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Research article

Development of a Low-Cost and Effective Trapping Device for Apple Maggot Fly (Diptera: Tephritidae) Monitoring and Control in Mexican Commercial Hawthorn Groves

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Abstract

Few efforts have been made in Mexico to monitor *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae) in commercial hawthorn (*Crataegus* spp.) crops. Therefore, the main objectives of this study were to evaluate infestation levels of *R. pomonella* in feral and commercial Mexican hawthorn and to assess the efficacy of different trap-lure combinations to monitor the pest. Wild hawthorn was more infested than commercially grown hawthorn at the sample site. No differences among four commercial baits (Biolure, ammonium carbonate, CeraTrap, and Captor + borax) were detected when used in combination with a yellow sticky gel (SG) adherent trap under field conditions. However, liquid lures elicited a slightly higher, although not statistically different, capture. Cage experiments in the laboratory revealed that flies tended to land more often on the upper and middle than lower-bottom part of polyethylene (PET) bottle traps with color circles. Among red, orange, green, and yellow circles attached to a bottle trap, only yellow circles improved fly captures compared with a colorless trap. A PET bottle trap with a red circle over a yellow background captured more flies than a similar trap with yellow circles. An SG adherent yellow panel trap baited with ammonium carbonate was superior to the improved PET bottle trap (red over a yellow background) baited with different liquid proteins, but a higher proportion of females and no differences in fly detection were measured in PET traps baited with protein lures. These trials open the door for future research into development of a conventional nonadherent trap to monitor or control *R. pomonella*.

Key words: *Rhagoletis pomonella*, *Crataegus*, population monitoring, trapping, polyethylene (PET) bottle trap

The apple maggot, *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae), is a tephritid pest native to North America. Different populations of the pest, breeding on different hosts (hawthorn and apple) across different geographical regions (eastern United States, western United States, and Mexico), are genetically distinct and exhibit behavioral differences in host odor recognition and partial reproductive isolation (McPheron et al. 1988, Berlocher et al. 1993, Berlocher 2000, Michel et al. 2007, Rull et al. 2010, Yee et al. 2014a). Unlike populations in the eastern United States, where this species is a key pest of apples (Prokopy et al. 2005), in the western United States and Mexico, it never or rarely occurs in commercial apples but is commonly found in wild hawthorns, *Crataegus* spp. (AliNiazee et al. 1987, Klaus 2003, Hernández-Ortíz et al. 2004, Yee 2008, Yee et al. 2014a). The taxonomic status of some Mexican populations of *R. pomonella* infesting *Crataegus* spp. is uncertain, as they differ morphologically and genetically from other species of the *Pomonella* group, suggesting that the Mexican populations could represent a distinct species (Smith and Bush 1997, Rull et al. 2010).

Mexican hawthorn, *Crataegus mexicana* Moc. & Sessé, fruit is widely commercialized in Mexico during November and December for religious and cultural celebrations, where it is used to prepare a drink called "ponche," or to produce concentrated pulp, jellies, and jams (Nuñez-Colín and Sánchez-Vidaña 2010). Additionally, Mexican hawthorn is attractive for the processed food industry because of its high pectin content and because such pectins are efficient emulsifying and stabilizing agents when used on their own, as they possess a balance between high molecular weight and hydrophobic contributing moieties (Cuevas-Bernardino et al. 2016).

The Mexican hawthorn market is also experiencing an increase in demand from Mexican emigrants living in the United States that represents a potentially profitable export market. However, *R. pomonella* infestations reduce production and limit exports (Karp 2010). Economic losses of hawthorns from the pest have not been accurately evaluated, but growers are concerned by the need to manage the fly so that they can obtain a high quality product for export or local market. Although there are few commercial plantations of this crop, small farmers of the Central Mexican Altiplano (highlands in states of Puebla, Tlaxcala, Morelos, Hidalgo, Estado de Mexico, and Veracruz) frequently have a few hawthorn trees as wind-break barriers or growing interspersed with apples, pears, and peaches in small groves. Pending development of more modern production systems, such growers would highly benefit from developing low-cost, low-maintenance trapping systems for both monitoring and control of *R. pomonella*.

Monitoring is an important tool to detect tephritid pests, which allows the timely implementation of control strategies. Fruit volatiles, derived from apples, have been effectively used in the eastern United States to attract *R. pomonella* in combination with a red sticky trap (Fein et al. 1982, Reissig et al. 1982, Aluja and Prokopy 1993, Reynolds and Prokopy 1997). In contrast, in the western United States, the same apple fruit volatiles are less attractive (Brunner 1987; Jones and Davis 1989; Kroening et al. 1989; Yee et al. 2005, 2014b) and ammonium carbonate with yellow sticky panels is the most widely used detection method (Klaus 2003; Yee et al. 2005, 2014b). In Mexico, there is little information on the attraction of this pest to different lures and trap types. Hydrolyzed protein lures are the main tool to monitor other tephritid pests in Mexico, mostly those in the genus *Anastrepha* (Anonymous 1999).

Recent studies comparing efficacy of commercial protein lures for capture of *Anastrepha* fruit flies have revealed that the use of a relatively new commercial product, CeraTrap, can result in greater fly captures and requires much less frequent rebaiting than several other protein-based lures (Lasa and Cruz 2014, Lasa et al. 2015). This lure, however, is not as effective in attracting *Zeugodacus* or *Bactrocera* (Royer et al. 2014, Shelly and Kurashima 2016), and has not been tested against flies within *Rhagoletis*. Previous studies have examined McPhail trap captures of *R. pomonella* over the hawthorn-growing season in the state of Puebla (Hernández-Ortíz et al. 2004) or have compared responses of Mexican populations to different trap types and color combinations using two commercial hydrolyzed protein lures (Nieto-Ángel et al. 2016).

