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1 Synergism of pheromone and host-plant volatile blends in the attraction of *Grapholita*
2 *molesta* (Busck) (Lepidoptera: Tortricidae) males

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21 **Short title** Pheromone and plant volatile synergism in *G. molesta*

23 **Keywords** – Insects, Oriental fruit moth, wind tunnel, aromatics, green leaf volatiles

Abstract

Control of *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae), a major pest of stone and pome fruits, is successfully achieved by mating disruption. Under these conditions, tools other than conventional pheromone dispensers are needed for flight monitoring. The objective of the present work was to determine whether plant volatiles synergize male *G. molesta* attraction to a suboptimal dose of synthetic sex pheromone. The plant blend (5VB), a mixture of three green leaf volatiles ((*Z*)-3-hexenyl acetate, (*Z*)-3-hexenol and (*E*)-2-hexenal), and two aromatics (benzaldehyde and benzonitrile), was added to the suboptimal pheromone dose (2 ng on filter paper) in log steps (up to 10,000x the pheromone dose) to test synergism of pheromone and plant blends. In addition, the effect of individual plant volatiles on male responses was investigated by adding to the suboptimal pheromone dose each of the four-compound plant-volatile blends resulting from eliminating a different plant volatile from the 5VB at a time, or each plant volatile alone. Flight behaviour and the time to reach the source were recorded. The 5VB alone was not attractive to *G. molesta* males, but at a ratio of 1:1000 (Ph:5VB) or higher increased the attractiveness of the suboptimal pheromone dose, to a level similar to that of the optimal pheromone dose (10 ng). All tested plant volatiles, except benzaldehyde, synergized the response to the pheromone when added individually, but only (*Z*)-3-hexenol and benzonitrile did so to a level not significantly different from the Ph:5VB blend. Aromatics had a stronger effect than GLVs, because their removal, but not the removal of GLVs, decreased landing responses. The addition of the 5VB decreased significantly the time males needed to reach the odour source. The observed enhanced male attraction to mixtures of pheromone and plant volatiles will facilitate the development of lures for *G. molesta* adult flight monitoring.

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1 Introduction

The oriental fruit moth, *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae), is an important pest of peach and apple worldwide (Il'ichev et al., 2004). The female sex pheromone is composed of (Z)-8 dodecenyl acetate (Z8-12:Ac), (E)-8 dodecenyl acetate (E8-12:Ac) and (Z)-8 dodecen-1-ol (Z8-12:OH) (Roelofs et al., 1969), and the blend of the three pheromone components is more effective than any single component in eliciting male responses (Baker & Cardé, 1979). The synthetic sex pheromone blend is widely used for *G. molesta* male flight monitoring and for mating disruption in stone and pome fruit orchards (Il'ichev et al., 2004; Stelinski et al., 2007). However, traps lured with conventional sex pheromone dispensers are not effective in mating disruption orchards, so the use of plant volatiles, mainly in combination with synthetic pheromones, has been suggested. As insect attractants, plant volatiles and sex pheromones can complement each other because the latter attract only one sex, males in the case of moths, whereas plant volatiles attract females, which are the ones that lay the eggs. Furthermore, plant volatiles may act as synergists, if the response elicited by the mixture of the pheromone and the plant volatiles is higher than the sum of the responses to the pheromone or the plant volatiles alone (Landolt & Phillips, 1997; Reddy & Guerrero, 2004). A recent meta-analysis of the published literature on the response of insects to individual plant volatiles or their mixtures in the field, shows that females are more attracted to plant volatiles than males (Szendrei & Rodriguez-Saona, 2010).

