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RESEARCH ARTICLE

COMPOUNDS FROM PEPPER FLOWERS AND FRUITS AS POTENTIAL ATTRACTANTS FOR THE CAPTURE OF PEPPER WEEVIL

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Abstract

Two field experiments were conducted to evaluate synthetic attractants derived from pepper flowers, flower buds and fruits, alone or in combination with the aggregation pheromone. The evaluation was carried out with the release and recapture of *Anthonomus eugenii* adults at different distances from the four cardinal points in separate trials. The volatility of the synthetic mixture and aggregation pheromone was determined by gas chromatographic analysis of the volatiles captured by dynamic headspace. The traps with synthetic mixture and essential oil captured insects at 10 m, while the aggregation pheromone trapped up to 60 m. The combination of synthetic mixture or essential oil with the aggregation pheromone did not increase the number of recaptures compared to the single pheromone. The synthetic mixture together with geranic acid recaptured adults up to 15 m, although they were not significantly different from the control. The exclusion of geranic acid from the aggregation pheromone significantly reduced the number of recaptured insects ($P<0.05$), while geranic acid alone failed to capture weevils. The results could be improved by increasing the concentrations of the compounds or by adding other compounds released during the reproductive stages of pepper. These results could guide future efforts for the development of tools based on synthetic plant volatiles for the monitoring of this pest.

Introduction:-

The pepper weevil (*Anthonomus eugenii* Cano) is the main problem of pepper (*Capsicum annuum* L.) in regions where this crop is produced. This pest is present in Mexico, southern United States, Central America and the Caribbean, as well as in Hawaii, French Polynesia, Dominican Republic and Puerto Rico (EPPO, 2019; Addesso *et al*., 2021). Its presence was also reported in greenhouses in saouthern Canada (Labbé *et al*., 2018), the Netherlands (Van Der Gaag and Looman, 2013) and Italy (Speranza *et al*., 2014: Anonimo, 2018) where they were eradicated.

Direct damage is caused by the larvae, as they develop endophytically, feeding on the placenta and seeds, resulting in severe damage to the fruit. Upon emergence, the adults damage reproductive structures of the plant, causing its

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abscission and reduction in the field ranging from 30 to 90% of production if timely action measures are not implemented (Campbell, 1924; Fernández *et al*., 2020). Traditional methods are difficult because part of its development takes place inside the fruit, which allows the insect to evade any chemical application. In Mexico, in open fields, up to 15 insecticide applications are performed per season (Avendaño-Meza, 2017), which generates negative effects for beneficial organisms (Rodríguez-Leyva *et al*., 2007; Labbé *et al*., 2020).

Since the discovery of the aggregation pheromone, it has been used effectively to monitor the insect in the field (Eller *et al*., 1994; Eller and Palmquis 2014), but this strategy is only efficient before flowering and at the end of the harvest, because the effect of the pheromone is diluted by the large number of volatiles released by flowers. Recent studies indicated that the pepper weevil responds to odors released by host plants (Addesso and McAuslane, 2009) and in particular by their reproductive structures (Bautista-San Juan *et al*., 2019). Several of these compounds have been identified, synthesized and evaluated in laboratory experiments; their combination with the aggregation pheromone showed synergism in the insect´s response in laboratory tests (Muñiz-Merino *et al*., 2014).

Although the compounds released by the reproductive structures of peppers have been identified, no studies have been conducted to determine their effectiveness for monitoring insects in the field. Therefore, this work aimed to evaluate the attraction of synthetic compounds derived from flowers, flower buds, fruits and the aggregation pheromone for the capture of pepper weevil adults under field conditions.

Materials And Methods:-

Insects

Insects of unknown age and mating status were collected in Ejido Vallejo (23.118245 º N, -100.545644 º W), municipality of Villa de Guadalupe, San Luis Potosí, Mexico, on serrano pepper cultivars during August 2018. The colony was established at the Colegio de Postgraduados, under controlled conditions of temperature (26±2 º C) and photoperiod (13:11 h light: dark). Every third day, emerged adults were removed and transferred to 3 L capacity containers, where they remained until use, continuously fed with developing jalapeño pepper fruits (≤ 30 mm in length). For field experiments, weevils more than 10 days old were used, separated by sex according to the characteristics described by Eller *et al*., (1995). The insects were left without food and water for 12 hours prior to field trials.