Key information on pest dynamics, stimuli related to host utilization, and efficient trapping devices are currently lacking. Testing responses to traps and lures by *R. pomonella* populations is important because differences in behaviors of flies among races are evident. Moreover, growers in Mexico are often reluctant to use sticky yellow rectangle traps coated with polybutene-based sticky gel (SG), because locally commercialized traps usually need to be treated with additional SG before use. The use of SG is unpleasant, as it leaves sticky residues on the hands, tools, and clothes, and is not easily removed by solvents (Yee 2011). Moreover, traps rapidly become saturated with nontarget insects and need to be replaced frequently by new ones.

Although other sticky traps, such as hot-melt sensitive adherent yellow traps, could be used for *Rhagoletis* species monitoring to avoid this problem (Yee 2011), these traps are currently expensive for use by Mexican growers and market demand appears to be insufficient to attract commercial companies and guarantee a steady supply of alternative trap types. Under these circumstances, growers require the development of an easy to handle, low-maintenance, effective and affordable trap, such as the simple, and virtually cost-free polyethylene (PET) bottle traps used to monitor other tephritid pests in Mexico (Lasa et al. 2015). Therefore, the main objectives of this study were first, to evaluate infestation levels of *R. pomonella* in feral and commercial Mexican hawthorn fruit, and second, to test the efficacy of several commercial lures used with yellow sticky traps compared with the standard ammonium carbonate lure. A series of tests were subsequently performed under caged conditions to examine the influence of color stimuli on responses to a virtually cost-free PET bottle trap. Finally, we compared the efficacy of a conventional sticky trap baited with ammonium carbonate with the cost-free PET bottle trap using several hydrolyzed proteins that are commercially available in Mexico.

Materials and Methods

Traps and Lures

Experiments were developed using two trap types: Adhesive Pherocon AM traps (Agrobioquim, Tamaulipas, Mexico) and a homemade 600-ml PET trap, with four equidistant 10-mm holes drilled at 2/3 of the trap height and with several color modifications (Fig. 1). RGB colors were identified throughout photos taken with a Canon EOS 7D camera with an Iwasaki Eye Color lamp (70 W 1.0 A, Venture Lighting Europe Ltd. Hertfordshire, United Kingdom) and the plugin Color Inspector 3D of Image J (Rasband 2015). Pherocon AM traps were similar to other Pherocon traps with sticky sides. When folded, they measured 23 cm in height by 14 cm in width without nonsticky borders and with a total surface area of 644 cm^2. Owing to the irregular coverage of adherent on traps, both sides of the rectangular cardboard were reimpregnated with a polybutylene stikem gel, Pstikem (Agrobioquim, Tamaulipas, Mexico), to increase trap effectiveness. Six different commercial odor lures were used in field experiments; three of these were liquid, chemically hydrolyzed proteins: 1) Captor 300 (Promotora Agropecuaria Universal, Mexico City, Mexico), 2) Flyral (Cuprosa, Guadalajara, Mexico), and 3) Winner 360 (Internacional Química de Cobre, Mexico City, Mexico). The other three were an enzymatically hydrolyzed protein CeraTrap (Bioibérica, Barcelona, Spain) proved to be very attractive to *Anastrepha* species (Lasa and Cruz 2014, Lasa et al. 2015, Rodriguez et al. 2015), and two dry lures: 1) Biolure (Suterra Inc., Bend, OR), a dry lure containing ammonium acetate and putrescine in individual sachets (Heath et al. 1997), and 2) ammonium carbonate (AC) powder ∼98% purity (Droguería Cosmopolita, Mexico City, Mexico). Hydrolyzed proteins (Captor, Flyral, and Winner 360) were prepared combining 10 ml of protein with 5 g of borax pentahydrate (J. T. Baker, Mexico City, Mexico) and 235 ml of water, as indicated in the Mexican standard formula for this type of fruit fly lure (Anonymous 1999).

Infestation of Wild and Commercial Hawthorns

Fruit from commercial and wild hawthorn trees were collected simultaneously in an orchard in San Miguel Tlaixpan, Estado de Mexico, Mexico (19° 30′9.99″ N, 98° 48′14.20″ W) on two dates during November 2014. Cultivated hawthorn plantations in the study area are >50 yr old and trees were grafted in the past to obtain an improved quality of hawthorn. Growers and researchers have no information relative to the specific rootstocks that were used to graft hawthorn varieties, although apple and pear were predominant (Nieto-Angel, personal communication). Wild hawthorn plants also coexist within cultivated plantations in this area. No control strategies had been implemented in the orchard within the 3–4 previous

