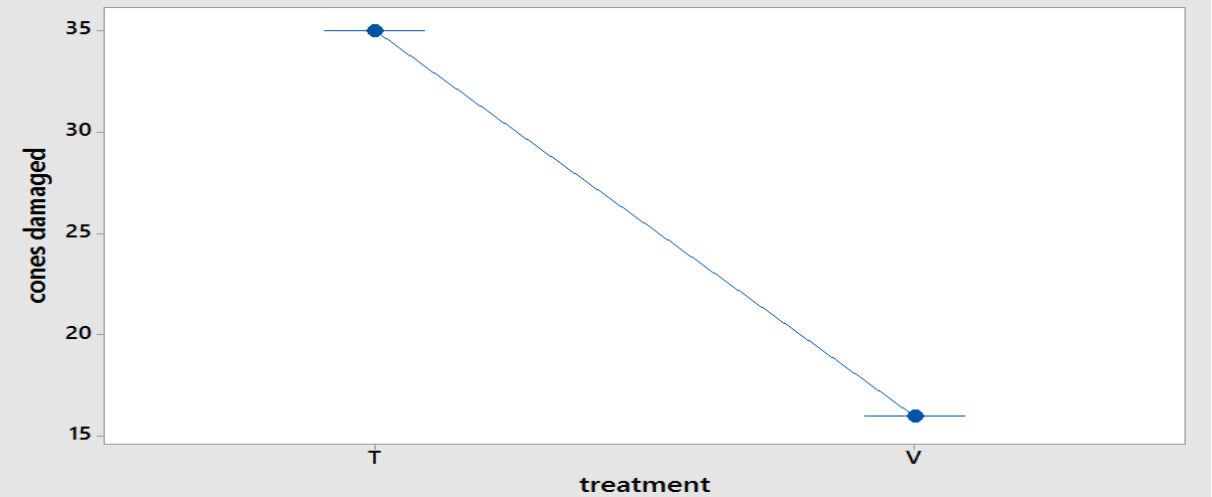


Fig. 11 Experimento 4: Interval plot of cones damaged for Verbenone versus control(T) (95% Cl for the Mean). The pooled standard deviation was used to calculate the intervals.

The anterior results are promisories but still supported upon preliminary tests, since for some of them were low sample sizes (For the case, Experiment 2 with low number of cones per treatment), in dependence to the number of possible number of cones per shoot and trees under conditions of cones abundance on all stands selected. Otherwise, so it appears likely that there are other important pheromone components involucred for Mexican cone borer species and semiochemicals from their hosts (pine native species) that nowadays have not been identified.

Acknowledgments

The author gives thanks to USDA Forest Service in Pacific Southwest Research Station(Albany, CA) for all semiochemicals tested and the experimental logistic, especially to Drs. Nancy Guillete and Silvia Mori.

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