Attractants

Compounds (Z)-β-ocimene, 2-Isobutyl-3-methoxypyrazine, (Z)-3-hexenyl acetate, terpinolene, geraniol and geranic acid were purchased from Sigma Aldrich®, while (E)-β-ocimene was purchased from Chemos®. The components of the aggregation pheromone of the pepper weevil Z Glandlure II, E Glandlure II and Glandlure III & IV mixture (1:1) were purchased from Bedoukian Research® and were formulated according to the concentrations reported by Eller *et al*., (1994). The essential oil was extracted by steam entrainment with 100 g of poblano pepper cv. flower buds using the methodology described by Zheljazkov *et al*., (2013), with some modifications. For the synthetic mixture and essential oil, microcentrifuge tubes were used as releasers, which were loaded with 500 mg (mixture or essential oil) diluted in mineral oil (Herschi Trading®) with a final volume of 1 mL for field evaluation. The treatments used during the 2018 and 2019 experiments are shown in Table 1.

Table 1:- Treatments used in the field for the pepper weevil during the experiments carried out.

	Treatment no.	Compound	Amount (%/lure)	Purity
Experiment 2018	1 (Synthetic mixture) SM*	(E)-β-ocimene	53	≥95 %
		(Z)-β-ocimene	6	≥90 %
		2-Isobutyl-3-methoxypyrazine	3	≥99 %
		(Z)-3-hexenyl acetate	25	≥98 %
		Terpinolene	13	≥90 %
	2[a] EO*	Flower bud essential oil	-	-
	3 (*A. eugenii* pheromone) AP*	Glandlure II Isomer Z	48	≥98.5 %
		Glandlure II Isomer E	32	≥97 %
		Glandlure III & IV [b]	4	≥95 %
		Geraniol	2	≥98 %
		Geranic acid	14	≥85 %
	4[c] SM + AP*	Synthetic mixture + *A. eugenii* pheromone		
	5[d] EO + AP*	Flower bud essential oil + *A. eugenii* pheromone	-	-
Experiment 2019	6 CO*	Control (no-lure)	-	-
	1 (Synthetic mixture + geranic acid) SM + GA*	(E)-β-ocimene	53	≥95 %
		(Z)-β-ocimene	6	≥90 %
		2-Isobutyl-3-methoxypyrazine	3	≥99 %
		(Z)-3-hexenyl acetate	25	≥98 %
		Terpinolene	13	≥90 %
		Geranic acid [e]	14	≥85 %
	2 (*A. eugenii* pheromone without geranic acid) RS*	Glandlure II Isomer Z	50	≥98.5
		Glandlure II Isomer E	41	≥97 %
		Glandlure III & IV	7	≥95 %
		Geraniol	2	≥98 %
	3 GA*	Geranic acid [e]	14	≥85 %
	4[f] AP*	*A. eugenii* pheromone	-	-
	5 CO*	Control (no-lure)	-	-

[a] Treatment 2 corresponds to the essential oil extracted from the pepper flower buds; [b] 1:1 Mixture; [c] Combination between treatments 1 and 3; [d] Combination between treatments 2 and 3; [e] The geranic acid was formulated separately and mixed with an equal amount of mineral oil, using microcentrifuge tubes as dispersers; [f] Same formulation as treatment 3 of the 2018 experiment. * Name of each treatment.

Field experiments

They were conducted in an area of the Colegio de Postgraduados, municipality of Texcoco, State of Mexico, Mexico (19.468861 º N, -98.898833 º W), in a field with a flat topography, without the presence of pepper cultivation to avoid interference from weevils coming from the field. Yellow traps (30.5 cm x 15 cm) impregnated with glue (Adhequim®) were used, where the treatments were placed. The traps were placed one day before starting the experiment at a height of 0.30 m in the direction of the prevailing winds. They were distributed in a completely randomized block design, with three replicates per treatment with a separation of 100 m between each replicate and treatment.