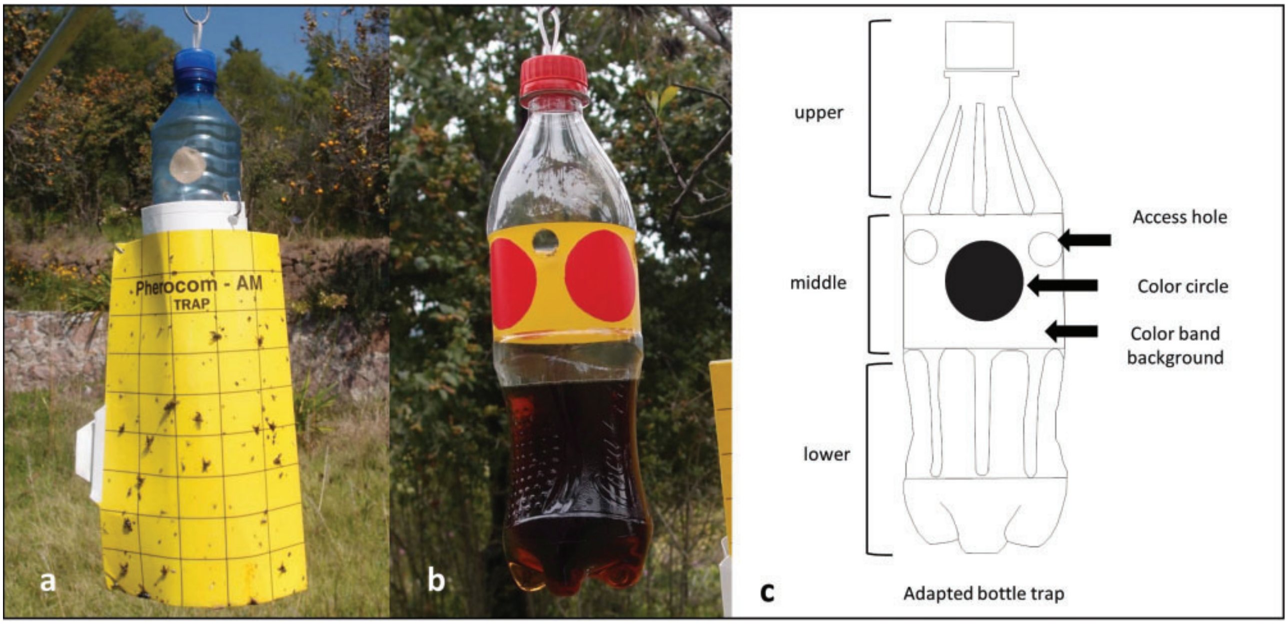

Fig. 1. Traps used in the *R. pomonella* trapping experiments: (**a**) Pherocon AM adherent trap with the inclusion of a bottle trap for lure emission, (**b**) Adapted bottle trap, and (**c**) Bottle sections considered for fly landing tests.

years. On one date, a sample of 50 ripening fruit (turning in color from green to reddish-yellow) was randomly collected from the canopy of three different trees (15–18 fruits per tree) of wild hawthorn and commercial hawthorn. Samples were collected in the same orchard (1.5 ha), although it mostly comprised cultivated hawthorn. Fruit were weighed and the equatorial and apical diameter measured before they were placed individually into plastic containers of 200 ml, provided with a layer of moist vermiculite, and stored in the laboratory at 23.5 ± 1 °C, 65 ± 10% relative humidity, and a photoperiod of 13:11 (L:D) h during 45 d to allow larval development and pupation. After this period, the percentages of fruit infested and the numbers of pupae per individual fruit were recorded for both fruit types. A second sample of several kilograms of ripening fruit was collected with a similar methodology 1 wk later, from both types of trees, and maintained in plastic crates (with all fruit held together). Pupae were recovered during 45 d as in the previous test. The weight of each sample and the number of pupae recovered were determined for each fruit type.

Attraction of *R. pomonella* to Lures Under Field Conditions

The capture of *R. pomonella* with four commercial lures was evaluated using adherent Pherocon AM Traps during eight consecutive weeks. The trial was performed in a crop area of *C. mexicana*, in San Miguel Tlaixpan, Estado de Mexico, Mexico (19° 30′28.58″ N, 98° 48′19.03″ W), from 14 September to 9 November 2014. The test area was divided into five blocks of ~0.6 ha each. Each block had four Pherocon AM traps. Each of these traps was wrapped around a PET bottle with three 10-mm holes covered with a 0.3-mm net in which the lures were introduced. The 0.3-mm net covering holes was included to allow emission of volatiles and prevent the entrance of insects (Fig. 1a). The PET bottles were baited with four different lures: 1) 20 g of ammonium carbonate (AC) as the standard lure, 2) Biolure, ammonium acetate, and putrescine supplied in two individual sachets, 3) 250 ml of Captor + borax, and 4) 250 ml CeraTrap. Captor + borax and ammonium carbonate were replaced every 7 d, whereas Biolure and CeraTrap were not replaced during the test. Each trap was placed on a tree at a height of 3–3.5 m, within the canopy, and spaced at a distance of 21–25 m between traps. Placement of traps within each block was randomized initially and trap position was rotated clockwise in the block every week. Traps were inspected every 7 d, and captured fruit flies were carefully removed from panels using entomological tweezers, counted, and identified to species and sex in the field. Owing to the capture of a large quantity of nontarget insects, panels had to be replaced with new panels every 2 wk. The number of flies per trap per day for each trap and lure combination was calculated for eight consecutive weeks. The proportion of females was calculated for traps that captured at least one fly.

Improved PET Bottle Trap for Trapping *R. pomonella*

Flies used for behavioral tests to develop improved PET bottle traps were obtained from infested hawthorn fruit, *C. mexicana*, collected during August and September 2014 in the villages of Chiconquiaco (19° 44′37.89″ N; 96° 49′42.48″ W, 2,069 m altitude) and Landero y Coss (19° 43′42.12″ N, 96° 50′37.65″ W, 2,006 m altitude) in the state of Veracruz, Mexico. Collected fruit were taken to the laboratory at the BioMimic Scientific and Technological Cluster, Instituto de Ecología A.C. in Coatepec, Veracruz, and pupae and adults reared following the methodologies previously described in Rull et al. (2006). Adults that emerged during intervals of 5 d were separated according to sex and placed in plastic cages of 3 liter capacity, provided ad libitum access to food (3:1 mix of sugar: hydrolyzed protein) and water until sexual maturity. Flies used in tests were 20–35 d old. Two experimental cages were constructed using a 0.6- by 0.6- by 0.9-m PVC tubular frame covered with a white 1-mm nylon mesh. The size of these cages is adequate for laboratory testing of visual responses of Tephritidae, which are known to occur at close range (a few cm; Prokopy and Owens 1983, Brévault and Quilici 2010). Similar-sized cages have been successfully used for recent visual tests on some species of *Bactrocera* and *Zeugodacus* (Piñero et al. 2017). Four elastic lines placed across the top of each cage were used to hang galvanized wires from which traps were suspended. At the top of each cage, a white light (960 lm) lamp was placed at 15 cm above the ceiling to increase light intensity. Three independent tests were performed in cages to evaluate and improve visual attraction of *R. pomonella* to simple modified PET bottle traps.

