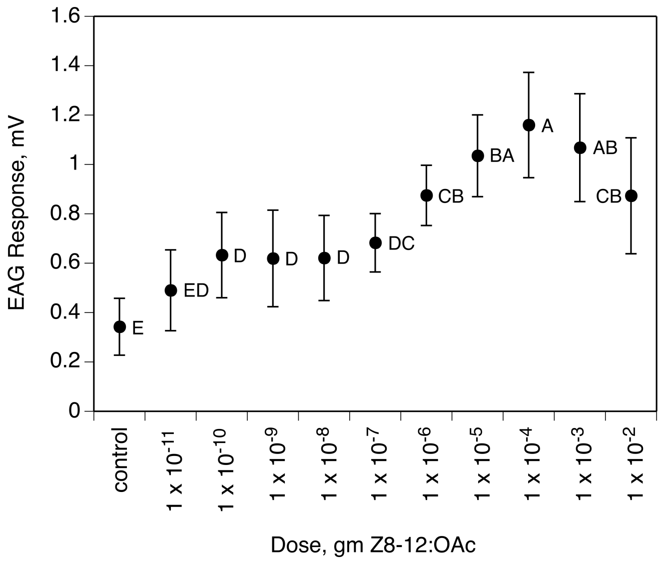
3. *Results*

3.1 *Pheromone and citral dose–EAG response.*

EAG response was affected by dose of the (*Z*)-8-12:OAc stimulus ($F_{10,90}$ = 33.8, $P < 0.0001$) (Fig. 32). A statistically significant response greater than the response to the control was detected using 1 x 10 $^{-10}$ g (i.e. 0.1 ng) of pheromone. There was no change in response with increased dose over the range 1 x 10 $^{-10}$ – 1 x 10 $^{-7}$ g of pheromone. Above this range, there was a trend of increase up to 1 x 10 $^{-4}$ g, and a sharp decline in response to the two highest doses of pheromone. There is no difference in the response to 1 x 10 $^{-5}$ and 1 x 10 $^{-4}$, and to 1 x 10 $^{-4}$ and 1 x 10 $^{-3}$ g (*Z*)-8-12:OAc. Consequentely the 1 x 10 $^{-5}$ g dose (i.e. 10 µg) was selected for use in experiments measuring treatment effect on the responsiveness of antennae. This same dose was used by Trimble and Marshall (2007; 2010) in their studies of sensory adaptation in OFM antennae.

Figure 32: Mean ±SD EAG response (mV) of male *Grapholita molesta* antennae after stimulation with increasing doses of *Z*8-12:OAc (g) at 1 min intervals. Different letters indicate statistically significant differences. ANOVA followed by Tukey test.



3.2 *Effect of citral on EAG response to pheromone.*

Net EAG response increased with the addition of increasing relative amounts of citral to the 10 µg (*Z*)-8-12:OAc stimulus ($F_{5,5} = 24.43$, $P = 0.002$) (Fig. 33a). Mean response (0.896 mV) to 10 µg (*Z*)-8-12:OAc plus 1000 µg of citral (i.e. 10,000%) was 2x greater than the mean response (0.462 mV) to 10 µg of (*Z*)-8-12:OAc alone. There was a weak relationship between net EAG response and Log_{10} (% citral) using both a linear (Net EAG response = 0.598 + 0.069 ($\log_{10}$ % citral), $F_{1,48} = 45.60$, $P < 0.0001$, $R^2 = 0.487$) and a quadratic polynomial (Net EAG response = 0.579 + 0.069 ($\log_{10}$ % citral) + 0.009 ($\log_{10}$ % citral)^2, $F_{2,47} = 23.44$, $P < 0.0001$, $R^2 = 0.499$) regression model. There was no change in net EAG response when citral was not added to the 10 µg (*Z*)-8-12:OAc stimulus ($F_{5,5} = 2.88$, $P = 0.14$) (Fig. 33b).

There was a very weak relationship between net EAG response and the time (Time, min) of stimulus application using a linear regression model (Net EAG response = 0.517 – 0.028 (Time, min), $F_{1,58} = 5.78$, $P < 0.02$, $R^2 = 0.09$) and no relationship when using a quadratic polynomial regression model (Net EAG response = 0.530 + 0.028 (Time, min) – 0.009 (Time, min – 3.5)^2, $F_{2,57} = 3.0$, $P = 0.06$, $R^2 = 0.1$).

The dose of citral alone, without *Z*8-12:OAc, affected mean EAG response ($F_{5,5} = 59.01$, df = 5,5, $P = 0.001$) (Fig. 34). Response to the control (0.501±0.067, mean±SD) and to the 0.1 (0.498±0.055), 1 (0.480±0.055) and 10 (0.498±0.072) µg doses were statistically similar ($P > 0.05$). The response to a 100 µg dose (0.644±0.095) was significantly greater than the response to the 10 µg dose ($P < 0.0001$) and the response to the 1,000 µg dose (0.867±0.125) was greater than the response to the 100 µg dose ($P < 0.001$). The mean±SD net EAG response was 0.143±0.073 mV using the 100 µg dose and 0.366±0.112 mV using the 1,000 µg dose of citral (Fig. 33c). Repeated stimulation with the control (i.e. 50 µL ethanol) seven times at one-minute intervals did not affect EAG response of antennae ($F_{6,4} = 2.78$, df = 6,4, $P = 0.17$).