In the first experiment (August-December 2018) treatments were evaluated at six distances in separate trials, each lasting 15 days. *A. eugenii* adults were released at the four cardinal points of each treatment from each distance used. For evaluation 1, 720 adults were used, 5 females and 5 males were released from 5 m. For evaluation 2, the same number of insects was released from a distance of 10 m. In evaluation 3, 864 weevils were used, 6 females and 6 males were released from 15 m. In evaluation 4, 1008 weevils were used, 7 females and 7 males were released from a distance of 30 m. In evaluation 5, 1296 adults were used, 9 females and 9 males were released from 60 m. The

second experiment was conducted during August-October 2019, where the treatments were evaluated at 5, 10 and 15 m. At each distance, 600 weevils were used, of which 10 adults (5 females and 5 males) were released at the four cardinal points of each treatment. The releases were carried out during 13:00 to 17: 00 hours in the afternoon, according to the period of greatest activity of females and males (Muñiz-Merino et al., 2014). The number of insects used was subject to their availability in the established colony. The traps were checked once a week, during the time each evaluation lasted (distance evaluated), while the volume level of each dispenser was checked every third day.

Collection of volatiles in dispersers

The compounds of the synthetic mixture and aggregation pheromone were captured by dynamic headspace on days 1, 7, 14, 21 and 28. The releasers were placed in a cylindrical glass flask with a 29/42 ground-glass neck, 21 cm high, 6 cm internal diameter and 500 mL capacity (Pyrex®). The flask had a ground-glass stopper (2942), with two glass tubes to which a Nalgene hose (3/16 ID) was attached, through which air was passed with an Elite 802 pump with a flow rate of 60 mL/min, regulated with a flow meter (Gilmont®).Three flasks were placed at the same time for each releaser, a 150 mm pasteur pipette (Brand®) was placed at each air inlet point, packed with 50 mg of Tenax TA 60/80 adsorbent (Sigma Aldrich®), which served as a filter. Another cartridge of the same type was placed in line at the outlet of each flask to collect the compounds from the disperser, with a capture time of 3 hours. The volatiles captured in each cartridge were eluted with 4 mL of HPLC grade hexane and brought to a concentration of 100 µL by a gentle stream of nitrogen. The resulting solution was placed in 3 mL amber vials (Agilent-Technologies®) and stored at – 4 °C until analysis by gas chromatography.

Chromatographic analysis of the samples of the releasers

1 µL of each concentrated sample was injected into a Hewlett Packard gas chromatograph (5890) with a flame ionization detector (GC-FID). Gas Chromatography conditions were: nitrogen as carrier gas, with a flow rate of 1 mL min^{-1}, the detector and injector temperature was 250 ° C. The run conditions were an initial temperature of 40 °C stable for 5 min, then increased by 5 °C min^{-1} until reaching 100 °C, then increased by 10 ° C min^{-1} until reaching 210 °C, maintained for 5 min, with a total run time of 33 min. Identification and confirmation of the compounds were obtained by comparing retention times with commercial standards.

Statistical analysis

Data from the 2018 and 2019 field experiments were analyzed using linear mixed models, treatments were used as a fixed factor and replicates nested in weeks as a random factor. A *post hoc* analysis with Bonferroni correction with a probability of 0.05 was performed to test for significant differences within each group of means. All analyses were performed using SPSS v. 25.0 for Windows (IBM Corp. 2019).

Results:-

Field experiment during September-December, 2018.