Experiment 1

Polyethylene Bottle Traps With Colored Circles

Five PET bottle trap devices modified by the inclusion of 40-mm-diameter colored circles were tested. Four circles, consisting of colored paper with adhesive on one side (LUSTRIN, IKW, Mexico), were stuck 11.5 cm from the trap base and between the access holes of each bottle trap (Fig. 1b). Treatments were 1) a colorless bottle trap, 2) a bottle trap with green circles (RGB: 10, 134, 49), 3) a bottle trap with yellow circles (RGB: 240, 188, 31), 4) a bottle trap with orange circles (RGB: 212, 83, 24), and 5) a bottle trap with red circles (RGB: 180, 15, 18). All traps were baited with 250 ml of Captor + borax. Devices were randomly suspended from the ceiling in the corners and the center of the cage and rotated clockwise for a total of 10 replicates (i.e., each trap was tested two times in each position) to avoid a position effect. Within each cage, 10 male and 10 sexually mature female flies, alternately marked according to sex on the back of the thorax with a water-based white paint (Politec, Vinci, CD Mexico), were released and observed. The insects were introduced into cages at 11:25 h, 5 min before two observers scanned and recorded behavioral events: 1) landing on trap; 2) landing site on trap, divided into upper, middle, and lower parts (Fig. 1c); 3) and the sex of the fly. A landing event or a visit to a trap was scored when the fly landed and stayed for at least 5 s. Observations were made until 1400 hours. Total fly captures were recorded 24 h after release. Two cages, one per observer, were evaluated simultaneously.

Experiment 2

Polyethylene Bottle Traps With Colored Circles Against a Green Background

Based on the results of the first experiment, colors that yielded the best visitation and capture results (yellow, green, and orange) were chosen for a second experiment. Yellow- and orange-colored disks placed against a 4.5-cm wide green paper band background to emulate ripening fruit contrasting against tree foliage were evaluated. These designs were compared with colored circles without the green contrasting color. As in Experiment 1, two observation cages and the same observation criteria were established for four simultaneous treatments applied to PET bottles: 1) yellow circles, 2) orange circles, 3) yellow circles over a green background, and 4) orange circles over a green background. In total, eight replicates (two positions per trap) were performed to avoid position effects.

Experiment 3

Considering the results of both previous experiments, the bottle trap with yellow circles was selected for a final comparison against a bottle trap with red circles over a yellow background. This color combination imitates the effect of the “Ladd trap” developed for *R. pomonella* in United States (Kring 1970), where the contrast of a dark (red) object over a light background (yellow) triggers visual response of flies (Schutze et al. 2016). In this case, there were two trap models compared: 1) yellow colored circles and 2) red colored circles over a yellow background. Traps were baited with 250 ml of Captor + borax. Devices were randomly suspended from opposite corners of the ceiling and their positions rotated. Capture of flies was recorded 24 h after flies were released.

Comparison of PET Bottle Trap Versus Adherent Trap Efficacy

A final test was conducted to compare the efficacy of the conventional adherent yellow Pherocon AM trap (Fig. 1a) and PET bottle traps with red circles over a yellow background (Fig. 1b). The improved PET bottle trap was used owing to the higher efficacy of this design in previous laboratory tests. Owing to the higher efficacy of the Captor + borax lure during the first experiment, additional commercial proteins were also evaluated. An experiment was performed in the same area as the first field experiment between 28 September and 26 October 2015. The test area was divided into six blocks of ~0.6 ha each, with a total of 24 traps. The treatments evaluated after were 1) Pherocon AM trap baited with 20 g ammonium carbonate (standard reference trap), 2) PET bottle trap with Captor + borax, 3) PET bottle trap with Winner 360 + borax, and 4) PET bottle trap with Flyral + borax. Bottle traps were baited with 250 ml liquid hydrolyzed proteins that were replaced every 7 d. Owing to low decomposition rate, ammonium carbonate only needed to be replaced every 14 d. Each trap was placed on a hawthorn tree at a height of 3–3.5 m, within the canopy, and spaced 21–25 m apart. Placement of traps within each block was randomized and trap positions moved sequentially each week. Traps were inspected every week and captured flies were removed as in the first field experiment. Adherent yellow panels were replaced with new panels every 2 wk. The number of flies per trap per day for each trap type was calculated each week and the percentage of females was calculated from traps that captured at least one fly. The number of zero captures was recorded for traps that failed to capture any fly during each weekly exposure period.

Statistical Analyses

Mean percentage of wild and commercial hawthorn infested by *R. pomonella* was compared by *t*-test, with values normalized by rank transformation. Mean numbers of pupae per fruit, fruit weight, and the apical and equatorial diameter of fruit were also compared between fruit types by *t*-test of rank-transformed values. Mean numbers of flies trapped per day (FTD) were rank transformed (Conover and Iman 1981) and subjected to a one-way analysis of variance (ANOVA) in both field experiments. The percentage of females in trap catches was calculated for all traps that captured at least one fly. Percentage values were normalized by arcsine root transformation and subjected to one-way ANOVA in the first field experiment and subjected to a nonparametric Kruskal–Wallis test in the second field test. In this case, differences among treatments were obtained through Mann–Whitney tests with Bonferroni correction for multiple comparisons. The proportions of traps with zero captures were compared by Z test. For laboratory behavioral tests, a three-way ANOVA on rank-transformed frequencies per replicate was used in Experiment 1 for fly visits to the traps, considering trap model, sex, and the position where flies landed. Attraction among traps with colored circles with, or without a colored background, was analyzed by three-way ANOVA, considering sex, circle color, and background color. For all laboratory tests, flies captured per replicate were normalized by $\sqrt{(x+0.5)}$ transformation and subjected to a two-way ANOVA including model trap and sex. Separation for all ANOVA analyses was performed by Tukey’s honestly significant difference. All statistical procedures were performed using STATISTICA software v.7 (StatSoft Inc. 2015).