Figure 33: Mean ±SD net EAG response (mV) of male *Grapholita molesta* antennae after six stimulations at 1 min intervals: (a) with 10 µg *Z*8-12:OAc alone and with increasing relative amounts (%) of citral; (b**)** with 10 µg *Z*8-12:OAc. Means followed by the same letter are not significantly different (Mulivariate repeated measures ANOVA followed by contrasts).

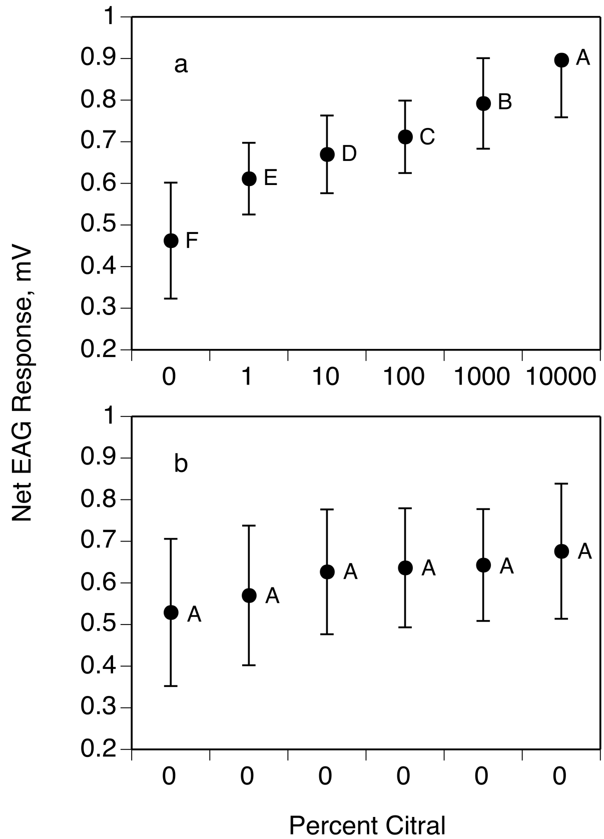
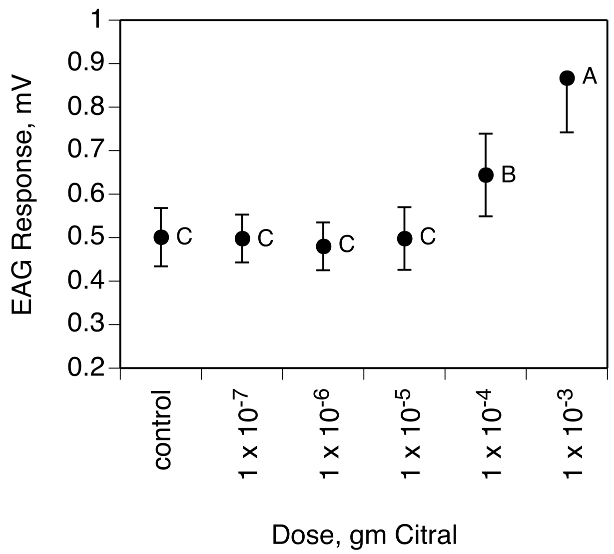


Fig. 34 Mean ±SD EAG response (mV) of male *Grapholita molesta* antennae after stimulation with increasing doses (gms) of citral at 1 min intervals. Means followed by the same letter are not significantly different (Mulivariate repeated measures ANOVA followed by contrasts).



3.3 *Effect of citral on sexual response of males to females and synthetic pheromone lure.*

The emission of 1,000 ng/min of citral approximately 1 cm down wind from a virgin calling female did not affect the number of males initiating the activation and take-off behaviors. The addition of citral to virgin calling female effluvia: a) reduced the number of male locking-on to the pheromone plume by 57.9%; b) reduced the close-in and touchdown phases of upwind flight behavior by 65.8% (Tab. 10); c) increase the time required for activation by 21.1% but did not affect the time required for the initiation of subsequent phases of upwind flight behavior (Tab. 11).
The emission of 1,000 ng/min of citral approximately 1 cm downwind from a Trécé Pherocon synthetic pheromone lure had no detectable effect both on the number of males initiating the activation or subsequent phases of upwind flight behavior (Tab. 10) and on the time required for onset of the five behavioral phases of upwind flight (Tab. 11).

Table 10: Number of male *Grapholita molesta* initiating successive phases of upwind flight behavior to two sources of pheromone in a flight tunnel with and without citral added to the pheromone plume and results of *Pearson's Chi-square* test (df = 1).