The best-known example of the use of a plant volatile in pest control is probably ethyl the case of the (E, Z)-2,4 decadienoate, the so-called pear ester. Identified from ripe Bartlett pears, it attracts mainly *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), but also other tortricid moths (Light et al., 2001; Schmidt et al., 2007; Molinari et al., 2010). In combination with the sex pheromone, the pear ester is used commercially in America (Knight et al., 2005) and Europe (Ioriatti et al., 2003) to monitor *C. pomonella* populations in apple, pear and

walnut orchards chemically treated or under mating disruption (Witzgall et al., 2008). As for *G. molesta*, a blend of five peach-shoot volatiles - three green leaf volatiles (GLVs): (Z)-3-hexenyl acetate, (Z)-3-hexenol and (E)-2-hexenal, and two aromatics: benzaldehyde and benzonitrile – has been reported to attract *G. molesta* females in laboratory tests carried out using a dual-choice olfactometer (Natale et al., 2003, 2004a, b; Piñero & Dorn, 2007), but the effect of plant volatiles, either alone or mixed with the pheromone, on the behaviour of *G. molesta* males has yet to be determined.

The main objective of this study was to determine whether host plant volatiles synergize the flight behaviour of *G. molesta* males towards the synthetic sex pheromone blend in the laboratory. This synergism could improve monitoring of male adult flight, especially in pheromone-treated orchards. To test this hypothesis we first established a pheromone dose-response curve and determined a suboptimal pheromone dose. Then we added different amounts of the plant volatile blend that attracts females to the suboptimal pheromone dose, to determine whether the response to this suboptimal blend could increase with plant volatiles. A suboptimal dose was used instead of the optimal one to increase the probability of detecting an effect of the plant volatiles and to test the possibility of reducing the amount of pheromone used in the field. Finally, we tested the effect of each single volatile when added to the pheromone, and the effect when subtracting it from the complete plant-volatile blend, on pheromone synergism.

Material and Methods

Insects

A colony of *G. molesta* was established at the University of Lleida (Spain) in September 2005 using individuals from a laboratory colony established from field-collected Italian insects (Dr. Fabio Molinari, Piacenza, Italy). Larvae were reared on a semi-artificial diet modified from

Ivaldi-Sender (1974) under a 16L:8D photoperiod at $25 \pm 1^\circ\text{C}$. Male pupae for experimental use were placed in a separate environmental chamber at $23 \pm 1^\circ\text{C}$ under a 16L:8D photoperiod. Male emergence was checked daily, and males were kept in ventilated plastic boxes with a 10% sugar-water solution. Two- to 3-day-old males were used in wind tunnel experiments.

Chemicals

The pheromone components of *G. molesta*, (Z)-8-dodecenyl acetate (Z8-12:Ac), (E)-8-dodecenyl acetate (E8-12:Ac) and (Z)-8-dodecenol (Z8-12:OH) (purity $\geq 99\%$) were purchased from Pherobank (Wageningen, The Netherlands). Plant compounds used for the experiments were the GLVs (Z)-3-hexenyl acetate (purity $\geq 98\%$), (Z)-3-hexenol (purity $\geq 98\%$) and (E)-2-hexenal (purity $\geq 95\%$), and the aromatics benzaldehyde (purity $\geq 99.5\%$) and benzonitrile (purity $\geq 99\%$), all purchased from Sigma-Aldrich Quimica SA (Madrid, Spain). The dilutions were prepared in *n*-hexane (purity $\geq 95\%$), and analyzed by GC-MS to detect possible contaminations or errors in blend composition.

Wind Tunnel

A 151 x 47 x 44 cm (length x height x width) glass wind tunnel without any pattern on the floor or the ceiling was used. The wind speed was maintained at 0.3 m s^{-1} and the temperature during tests was of $24 \pm 2^\circ\text{C}$. The wind tunnel was illuminated from above with white incandescent light bulbs producing approximately 100 lux inside the tunnel. Incoming room air was blown from a 30-cm-diameter fan, and the plume was extracted to the exterior of the building at the downwind end of the wind tunnel through an exhaust duct.