Table 1. Mean weight (± SE), apical and equatorial diameter (± SE), and percentage of infestation of commercial and wild hawthorn (*C. mexicana*) fruits and mean (± SE) numbers of pupae recovered from fruits of wild and commercial hawthorn

	Weight (g)	Diameter (mm)		% Infested fruits	Mean pupae per infested fruit[a]
		Apical	Equatorial		
Commercial hawthorn	20.1 ± 0.4a	32.1 ± 0.3a	35.1 ± 0.3a	36b	1.17 ± 0.1a
Wild hawthorn	6.5 ± 0.2b	23.7 ± 0.4b	23.6 ± 0.2b	66a	1.27 ± 0.1a

Values followed by the same letters are not significantly different (*t*-test, $P > 0.05$).
[a] Mean pupae considering infested fruits.

Table 2. Mean number of *R. pomonella* flies per trap per day (FTD ± SE), proportion of females, and total flies captured with different lures during an 8-wk field evaluation period

Lure	Mean FTD ± SE	Females (%) ± SE	Total flies trapped	No. of zero captures ($n = 40$)[a]
AC	1.05 ± 0.28a	80.5 ± 3.9a	294	8ab
Biolure	1.10 ± 0.36a	85.7 ± 4.5a	308	12b
CeraTrap	1.16 ± 0.30a	77.7 ± 4.3a	325	12b
Captor + Borax	1.74 ± 0.39a	87.8 ± 14.4a	487	3a

Values followed by the same letters are not significantly different among lures in the orchard ($P > 0.05$).
[a] Number of traps that did not capture any *R. pomonella* considering all traps during the whole period. Different letters indicate significant differences among lures in the orchard (Z-test, $P < 0.05$).

Results

Infestation of Wild and Commercial Hawthorns

Fruits stemming from commercial hawthorn orchards had a significantly higher weight ($t = 17.26$; df = 98; $P < 0.001$), apical diameter ($t = 14.90$; df = 98; $P < 0.001$), equatorial diameter ($t = 17.15$; df = 98; $P < 0.001$), and lower infestation ($t = 3.18$; df = 98; $P = 0.002$) than wild hawthorns (Table 1). Similarly, for samples that were collected and maintained in groups, a total of 113 pupae were recovered from 4.3 kg of commercial hawthorn (26.3 pupae per kg) compared with 272 pupae recovered from 2.7 kg of wild hawthorn (100.8 pupae per kg).

Attraction of *R. pomonella* to Lures Under Field Conditions

In total, 1,442 *R. pomonella* were captured during the experiment. Only 1.9% of trapped flies (28) could not be sexed because individuals had suffered damage and had lost their apical part. Among the sexed flies, 79.6% (1,126) were females and 20.4% males (276). During the 8-wk assessment, the effect of treatment was not significant ($F = 2.19$; df = 3,140; $P = 0.092$; Table 2). The proportion of females captured was also statistically similar among treatments ($F = 0.798$; df = 3,102; $P = 0.497$). A significantly lower number of zero captures was observed among lures ($\chi^2 = 18.92$; df = 3; $P < 0.01$), with Captor + borax being significantly more efficient than Biolure or CeraTrap, but statistically similar to ammonium carbonate.

Improved PET Bottle Trap for Trapping *R. pomonella*

Experiment 1

In total, 378 fly visits to PET bottle traps were observed during the experiment. The inclusion of yellow and green colored circles on bottle traps significantly increased fly visits versus a colorless bottle trap ($F = 4.29$; df = 4,490; $P = 0.002$). Red and orange circles resulted in intermediate attractiveness (Fig. 2a). Bottle traps with green circles were the most visited device (24.60%), followed by bottles with orange circles (23.28%), yellow circles (21.69%), red circles (16.93%), and colorless bottles (13.60%). Significant differences were observed among traps according to the part of the bottle where flies landed ($F = 15.243$; df = 2,490; $P < 0.001$). Flies landed more frequently on the middle (42.59%) and upper part (42.59%) of the bottle than on the lower section (14.81%; Fig. 2a). No significant differences in the proportion of females versus males landing on traps were observed ($F = 0.202$; df = 4,490; $P = 0.653$; females, 48.68%; males, 51.32%). The final capture of flies after 24 h differed significantly among treatments ($F = 2.605$; df = 4,80; $P = 0.04$), with bottle traps with yellow circles showing a higher capture than the colorless trap, but there were no differences among the other colors (Fig. 2b). No significant differences were observed between sexes ($F = 0.010$; df = 1,80; $P = 0.919$), with females (50.79%) and males (49.21%) captured at similar frequencies.

Experiment 2

Traps with circles over a green colored background were not significantly more attractive ($\chi^2 = 0.740$; df = 3; $P = 0.864$) than traps with circles without a colored background. Attraction was not significantly influenced by sex ($\chi^2 = 2.706$; df = 1; $P = 0.099$). However, as observed in the previous experiment, flies were significantly more attracted to the middle and upper part of the trap ($\chi^2 = 8.919$; df = 2; $P = 0.012$) than to the lower section, with mean percentages of landing of 43.2%, 43.9%, and 12.9% on the upper, middle, and lower sections, respectively (Fig. 3a). Furthermore, no significant differences were observed in the capture among traps ($F = 2.026$; df = 3,48; $P = 0.123$) and between sexes ($F = 0.303$; df = 1, 48; $P = 0.585$). Although there were no significant differences, traps with yellow circles had the highest mean percentage of capture, followed by traps with yellow circles over a light green background, traps with orange circles, and finally the trap with orange circles over a green background (Fig. 3b).