Traps placed at 5 m distance showed a significant difference in the mean number of recaptured weevils ($F=31.558$; $df= 5, 48$; $P < 0.05$). AP (8.55±1.02), SM: AP (7.33±1.02) and EO: AP (7.33±1.02) treatments recorded the highest means, while SM and EO presented much lower values, but higher compared to CO. In *post hoc* comparisons, the mean difference between AP compared to SM (8.11±1.02), AE (7.88±1.02) and CO (8.55±1.02) recorded significantly higher mean differences ($P<0.05$); while SM: AP and EO: AP presented similar mean differences compared to SM (6.88±1.02), EO (6.66±1.02) and CO (7.33±1.02). In the second evaluation at 10 m distance, a significant difference was also obtained among the treatments used ($F=52.509$; $df= 5, 40$; $P < 0.05$). The control (CO) recorded lower values compared to SM and EO, while AP, SM: AP and EO: AP presented the highest means. In *post hoc* tests, the mean differences between AP and SM (7.88±0.78), EO (8.22±0.78) and CO (9.00±0.78); SM: AP and SM (6.00±0.78), EO (6. 33±0.78) and CO (7.11±0.78); as well as EO: AP and SM (6.22±0.78), EO (6.55±0.78) and CO (7.33±0.78) were significantly higher ($P<0.05$).Results at 15 m ($F=124.310$; $df=5, 40$; $P<0.05$), 30 m ($F= 137.277$; $df=5, 40$; $P<0.05$) and 60 m ($F=41.353$; $df=5, 40$; $P<0.05$) distances indicated an effect of the treatments, which differed statistically in the mean number of recaptures. The means in SM, EO and CO (0.70±0.11) were similar in these evaluations, while AP, SM: AP and EO: AP presented the highest means. In *post hoc* tests, the mean difference between AP, SM: AP and EO: AP was significantly higher compared to SM, EO and CO ($P<0.05$). Univariate tests showed a significant difference in these distances, which indicated that the proposed model was acceptable ($P<0.05$).

Field experiment during August-October, 2019.

The mean number of adults recaptured at 5 m distance showed a significant difference among the evaluated treatments (F=23.024; df= 5, 40; P < 0.05). The mean difference in AP (2.48±0.14) was statistically higher than the remaining treatments (Figure 1; 5 m). SM: GA with GA (0.62±0.14), AP (-0.57±0.14) and CO (0.74±0.14); RS with GA (0.70±0.14), AP (-0.49±0.14) and CO (0.81±0.14); GA with SM: GA (-0.62±0.14), RS (-0.70±0.14) and AP (-1.19±0.14); AP with SM: GA (0. 57±0.14), RS (0.49±0.14), GA (1.19±0.14) and CO (1.31±0.14); as well as CO with SM: GA (-0.74±0.14), RS (-0.81±0.14) and AP (-1.31±0.14) treatments recorded a significant difference according to *post hoc* comparisons (P<0.05). At 10 m, a significant difference was again found in the mean number of recaptured adults among the evaluated treatments (F = 8.895; df = 5, 40; P < 0.05). Means between AP (1.61±0.12) and SM: GA (1.31±0.12) were higher compared to CO (0.86±0.12) used as a control (Figure 1; 10 m). In *post hoc* Bonferroni comparisons, RS (-0.48±0.12), GA (-0.63±0.12) and CO (-0.75±0.12) treatments presented a significantly lower mean difference than AP (P<0.05), while AP was higher compared to RS (0.48±0.12), GA (0.63±0.12) and CO (0.75±0.12). On the other hand, SM: GA was significantly higher than CO (0.45±0.12). For the evaluation at 15 m, a significant effect among treatments on the number of recaptures was also observed (F=10.612; df=5, 40; P<0.05). *Post hoc* tests in SM: GA (-0.36±0.08), RS (-0.48±0.08), GA (-0.48±0.08) and CO (-0.48±0.08) were significantly lower (Figure 1; 15 m), compared to AP (P<0.05); while AP was significantly higher than SM: GA (0.36±0.08), RS (0.48±0.08), GA (0.48±0.08) and CO (0.48±0.08).