Bioassay Procedure

Five hours prior to the experiment, each male was placed in a cylindrical glass tube (15 cm long x 2.5 cm diameter), and the open ends were closed by rigid gauze. The glass tubes were left in the environmental chamber and 3 h before scotophase they were transported to the wind tunnel room for acclimatization. All bioassays were performed during the two hours preceding the onset of the scotophase. The odour source (10 μ l of *n*-hexane dilution loaded on a 1 x 1.5 cm hexane-rinsed filter paper) was fixed on a 15-cm-high wire platform placed at the upwind end of the wind tunnel and centred between the two lateral walls, less than 5 s before the insect was introduced. The insect tube was held horizontally, parallel to the air flow vector, on a similar wire platform located 135 cm downwind from the odour source. The gauze was removed from both ends of the tube, and the male was allowed 2 min to respond. Those males that did not fly within this time were gently touched to check for their ability to fly, and if incapable of flying they were excluded from the analysis. Males were tested individually and only once. The total number of individuals was 35 for each treatment. During the 2-h test period no more than ten males per treatment were tested per day. Two or more odour sources were tested per day. Between two odour sources, the walls of the wind tunnel were cleaned with ethanol while the air was running.

To establish dose-response curves, the treatments were tested in order of increasing concentration. For the other tests the order of presentation of the treatments was randomized on each experimental day. The following five behavioural responses were recorded: activation (Act: wing-fanning and walking in the release tube), take-off (TO: beginning of flight in any direction), oriented-flight (OF: upwind zigzagging flight), close-in (CI: oriented flight up to at least 15 cm from the source), and landing (L: touchdown at the source).

The interior of the wind tunnel was cleaned with ethanol and the exhaust fan continued to run for at least 1 h after the end of each experimental day. Insect tubes and wire platforms were thoroughly washed with acetone and oven-dried at 200°C over-night.

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6 2 *Pheromone dose-response*
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8 3 A pheromone dose-response curve was established to determine a suboptimal pheromone
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10 4 dose under our wind tunnel conditions. The pheromone blend consisted of Z8-12:Ac, E8-
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12 5 12:Ac, and Z8-12:OH at a ratio of 100:6:10, respectively (Baker et al., 1983; Linn & Roelofs,
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14 6 1983). The doses tested (0.1, 1, 2, 5, 10, 100, 500, and 1000 ng) were applied in 10 µl of *n*-
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16 7 hexane on a clean filter paper, and the control treatment consisted of a clean filter paper with
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18 8 10 µl of *n*-hexane.
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24 10 *Effect of pheromone plus the complete plant volatile blend*
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27 11 To test synergism between pheromone and plant compounds, we used the five plant-volatile
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29 12 blend (5VB) that has been shown to attract *G. molesta* females in olfactometer tests (Piñero &
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31 13 Dorn, 2007), i.e. a 70:14:2:13:1 blend of (Z)-3-hexenyl acetate (Z3Ac), (Z)-3-hexenol
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33 14 (Z3OH), (*E*)-2-hexenal (E2Ald), benzaldehyde (BZA), and benzonitrile (BZN), respectively,
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35 15 which corresponds to the natural ratio of these compounds in the headspace of peach shoots
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37 16 (Natale et al., 2003). Different quantities of the 5VB were added to a constant 2 ng
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39 17 suboptimal pheromone dose (Ph) (chosen from the results of the previous experiment), to
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41 18 prepare 1:10, 1:100, 1:1000, and 1:10,000 solutions of the pheromone: five-volatile blend
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43 19 (Ph5VB) (Table 1). The 5VB at a dose of 10,000 ng, and the Ph at a dose of 2 ng were used as
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45 20 controls.
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53 22 *Effect of pheromone plus single plant volatiles, or their absence from the Ph5VB*
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55 23 The ratio 1:10,000 of pheromone:plant-volatile blend was chosen for this experiment
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57 24 according to the results obtained in the previous experiment. The suboptimal 2 ng pheromone
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59 25 dose was maintained for all tests, and the plant-volatile blend added to it was modified from
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the 5VB by either using the plant compounds individually (1-compound blends) or by removing each of them, one at a time, from the 5VB (4-compound blends) (Table 2). The quantity of each compound in the 1- and 4-compound plant blends was the same as in the 5VB, and therefore the relative proportion of compounds in the plant blends depended on blend composition (Table 2). The Ph and Ph5VB (1:10,000) treatments were used as controls. Additionally, the duration of the upwind flight (from the beginning of oriented flight to landing) was recorded in this experiment.