Experiment 3

Traps with yellow circles and no colored background, representing the best trap-color combination in the previous experiments, captured a significantly lower number of flies ($F = 4.473$; df = 1,44; $P = 0.040$) than traps with red circles over a yellow background

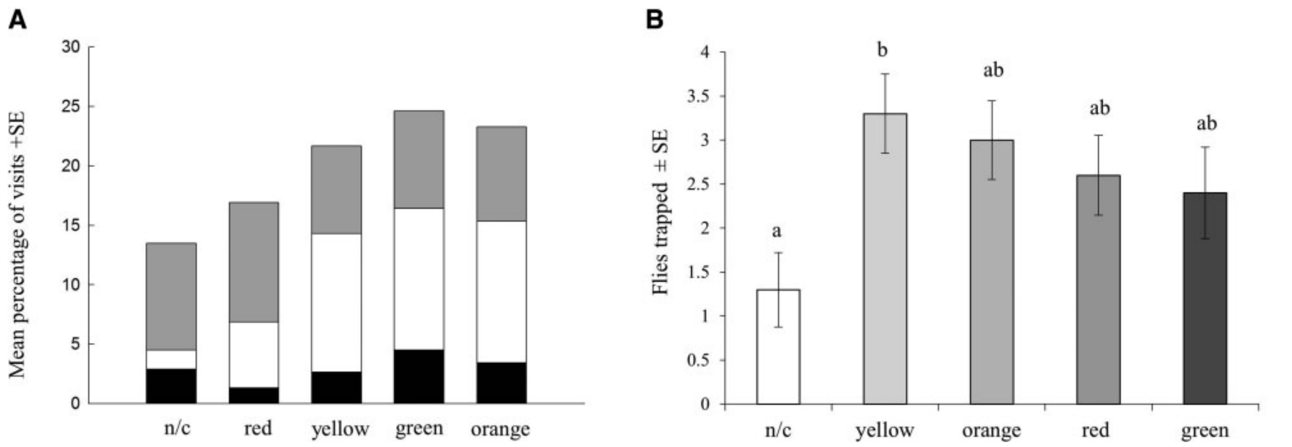

Fig. 2. Mean percentage of landing (**a**) and mean trapped *R. pomonella* flies ± SE (**b**) in colorless trap (n/c) and traps with different colored circles. Percentage of visits was also divided in reference to the lower (black), middle (white), and upper (gray) part of the bottle trap. Nontransformed means are shown. Columns headed by different letters indicate significant differences among model traps (Tukey, $P < 0.05$).

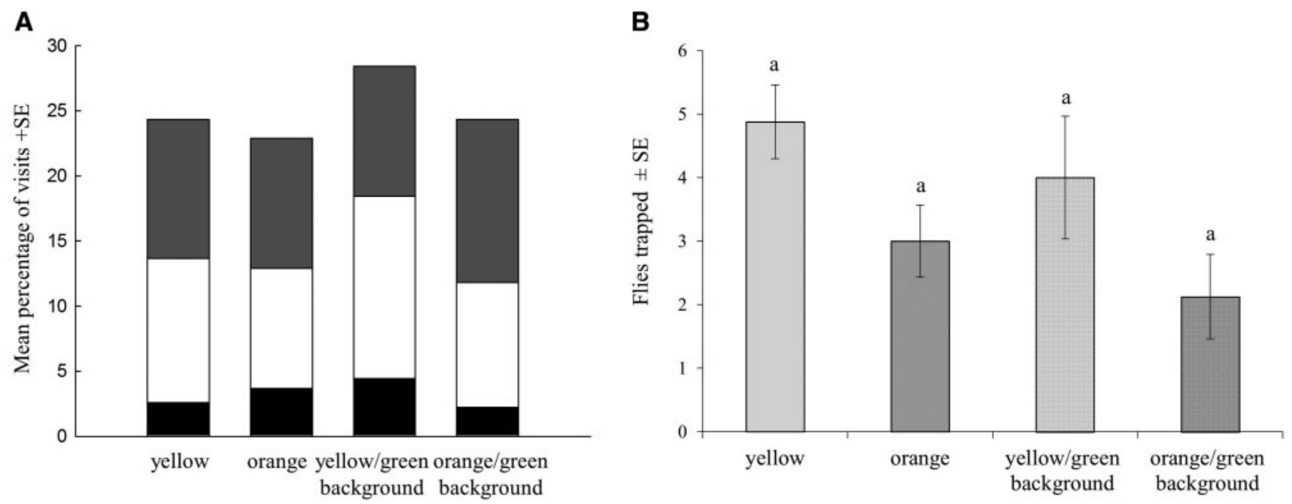

Fig. 3. Mean percentage of landing (**a**) and mean trapped *R. pomonella* flies ± SE (**b**) in different bottle traps with color circles with, or without, colored background. Percentage of visits was also divided in reference to the lower (black), middle (white), and upper (gray) part of the bottle trap. Nontransformed means are shown. Similar letters indicate no significant differences among model traps (Tukey, $P < 0.05$).

(Fig. 4). Again, no significant differences were observed between sexes ($F = 0.228$; df = 1, 44; $P = 0.635$), with females (49.47%) and males (50.53%) captured in similar frequencies.