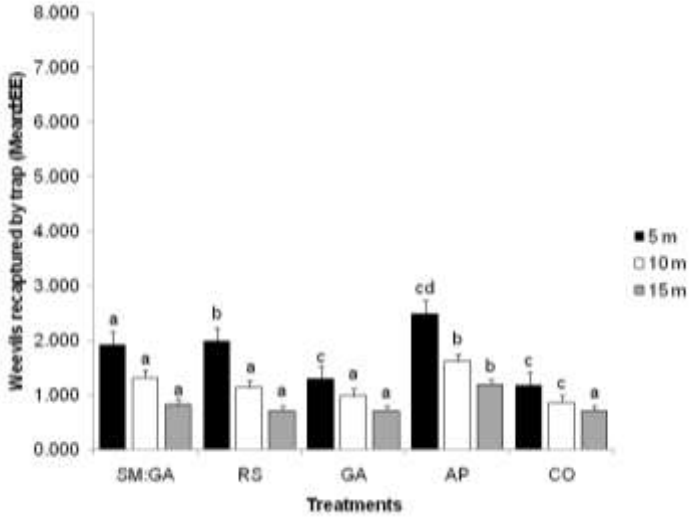

Figure 1:- Average number of weevils recaptured at 5, 10 and 15 meters away during 2019. Treatments: SM (Synthetic mixture), EO (Flower bud essential oil), AP (*A. eugenii* pheromone), SM: AP (Synthetic mixture + *A. eugenii* pheromone), EO: AP (Flower bud essential oil + *A. eugenii* pheromone) and CO (Control). Means with different letters are significantly different at $\alpha = 0.05$ (Bonferroni test).

Volatility of compounds in the field

Chromatographic analysis of the synthetic mixture with E-β-ocimene and Z-3-hexenyl acetate recorded the highest areas, while 2-isobutyl-3-methoxypyrazine presented the lowest area during field exposure time in microcentrifuge tubes. In contrast, in the aggregation pheromone, compounds (Z)-2-(3, 3-dimethylcyclohexylidene) ethanol, (E)-2-(3, 3-dimethylcyclohexylidene) ethanol and (E)-3, 7-dimethyl-2, 6-octadienoic acid recorded the highest volatility. Five of the compounds remained up to 28 days of exposure in the field, while (E) - 3, 7-dimethyl-2, 6-octadienoic acid only remained in the disperser for up to 14 days.

Discussion:-

Traps with the synthetic mixture captured *A. eugenii* adults up to 10 m away, although they were not significantly different from the control. The number of recaptures was lower in the synthetic mixture compared to the aggregation pheromone. Similarly, *A. rubi* is weakly attracted to traps baited with pheromone, but does not respond to traps baited only with host plant volatiles (Wibe *et al*., 2014). Previous studies (Muñiz-Merino *et al*., 2014; Bautista-San-Juan *et al*., 2019), demonstrated through laboratory bioassays by olfactometry that the response of males and females of *A. eugenii* was unequivocal towards the volatiles of the mixture used. Probably, the number of volatiles released in the field was below the reception threshold; this was suggested by the results of the chromatographic

analysis, where the compounds of the synthetic mixture showed higher volatility compared to the components of the aggregation pheromone. This may have caused the loss of attraction during the exposure time, so it would be necessary to increase the concentrations of the mixture in the dispersers or to add other compounds present in the pepper.

The essential oil had a limited longevity in the field, since its volume decreased more than 50 % in five days. The short permanence of the extract in the field may have caused the low captures in the traps, since the compounds essential for attraction were not present. Perhaps, the higher release is due to the high vapor pressure of the compounds, linked to the ambient temperature recorded as suggested by Mette-Cecilie *et al.*, (2019), who mention that a substance with a higher vapor pressure volatilizes more easily. The study of essential oils as attractants in the genus *Anthonomus* is scarce (McKibben *et al*., 1997), most have focused on repellent and insecticidal activity (Brito *et al*. 2021). Kendra *et al*., (2018) demonstrated the potential of essential oils for attracting *X. glabratus*, so their implementation in insect management is an option that would be worth exploring.

The combination of aggregation pheromone and synthetic mixture from the host plant did not increase the number of recaptures of *A. eugenii* compared to the single pheromone. Szendrei *et al*., (2011) in field experiments observed that the addition of Z-3-hexenyl acetate and hexyl acetate with pheromone components from *A. musculus* did not improve attraction. Possibly, the ratio of the release of the components in the mixture used was not sufficient to attract the weevil and cause synergism, as happened with *A. rubi*, where the addition of 1, 4-dimethoxybenzene, together with the aggregation pheromone caused higher captures than with the single pheromone (Wibe *et al*., 2014; Mozūraitis *et al*., 2020). Although this combination failed to improve attraction, this work provides the first evaluation of pepper volatiles together with the pheromone, which in future studies could be refined to increase attraction.