Data analysis

For each behavioural category, a single Fisher Exact Test (McDonald, 2009) was used in each of the 3 experiments to test the hypothesis of independence of the response on the factor considered (amount of pheromone, ratio of Ph:5VB, and presence or absence of each individual compound, respectively). When the hypothesis of independence was rejected ($P < 0.05$), the Fisher Exact Test was used to perform comparisons among treatment pairs. All possible pairwise comparisons were carried out in the pheromone-dose and in the pheromone-plant volatile ratio experiments (36 and 15 pairwise comparisons, respectively), applying the Bonferroni correction (corrected significance levels $P < 0.0014$, and $P < 0.0033$, respectively). In the third experiment, instead of performing all possible pairwise comparisons, the response to each pheromone + 1- and + 4-compound plant blend was compared only with the response to the Ph and 5VB controls, applying also the Bonferroni correction (21 pairwise comparisons, $P < 0.0024$). To compare the flight time among different blend compositions in the last experiment, an ANOVA followed by a Duncan's Multiple Range Test with a significance level of 0.05 were carried out, using the SAS System for Windows V8 (version 8).

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Results

Pheromone dose response

For the five behavioural categories recorded, male response to the synthetic sex pheromone was dependent on the dose (Fisher Exact Test, $P < 0.05$). In general, the response first increased with the dose, reached a plateau or a peak, and decreased at the highest concentrations (Figure 1, activation and take-off data not shown). The highest values observed for each behavioural category were 100 % (activation), 97 % (take-off), 88 % (oriented-flight and close-in), and 86 % (landing). No males oriented to a filter paper loaded with 10 μ l of *n*-hexane. All doses showed significant differences from the *n*-hexane control for activation and take-off (data not shown). Only the lowest dose tested (0.1 ng) showed no statistical differences from the *n*-hexane control with respect to the percentage of oriented-flight, and close-in (Figure 1), but significantly more males activated, and took flight when exposed to the 0.1 ng dose than to the *n*-hexane (data not shown). In general, the percentages of close-in and landing were higher to 10, 100 and 500 ng than to the rest of the doses, but there were not significant differences among them (Figure 1). Only at the 10 ng dose, all males that started oriented flight also landed (Figure 1). At the highest dose (1000 ng), 71% of males oriented, but only 35% approached the source, and 17% landed on it. As no significant differences among 1, 2 and 5 ng were found, the intermediate dose of 2 ng (that elicited a 31 % of landing) was selected as the suboptimal dose for the subsequent experiments.

Effect of pheromone plus the complete plant volatile blend

For each behavioural category, there was a significant effect of the dose of 5VB added to the Ph (Fisher Exact Test, $P < 0.05$). The 5VB alone at a dose of 10,000 ng was not attractive to *G. molesta* males, as no male oriented to this treatment (Figure 2). At the 1:10 and 1:100 Ph:5VB ratios, no behavioural response was significantly different from Ph (Figure 2, Fisher

Exact Test, $P > 0.0033$), whereas at ratios of 1:1000 and 1:10,000 (that correspond to a dose of 5VB of 2000 and 20,000 ng, respectively), close-in and landing increased significantly compared to Ph (Figure 2, Fisher Exact Test, $P < 0.0033$). At the 1:10,000 ratio, all the males that started an oriented flight also landed at the source, so we chose this plant-volatile dose (2 ng of pheromone : 20,000 ng of 5VB) for subsequent experiments (Figure 2).

Effect of pheromone plus single plant volatiles or their absence from the Ph5VB

The complete pheromone-plus-plant volatile blend (Ph5VB) elicited 91% landing, whereas the suboptimal pheromone dose (Ph) only elicited 34% landing (Figure. 3A). Landing was significantly higher for all treatments when compared to Ph alone ($P < 0.05$), except for the addition of benzaldehyde (PhBZA) (Figure 3A). When individual aromatic compounds were added to Ph, BZN was as effective on landing as Ph5VB, but BZA had no effect. When individual GLVs were added to Ph, Z3OH was as effective on landing as Ph5VB, whereas the percentage of landing for Z3Ac and E2Ald was higher than Ph, but not as high as for Ph5VB (Figure 3A). The removal of any of the three GLVs (Z3Ac, Z3OH, and E2Ald) from the Ph5VB did not significantly affect any behavioural component, whereas the removal of the aromatic compounds BZA or BZN decreased landing significantly (Figure 3A). In only four treatments (Ph5VB, PhE2Ald, PhBZN, and PhmZ3Ac) all males that started an oriented flight landed at the source. No significant differences were seen in activation and take-off (data not shown).