Comparison of PET Bottle Trap Versus Adherent Trap Efficacy

In total, 922 *R. pomonella* adult flies were captured during the 4-wk experiment. Of these, 55.3% (510) were females and 44.7% were males (412). The mean FTD for the adherent trap baited with ammonium carbonate was significantly higher than captures with bottle traps baited with protein lures ($F = 21.46$; df = 3,72; $P < 0.001$). However, the proportion of females captured by the adherent trap was significantly lower than that observed for bottle traps with protein lures ($\chi^2 = 18.92$; df = 3; $P < 0.01$; Table 3). Among protein lures, the effect of treatment was not significant (Table 3). The number of zero captures differed significantly among lures ($\chi^2 = 9.905$; df = 3; $P < 0.019$). However, adherent traps baited with ammonium

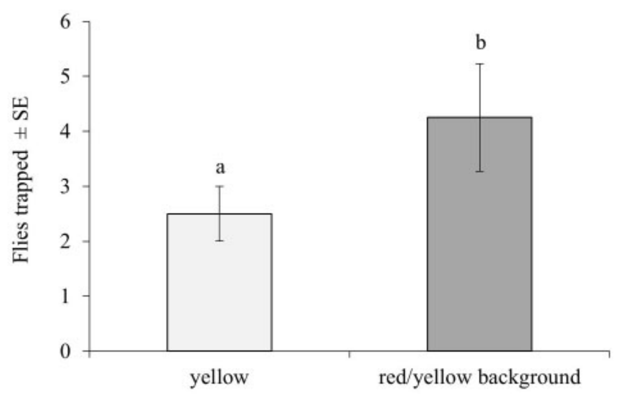

Fig. 4. Mean trapped *R. pomonella* flies ± SE in PET bottle trap with yellow colored circles and red circles with a yellow background (Fig. 1b). Different letters indicate significant differences among model traps (Tukey, $P < 0.05$).

Table 3. Mean number of *R. pomonella* flies per trap per day (FTD ± SE), proportion of females, and total flies captured with different lures during a 4-wk evaluation period

Lure	Mean FTD ± SE	Females (%) ± SE	Total flies trapped	No. of zero captures (n = 24)[a]
Pherocom AM trap—AC	3.0 ± 0.5a	49.5 ± 3.3a	511	0a
Bottle—Captor + Borax	0.9 ± 0.2b	75.7 ± 4.3b	153	2ab
Bottle—Flyral + Borax	0.4 ± 0.1b	77.5 ± 6.2b	67	7b
Bottle—Winner + Borax	1.1 ± 0.3b	67.9 ± 5.0b	191	3ab

Values followed by the same letters are not significantly different among lures in the orchard ($P > 0.05$).

[a] Number of traps that did not capture any *R. pomonella* considering all traps during the whole period. Different letters indicate significant differences among trap lure combination in the orchard (Z-test, $P < 0.05$).

carbonate were not significantly different in the number of zero captures than bottle traps baited with liquid Captor + borax (Z test, $P = 0.149$) or Winner + borax (Z test, $P = 0.68$), but had significantly lower zero catches than the trap with Flyral + borax (Z test, $P = 0.004$; Table 3).

Discussion

Wild hawthorn fruits were more heavily infested by *R. pomonella* than cultivated hawthorn fruits at the sample site. Flies in the genus *Rhagoletis* commonly exhibit marked preferences for some host species and cultivars over others (Stamenkovic et al. 1993, Liburd et al. 1998, Guillén et al. 2011). Cultivar susceptibility can be mediated by long distance attraction to fruit volatiles (Carle et al. 1987), greater visual stimulation for landing according to fruit color, size, or shape (Rull and Prokopy 2004a), greater egg-laying propensity of visiting females (Rull and Prokopy 2004b), and better larval performance (Dirks 1935), or as has been shown in the case of *Anastrepha ludens* (Loew) infesting apples, by the polyphenol content of the fruit, which is invariably higher in wild than cultivated apples (Aluja et al. 2014). Because there is a noticeable difference in the size of the fruit and variation in color, differences in responses by *R. pomonella* could be likely associated with visual stimuli. Alternatively, volatiles emitted by wild fruits may be more attractive to flies of both sexes. Regardless of the underlying cause, wild fruits were found to have a significantly greater probability of being infested at the sample site. Such a pattern has been informally observed over the course of extensive hawthorn sampling in Central Mexico (Rull et al. 2006), with large fruited domesticated hawthorn cultivars yielding substantially fewer larvae than their wild relatives. Unfortunately, these fruit collections were only identified to the hawthorn species level. Future research is therefore needed to document the tendency over several production areas across different seasons and considering other fruit properties such as ripening stage or sugar content as explanatory variables. Results of such studies can be exploited for the design of management strategies based on cultivar tolerance or trap cropping.

Another possibility explaining low commercial hawthorn infestation could be related to resistance transmission by rootstock through greater production of secondary metabolites. Aluja et al. (2014) determined that a lower production of *A. ludens* offspring from several apple cultivars was closely related to the presence of higher concentrations of phenolic compounds, and that high levels of these compounds could completely inhibit larval development, as previously reported for *R. pomonella* in crab apples (Pree 1977). From a management perspective, differential cultivar susceptibility can be exploited for trap-cropping, increased sensitivity to early detection, biological control, and directed chemical control (Aluja and Rull 2009).