The synthetic mixture and geranic acid placed in the same trap captured insects at 15 m distance, although they were not significantly different from the control. Rodriguez-Saona *et al*., (2020) found that the response of *A. musculus* is not affected by the addition of geranic acid to the aggregation pheromone; furthermore, the addition of the sex pheromone of *L. rugulipennis* and 1, 4 dimethoxybenzene to the aggregation pheromone of *A. rubi* did not cause significant changes in the number of captures (Baroffio *et al*., 2018). In the case of *A. eugenii*, recaptures decreased significantly when adults were released at greater distances from the release point, similarly Dissanayaka *et al*., (2020) observed a decrease as the release distance of *R. dominica* increased. Perhaps this is because as distance increases, the concentration of the compound decreases, reducing insect attraction.

The aggregation pheromone (without geranic acid) captured fewer insects than the complete pheromone. In this respect, Eller *et al*., (1994) in field tests observed higher attraction of *A. eugenii* adults in traps baited with pheromone with the mixture of six compounds than with the mixture of five. Surely, the absence of geranic acid caused a reduction in the attraction of the insect, however, the study of the effect of different doses of geranic acid would help improve the attractiveness of the pheromone in the field. Geranic acid was ineffective in attracting *A. eugenii* adults, since traps with this compound showed lower captures compared to the control. Perhaps, as suggested by Eller *et al*., (1994), geranic acid is inactive when used individually and only works when the complete pheromone is present. In addition, the rapid volatility in the field may have caused a null response, since according to chromatographic analysis, this compound was only present for up to 14 days.

Conclusion:-

The traps with the mixture of (E)-β-ocimene, (Z)-β-ocimene, 2-Isobutyl-3-methoxypyrazine, (Z)-3-hexenyl acetate and terpinolene presented insects at short distance, similar to the essential oil. Improvement in detection systems for *A. eugenii* with the synthetic mixture should be possible by increasing the concentration of the components or by adding other compounds present in pepper. It is clear that the aggregation pheromone is an effective strategy for capturing *A. eugenii* adults, as long as it is placed before flowering and after harvest to capture as many adults as possible on alternate hosts. Further studies are required to develop a formulation that increases the efficacy of the synthetic mixture with the aggregation pheromone evaluated here.