The duration of the upwind flight also differed among treatments ($F = 5.05$, $P < 0.001$, $df = 11, 285$). Only the addition of the 5VB to the suboptimal dose of the pheromone decreased significantly the time males needed to reach the odour source (Figure 3B). When individual aromatic compounds were added to Ph, the addition of BZA showed no significant differences with the Ph5VB, whereas the addition of BZN did. When individual GLVs were

added to pheromone, the addition of Z3Ac showed no significant differences with the Ph5VB, whereas the addition of Z3OH and E2Ald did (Figure 3B). Removal of any of the three GLVs Z3Ac, Z3OH and E2Ald from the Ph5VB significantly increased the time males needed to reach the odour source compared with the Ph5VB, whereas in the case of the aromatic compounds only the removal of BZN did.

When taking into account both landing and flight time, the best result was obtained with the Ph5VB treatment, which elicited the highest landing rate in the shortest time, compared to all other treatments (Figure 3).

Discussion

Our data show that, from a dose of 2000 ng, the presence of host plant volatiles increases *G. molesta* male attraction to a sub-optimal dose (2 ng) of synthetic sex pheromone in a wind tunnel. Male landing in the wind tunnel significantly increased more than twofold when plant volatiles were added to the suboptimal pheromone dose, whereas no response was obtained with the plant volatile blend alone at the tested dose (10,000 ng, higher than the lowest dose that increased the response to the suboptimal pheromone dose). Thus, there is a synergistic effect of the five-plant-volatile blend on the male response to the sex pheromone. As for individual compounds, their reported effect on the response of lepidopteran males to the pheromone depends on the species, but the three GLVs that we have tested have already been reported to act as synergists (Reddy & Guerrero, 2000; Schmidt-Büsser et al., 2009).

The aromatic compound BZA had no effect when added to the pheromone, but its removal from the Ph5VB blend (PhmBZA) reduced synergism in *G. molesta*. This observation suggests that plant volatile-blends act as a unit, as it has been shown for the sex pheromone blends (Linn et al., 1987). The amount of BZA in peaches and nectarines is known to increase during maturation and can be translocated from the leaves to the fruit

(Chapman et al., 1991), and thus it could signal *G. molesta* males the proximity of females. Of all the compounds that we tested, BZN showed the strongest effect overall by being the only plant volatile that caused an effect both when added to the pheromone and when subtracted from the Ph5VB blend. This is especially significant because BZN constituted only 1% of the plant blend. We have not found any example in the literature for BZN having an effect on pheromone response, but this compound is an essential component in the plant blend that attracts females, where it activates a glomerulus in the antennal lobe that shows specific activity to the blend containing BZN (Piñero et al., 2007; Najar-Rodriguez et al., 2010).

The addition of the 5VB to the suboptimal dose of the pheromone clearly reduced the time it took males to complete their flight, meaning that they flew either faster or straighter towards the pheromone source when it was mixed with the complete plant blend than with the incomplete plant blends. Neither of the 4-compound plant blends, nor the individual plant compounds mixed with pheromone, shortened the flight time with respect to pheromone alone, but two individual compounds (PhZ3Ac and PhBZA), and one 4-compound blend (PhmBZA) reduced the time to reach the source to a level that was not different from the pheromone plus the complete plant blend (Ph5VB). Interestingly, the treatments that reduced flight time also had low scores of synergism. This is just the opposite of what we expected, that is, that the more synergistic treatments also reduce flight time, as it was reported in *E. ambiguella* (Schmidt-Büsser et al., 2009). In this last species two compounds, methyl salicylate and (*E*)- β -caryophyllene significantly reduced the time it took males to reach the pheromone source, and these two compounds also increased the percentage of males that responded to the pheromone. Furthermore, (*E*)- β -caryophyllene and (+)-terpinen-4-ol also induced a faster activation (Schmidt-Büsser et al., 2009). Although the effects of individual compounds and incomplete blends on flight time of *G. molesta* were heterogeneous, it is clear that some plant volatiles reduced the time it took males to reach the pheromone source, which