Trap efficacy among tephritid flies has been demonstrated to be influenced by biotic factors such as gender, age, mating condition, nutritional status, host species, and phenology, in addition to abiotic factors such as climatic conditions, water, thermal stress, and the position of the trap, among others (reviewed by Díaz-Fleicher et al. 2014). However, chemical and visual cues of traps are determinant in their efficacy (Aluja and Prokopy 1993, Epsky and Heath 1998, Piñero et al. 2006, Brévault and Quilici 2010). In contrast to tephritid pest species in other genera, for which chemical stimuli prevail over visual stimuli, flies of the genera *Rhagoletis* usually exhibit strong responses to visual stimuli and have been considered to be visual specialists (Morrison et al. 2016). This may be because flies in the genus *Rhagoletis* mate on host fruit, unlike most species of *Anastrepha*, *Ceratitis*, and *Bactrocera* (Prokopy and Papaj 2000). Such behavior may exert stronger selection on attraction to fruit because it is both a mating arena and a site for offspring (larval) development. A second implication of the *Rhagoletis* mating system is that both sexes commonly respond to host-associated stimuli in a similar way. Nevertheless, as for tephritids in other genera, sexually immature female *R. pomonella* have oogenesis-related protein requirements. Males also have a need for protein, but to a lesser extent, so females may be more responsive to food-based lures (Rull and Prokopy 2000). In this respect, female *R. pomonella* exhibited a stronger response to protein-based lures in the field (typically ~80% of captures in the present study) than males, yet responses to visual stimuli in the laboratory were in all cases similar for both sexes.

The role of vision in attraction to traps by *R. pomonella* has been examined (Prokopy and Owens 1983), and in addition to color, the size and shape of stimuli are the two features that have received special attention. It has been hypothesized that *R. pomonella* adults react to yellow panels on the basis of true color discrimination, possibly because yellow surfaces represent a supernormal foliage type stimulus that elicits feeding behavior (Prokopy 1972). In contrast, the response to red spheres, in the case of flies infesting apples, constitutes a supernormal fruit type stimulus that elicits mating and egg-laying behaviors (Prokopy 1977). Combining an olfactory attractant with traps that elicit feeding-type reactions was shown to improve the capture of flies on yellow rectangles (Prokopy 1972, Yee et al. 2014b), but to a much lesser degree on red spheres (Rull and Prokopy 2000).

Under caged conditions, Mexican *R. pomonella* flies respond better to transparent traps with yellow color circles than to red traps, with a middle attraction to orange and green. Those results are in accordance with an electrophysiological study that revealed that *R. pomonella* flies responded to monochromatic light stimuli with a peak response from 450 to 570 nm (blue-green to yellow) and with a reduction response from 570 to 625 nm (orange to red;

Agee 1985). However, Prokopy (1968) found that *R. pomonella* flies exhibited a preference for red, blue, violet, and black apple-sized spheres, but when the size of spheres increased, there was a shift in color preference to yellow and shape became unimportant. No improvement of yellow and orange circle colors was observed when they were used in traps against a green background band. This could be owing to a reduced response to low contrast between both these colors. Owens and Prokopy (1984) observed that colored spheres were more attractive when the background was dark green than when it was light green or sky blue. When dark-red spheres were used, they were more attractive over a sky blue background than over darker foliage, indicating that for visual detection, the contrast of the object with the background could be more important than the color per se. Similar findings have been reported during field evaluations of Ladd traps and visually equivalent designs (red circles printed over a yellow rectangular cardboards) for *Bactrocera tryoni* (Froggatt) (Schutze et al. 2016). This contrast probably explains the stronger response observed in our study under laboratory conditions, using the bottle traps with red colored circles over a yellow background when compared with the simple yellow circle, despite the fact that red colored circles alone were initially less attractive than other colors.

In the first experiment, no significant differences were observed among different lures using yellow adherent panels, but numerically slightly higher captures were observed with liquid hydrolyzed proteins. In the case of CeraTrap, a lure that proved to be much superior to Captor + borax in attracting pest species in the genus *Anastrepha* (Lasa and Cruz 2014, Lasa et al. 2015, Rodriguez et al. 2015), the response of *R. pomonella* was equal or slightly lower than observed for other lures. In the second field experiment, no significant differences were observed among different chemical protein lures. Under field conditions, a significantly lower number of flies were captured in bottle traps baited with liquid protein lures than in the control Pherocon adherent trap baited with ammonium carbonate. Reynolds et al. (1996) found that *R. pomonella* females from eastern United States were very reluctant to enter openings in perforated red spheres containing lures and feeding stimulants. This could explain the greater performance of panels over bottle traps, but would require confirmation through behavioral observations in the field. However, a significantly higher proportion of females were trapped in the bottle traps. Tephritid females are in general more responsive than males to protein-based lures, probably because they have greater protein requirements for ovarian development (Díaz-Fleicher et al. 2014). It is difficult to explain why this capture pattern was not reproduced with yellow panels. Perhaps, it can be attributed to a greater effect of the visual stimulus (where no sex related differences exist) over chemical attraction.

Although tests performed with visual stimuli in traps were not very conclusive, further experiments could yield more useful results, given the small difference observed among adherent and nonadherent traps. For example, because *R. pomonella* flies tend to land on the middle and upper part of the traps, some traps with wider yellow stimuli and lateral access holes that proved effective for *Anastrepha* flies (Lasa and Cruz 2014, Lasa et al. 2014), could be a promising option for additional experiments seeking to replace McPhail traps where access is through the bottom of the trap (Hernández-Ortíz et al. 2004).

This study contributes new information on hawthorn infestation and the response of Mexican *R. pomonella* populations to commercial lures widely used in Mexico to monitor tephritid flies. Although a lower response was observed using bottle than panel traps, several variables could be improved in future studies. For example, studies on the response of flies to bottle traps with a higher yellow surface stimuli, other improvements in odor cues, and design of entry holes could be performed to develop a more effective, simple, and easy-to-use nonadherent monitoring trap that constitutes a necessary initial step toward rational pest management by commercial hawthorn growers. Indeed, because wild hawthorn is an important reservoir for the pest, it should also be monitored for *R. pomonella* activity in the periphery of commercial crops. Wild plants surrounding orchards could be used as trap-plants following control strategies, either collecting infested fruits or selectively applying insecticides to the fallen fruit or soil, to reduce fly emergence from one year to another. However, additional studies are required to better understand the specific attraction of Mexican *R. pomonella* populations to hawthorn species and varieties.

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