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References:-

1. Addesso, K. M. and McAuslane, H. J. (2009). Pepper weevil attraction to volatiles from host and nonhost plants. Environmental Entomology, 38 (1), 216-224. Doi: https://doi.org/10.1603/022.038.0127
2. Avendaño-Meza, F. (2017). Efectividad biológica de insecticidas para el manejo de la resistencia del picudo del chile. Revista Iberoamericana de las Ciencias Biológicas y Agropecuarias, 6 (11), 23-38. Doi: https://doi.org/10.23913/ciba.v6i11.61
3. Anonimo, 2018. Relazionesull'infestatatione da *Anthonomuseugenii*(Cano) nel territorio Laziale (2013–2018). Available at: http://www.agricoltura.regione.lazio.it/binary/prtl_sfr/tbl_misure/ANTHEU_REL_2013_2018.pdf. Cited: April 5, 2020.
4. Addesso, K. M., Alborn, H. T., Bruton, R. R. and McAuslane, H. J. (2021). A multicomponent marking pheromone produced by the pepper weevil, *Anthonomus eugeni* (Coleoptera: Curculionidae). Chemoecology, 31 (2). Doi: https://doi.org/10.1007/s00049-021-00347-3
5. Bautista-San Juan, A., Cibrián-Tovar, J., López-Romero, R. M., Martínez-Bautista, N. y Gómez-Domínguez, N. S. (2019). Atracción de adultos de *Anthonomus eugenii* (Cano) a mezclas de compuestos volátiles sintéticos. Southwestern Entomologist, 44 (3): 743-754. Doi: https://doi.org/10.3958/059.044.0319
6. Baroffio, C. A., Sigsgaard, L., Ahrenfeldt, E. J., Borg-Karlson, A. K., Bruun,S. A., Cross, J. V., Fountain, M. T., Hall, D., Mozuraitis, R., Ralle, B., Trandem, N. and Wibe, A. (2018). Combining plant volatiles and pheromones to catch two insect pests in the same trap: Examples from two berry crops. Crop Protection, 109: 1-8. Doi: https://doi.org/10.1016/j.cropro.2018.02.025
7. Brito, V. D., Achimón, F., Pizzolitto, R. P., Sánchez, A. R., Gómez, T. E. A., Zygadlo, J. A. and Zunino, M. P (2021). An alternative to reduce the use of the synthetic insecticide against the maize weevil *Sitophilus zeamais* through the synergistic action of *Pimenta racemosa* and *Citrus sinensis* essential oils with chlorpyrifos. Journal of Pest Science. 94:409–421. Doi: https://doi.org/10.1007/s10340-020-01264-0
8. Campbell, R.E. (1924). Injuries to pepper in California by *Anthonomus eugenii* Cano. Journal of Economic Entomology, 17: 645-647. Doi: https://doi.org/10.1093/jee/17.6.645
9. Dissanayakaa, D. M. S. K., Sammani, A. M. P., Wijayaratne, L. K. W., Rajapakse, R. H. S., Hettiarachchi, S. and Morrison, W. R. 2020. Effects of aggregation pheromone concentration and distance on the trapping of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) adults. Journal os Stored Products Research, 88 (1): 1-8. Doi: https://doi.org/10.1016/j.jspr.2020.101657
10. Eller, F. J., Bartelt, R. J., Shasha, B. S., Schuster, D. J., Riley, D. G., Stansly, P. A., Mueller, T. F., Shuler, K. D., Johnson, B., Davis, J. H. and Sutherland C. A. (1994). Aggregation pheromone for the pepper weevil, *Anthonomus eugenii* Cano (Coleoptera: Curculionidae): Identification and field activity. Journal of Chemical Ecology, 20 (7): 1537-1555. Doi: https://doi.org/10.1007/BF02059879
11. Eller, F. J. (1995). A previously unknown sexual character for the pepper weevil (Coleoptera: Curculionidae). Florida Entomologist, 78 (1): 180-183. Doi: https://doi.org/10.2307/3495683
12. Eller, F. J. and Palmquist, D. E. (2014). Factors affecting pheromone production by the pepper weevil, *Anthonomus eugenii* Cano (Coleoptera: Curculionidae) and collection efficiency. Insects, 5 (4): 909-920. Doi: 10.3390 / insects5040909
13. European and Mediterranean Plant Protection Organization (EPPO). May 18, 2021. EPPO Global Database, *Anthonomus eugenii*. Available at: https://gd.eppo.int/taxon/ANTHEU/distribution. Cited: may 18, 2021.
14. Fernandez, D. C., VanLaerhoven, S. L., McCreary, C. and Labbé, R. M. 2020. An Overview of the pepper weevil (Coleoptera: Curculionidae) as a pest of Greenhouse peppers, Journal of Integrated Pest Management, 11(1): 26; 1–11. Doi: 10.1093/jipm/pmaa029
15. IBM Corp. (2017) IBM SPSS Statistics to Windows, Versión 25.0. Armonk, Nueva York: IBM Corp
16. Kendra, P. E., Montgomery, W. S., Niogret, J., Tabanca, N., Owens, D. and Epsky, N. D. (2018). Utility of essential oils for development of host-based lures for *Xyleborus glabratus* (Coleoptera: Curculionidae: Scolytinae), vector of laurel wilt. Open Chemistry, 16 (1): 393-400. Doi: https://doi.org/10.1515/chem-2018-0045
17. Labbé, R., Hilker, R., Gagnier, D., McCreary, C., Gibson, G. A. P., Fernández-Triana, J., Mason, P. G. and Gariepy, T. D. 2018. Natural enemies of *Anthonomus eugenii* (Coleoptera: Curculionidae) in Canada. Canadian Entomologist. 150 (3): 404–411. Doi: doi:10.4039/tce.2018.3