could provide an advantage to encounter mates under natural conditions. Whether this effect would have been observed if the optimal pheromone dose would have been tested is arguable, as probably male flight behaviour is already at its maximum.

Males and females differ in their response to the same plant volatiles (Szendrei & Rodríguez-Saona, 2010). There are examples where plant volatiles attract males but not females (Coracini et al., 2004) and plant volatiles that attract both sexes. Males and females of *C. pomonella* are attracted to fruit odours (Landolt & Guédot, 2008), and both sexes of several tortricid species are attracted to pear ester (Schmidt et al, 2007). The pear ester, which attracts male and female *C. pomonella*, is released by ripe pears, which are not an adequate oviposition substrate but could advertise a food source (Goff & Klee 2006; Landolt & Guédot, 2008). In the case of *G. molesta*, females are not attracted to Z3Ac, or BZN (Natale et al., 2003; Piñero & Dorn, 2007), whereas in our study these same compounds synergized male response to the pheromone. Four-compound plant blends without E2Ald, BZA or BZN are not attractive to females (Piñero & Dorn, 2007), whereas the equivalent blends in our experiment (mE2Ald, mBZA and mBZN) synergized male response to the pheromone. Perhaps the most drastic difference between sexes is that the plant blend did not attract males at all, but it is as attractive to females as peach shoots, the plant tissue from where the volatile blend was identified (Piñero & Dorn, 2007; Piñero et al., 2007). However, it has to be taken into account that the response of females and males was studied using different methodologies; an olfactometer and a wind tunnel, respectively. A similar result was observed in *Lobesia botrana* Den. & Schiff., where mated females are attracted to grapevine plants, but males are not (Masante-Roca et al., 2007), and in *C. pomonella*, where females are more attracted to α -farnesene than males (Coracini et al., 2004). Males and females look for food and hiding places, but only females must find a suitable oviposition site for their larval progeny. The particular needs of each sex could explain the different responses of males and

females to plant volatiles. In addition, females are usually more responsive to plant volatiles when they are mated (Natale et al., 2004a; Masante-Roca et al., 2007), so the physiological stage also affects plant-volatile responses.

In conclusion, we have proved that a five plant-volatile blend (5VB) from peach shoots synergize in the laboratory the responses of *C. molesta* males to a suboptimal pheromone dose, increasing it to a level similar to that of the optimal pheromone dose (10 ng). All tested plant volatiles, except benzaldehyde, synergized the response to the pheromone when added individually, being the effect of aromatics compounds stronger than the one of GLVs. The next step is to study the effect of the 5VB and the individual volatiles in the field, in order to facilitate the development of lures for male *G. molesta* adult flight monitoring, as it is currently done for *C. pomonella*.

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Table 1. Amount (μg) of individual plant-volatile compounds added to 2 ng of the pheromone blend of *Grapholita molesta* in order to obtain the desired Ph5VB blend while maintaining the natural five-volatile-blend ratio (70:14:2:13:1).

Ratio Ph : 5VB	Pheromone	Volatile					
		Volatile blend	(Z)-3- hexenyl acetate	(Z)-3- hexenol	(E)-2- hexenal	Benza- ldehyde	Benzo- nitrile
0:10,000	0.000	10.00	7.000	1.4000	0.2000	1.3000	0.1000
1:0	0.002	0.00	-	-	-	-	-
1:10	0.002	0.02	0.0140	0.0028	0.0004	0.0026	0.0002
1:100	0.002	0.20	0.1400	0.0280	0.0040	0.0260	0.0020
1:1000	0.002	2.00	1.4000	0.2800	0.0400	0.2600	0.0200
1:10,000	0.002	20.00	14.000	2.8000	0.4000	2.6000	0.2000

Table 2. Composition of the treatments, in μg and %, used to test the effect of the addition of individual plant compounds to the pheromone of *Grapholita molesta* and their subtraction from the Ph5VB, at a Ph:5VB ratio of 1:10,000. Treatments PhZ3Ac, PhZ3OH, PhE2Ald, PhBZA and PhBZN are a mixture of the pheromone blend and one plant volatile. Treatments PhmZ3Ac, PhmZ3OH, PhmE2Ald, PhmBZA and PhmBZN result from extracting one plant volatile from the pheromone plus complete plant volatile blend (5VB).

Treatment	Compound											
	Pheromone		(Z)-3-hexenyl acetate		(Z)-3-hexenol		(E)-2-hexenal		Benzaldehyde		Benzonitrile	
	μg	%	μg	%	μg	%	μg	%	μg	%	μg	%
Ph	0.002	100	–	–	–	–	–	–	–	–	–	–
5VB	–	–	14	70	2.8	14	0.4	2	2.6	13	0.2	1
Ph5VB	0.002	0.01	14	70	2.8	14	0.4	2	2.6	13	0.2	1
PhZ3Ac	0.002	0.01	14	99	–	–	–	–	–	–	–	–
PhZ3OH	0.002	0.07	–	–	2.8	99	–	–	–	–	–	–
PhE2Ald	0.002	0.50	–	–	–	–	0.4	99	–	–	–	–
PhBZA	0.002	0.08	–	–	–	–	–	–	2.6	99	–	–
PhBZN	0.002	0.99	–	–	–	–	–	–	–	–	0.2	99
PhmZ3Ac	0.002	0.03	–	–	2.8	47	0.4	7	2.6	43	0.2	3
PhmZ3OH	0.002	0.01	14	81	–	–	0.4	2	2.6	15	0.2	1
PhmE2Ald	0.002	0.01	14	80	2.8	16	0.4	2	–	–	0.2	1
PhmBZA	0.002	0.01	14	71	2.8	14	–	–	2.6	13	0.2	1
PhmBZN	0.002	0.01	14	71	2.8	14	0.4	2	2.6	13	–	–

Dose-dependent responses of *Grapholita molesta* males to the sex pheromone blend (Z8-12:Ac, E8-12:Ac, Z8-12:OH at a ratio of 100:6:10, respectively) loaded on filter paper in the wind tunnel. The percentage of individuals responding with oriented-flight (empty bars), close-in (grey bars), and landing (black bars) is indicated for each treatment. Within a given behavioural category, different letters represent significant differences (Fisher Exact Test, $P < 0.0014$, $n = 35$).
120x67mm (300 x 300 DPI)

Figure 2. Responses of *Grapholita molesta* males to different ratios of a suboptimal dose of pheromone (Ph, 2 ng) and a five plant volatile blend (5VB) (see composition in Table 1) loaded on filter paper in the wind tunnel. The percentage of individuals responding with oriented-flight (empty bars), close-in (grey bars), and landing (black bars) is indicated for each treatment. Within a given behavioural category, different letters represent significant differences (Fisher Exact Test, $P < 0.0033$, $n = 35$).

114x65mm (300 x 300 DPI)

Figure 3. Responses of *Grapholita molesta* males to blends of a suboptimal dose of pheromone (2 ng) and several plant volatiles combinations: individual plant compounds, 4-compound plant blends, and the 5-compound blend (see composition in Table 2). A: The percentage of individuals responding with oriented-flight (empty bars), close-in (grey bars) and landing (black bars) is indicated for each treatment. Within a given behavioural category, different lower-case letters indicate significant differences from the pheromone blend, while different upper-case letters indicate significant differences from the Ph5VB blend (Fisher Exact Test, $P < 0.05$). B: Mean time (in seconds) that males took from the start of the oriented flight until landing. Different letters represent significant differences among treatments (ANOVA and Duncan's Multiple Range Test, $P < 0.05$, $n = 35$).

1375x1062mm (96 x 96 DPI)