18. Labbé, R. M., Gagnier, D., Rizzato, R., Tracey, A. and McCreary, C. (2020). Assessing New Tools for Management of the Pepper Weevil (Coleoptera: Curculionidae) in Greenhouse and Field Pepper Crops. Journal of EconomicEntomology, 113 (4): 1903-1912. Doi: 10.1093 / jee / toaa092
19. Muñiz-Merino, M., Cibrián-Tovar, J., Hidalgo-Moreno, C., Bautista-Martínez, N., Vaquera-Huerta, H. y Aldama-Aguilera, C. (2014). Compuestos volátiles atraen al picudo (*Anthonomus eugenii* Cano) del chile (*Capsicum*spp.) y presentan sinergia con su feromona de agregación. *Agrociencia*, 48 (8): 819-832. Doi: https://www.redalyc.org/articulo.oa?id=30232982005
20. McKibben, G. H., Mitchell, E. B., Scott, W. P. and Hedin, P. A. (1977). Boll weevils are attracted to volatile oils from cotton plants. Environmental Entomology, 6 (6): 804-806. Doi: https://doi.org/10.1093/ee/6.6.804
21. Mette-Cecilie, N., Sansom, C. E., Larsen. L., Worner, S. P., Rostás, M., Chapman, R. B., Butler R. C., De Kogel, W. J., Davidson, M. M., Perry, N. B. and Teulon, D. A. J. (2019). Volatile compounds as insect lures: factors affecting release from passive dispenser systems. New Zealand Journal of Crop and Horticultural Science, 47 (3): 208-223. Doi: https://doi.org/10.1080/01140671.2019.1604554
22. Mozūraitis, R., Hall, D., Trandem, N., Ralle B., Tunström, K., Sigsgaard, L., Baroffio, C., Michelle, F., Cross, J., Wibe, A. and Borg-Karlson, A. K. (2020). Composition of Strawberry Floral Volatiles and their Effects on Behavior of Strawberry Blossom Weevil, *Anthonomus rubi*. Journal of Chemical Ecology, **46 (11-12):** 1069–1081. Doi: https://doi.org/10.1007/s10886-020-01221-2
23. Rodriguez-Leyva, E., Stansly, P. A., Schuster, D. J. and Bravo-Mosqueda, E. (2007). Diversity and distribution of parasitoids of *Anthonomus eugenii* (Coleoptera: Curculionidae) from México and prospects for biological control. Florida Entomologist, 90 (4): 693-702. Doi: 10.1653 / 0015-4040 (2007) 90 [693: DADOPO] 2.0.CO; 2
24. Rodriguez-Saona, C., Alborn, H. T., Oehlschlager, C., Calvo, C., Kyryczenko - Roth, V., Tewari, S., Sylvia, M. M. and Averill, A. L. (2020). Fine-tuning the composition of the cranberry weevil (Coleoptera: Curculionidae) aggregation pheromone. Journal of Applied Entomology, 144 (5): 1–5. Doi: https://doi.org/10.1111/jen.12752
25. Szendrei, Z., Averill, A., Alborn, H. and Rodríguez-Saona, C. (2011). Identification and field evaluation of attractants for the Cranberry weevil, *Anthonomus musculus* Say. Journal of Chemical Ecology, 37 (4): 387-397. Doi: 10.1007 / s10886-011-9938-z
26. Speranza, S., Colonnelli, E., Garonna, A. P. and Laudonia, S. 2014. First record of *Anthonomus eugenii* (Coleoptera: Curculionidae) in Italy. Florida Entomologist, 97: 844–845. Doi: https://doi.org/10.1653/024.097.0275
27. Wibe, A., Borg-Karlon, A. K., Cross, J., Bichao, H., Fountain, M., Liblikas, I. and Sigsgaard L. (2014). Combining 1, 4-dimethoxybenzene, the major flower volatile of wild strawberry Fragaria vesca, with the aggregation pheromone of the strawberry blossom weevil *Anthonomus rubi* improves attraction. Crop Protection, 64: 122-128. Doi: https://doi.org/10.1016/j.cropro.2014.06.016
28. Zheljazkov, V. D., Cantrell, C. L., Astatkie, T. and Jeliazkova, E. (2013). Distillation time effect on lavender essential oil yield and composition. Journal of Oleo Science, 62 (4): 195-199. Doi: https://doi.org/10.5650/jos.62.195
29. Van Der Gaag, D. J. and Looman, A. 2013. Pest risk analysis for *Anthonomus eugenii*. Version 3.0. Netherlands Food and Consumer Product Safety Authority, Utrecht, 64 pp.