Treatment	Activation	Take-off	Lock-on	Close-in	Touchdown
Virgin calling *G. molesta* female					
Calling Female + Citral	39	38	16	13	13
Calling Female	40	40	38	38	38
χ^2	1.01	2.1	27.6	33.8	33.8
P	0.3	0.2	< 0.001	< 0.001	< 0.001
Synthetic sex pheromone lure					
Synthetic Pheromone + Citral	40	35	13	5	1
Synthetic Pheromone	40	35	8	6	1
χ^2	0.0	0.0	1.6	0.1	0.0
P	–	1.0	0.2	0.8	1.0

The effect of each treatment was tested on the response of 40 males.

Table 11: Mean (±SD) time (sec) required for male *Grapholita molesta* to initiate successive phases of upwind flight to two sources of pheromone in a flight tunnel with and without citral added to the pheromone plume and results of *Kruskal-Wallis* test (df = 1).

Treatment	Activation	Take-off	Lock-on	Close-in	Touchdown
Virgin calling *G. molesta* female					
Citral	10.4±8.2 (39)	76.2±85.6 (38)	5.2±3.0 (16)	3.4±2.5 (13)	1.9±1.0 (13)
Control	7.0±6.1 (40)	70.0±106.1 (40)	10.3±8.6 (38)	4.0±2.2 (38)	2.3±1.8 (38)
χ^2	5.7	0.5	3.4	1.7	0.1
P	0.01	0.5	0.07	0.2	0.8
Synthetic sex pheromone lure					
Citral	4.9±3.7 (40)	115.8±90.4 (35)	15.3±15.0 (13)	6.0±3.2 (5)	8.2±0.0 (1)
Control	7.0±11.8 (40)	116.1±67.2 (35)	20.9±22.3 (8)	4.7±1.6 (6)	3.6±0.0 (1)
χ^2	0.5	0.2	0.02	0.2	-
P	0.5	0.6	0.9	0.7	-

3.4 *Effect of pre-exposure of antennae to citral and citral combined with pheromone on EAG response to pheromone.*

Mean EAG response to the control stimulus (i.e. 50 µL ethanol) was reduced by 34.7, 38.8, 36.1, 40.8 and 23.7% after 15 min exposure to the air, (*Z*)-8-12:OAc, citral, (*Z*)-8-12:OAc + citral (1:1) and to the (*Z*)-8-12:OAc + citral (1:100) treatments, respectively (Tab. 12). Net EAG response to the 10 µg (*Z*)-8-12:OAc stimulus significantly declined after 15 min of exposure to the (*Z*)-8-12:OAc and citral treatments, but not after the same duration of exposure to the air and air + ethanol treatments (Tab. 13). The average percentage reduction in net EAG response was similar ($F_{3,16}$ = 0.5, P = 0.7) after 15 min of exposure to the (*Z*)-8-12:OAc (46.7±26.4, mean±SD), citral (62.5±24.3), (*Z*)-8-12:OAc + citral (1:1) (56.8±9.3) and to the (*Z*)-8-12:OAc + citral (1:100) (58.8±22.2) treatments.

Table 12: Mean±SD electroantennogram response (mV) of male *Grapholita molesta* antennae to a control stimulus before and after 15 min of continuous exposure to five treatments and results of *Paired t-test* (df = 4).

Treatment	Pre-exposure	Post-exposure	*t*	*P*
Control 1 (Air)	0.147±0.030	0.096±0.019	– 6.5	0.002
Control 2 (Air + Ethanol)	0.131±0.037	0.119±0.053	– 1.0	0.2
Z8-12:OAc	0.165±0.035	0.101±0.034	– 4.1	0.007
Citral	0.133±0.034	0.085±0.032	– 2.6	0.03
*Z*8-12:OAc + Citral (1:1)	0.147±0.040	0.087±0.019	– 5.1	0.004
*Z*8-12:OAc + Citral (1:100)	0.131±0.023	0.100±0.010	– 2.5	0.04

Five antennae were exposed to each treatment. Antennae were exposed to 6.25 µL ethanol/mL air in the Control 2 and pheromone/pheromone + citral treatments. Antennae were also exposed 1.0 x 10 $^{-6}$ ng *Z*8-12:OAc/mL air in the *Z*8-12:OAc treatment, 1.0 x 10 $^{-6}$ ng citral/mL air in the citral treatment, 1.0 x 10 $^{-6}$ ng *Z*8-12:OAc + 1.0 x 10 $^{-6}$ ng citral/mL air in the *Z*8-12:OAc + citral (1:1) treatment, and to 1.0 x 10 $^{-6}$ ng *Z*8-12:OAc/mL air + 1.0 x 10 $^{-3}$ ng citral/mL air in the *Z*8-12:OAc + citral (1:100) treatment.

Table 13: Mean±SD net electroantennogram response (mV) of male *Grapholita molesta* antennae to a 10 µg *Z*8-12:OAc stimulus before and after 15 min of continuous exposure to five treatments and results of *Paired t-test* (df = 4).

Treatment	Pre-exposure	Post-exposure	*t*	*P*
Control 1 (Air)	0.526±0.171	0.566±0.138	0.9	0.8
Control 2 (Air + Ethanol)	0.507±0.130	0.539±0.166	0.6	0.7
*Z*8-12:OAc	0.486±0.184	0.271±0.174	– 4.9	0.004
Citral	0.514±0.172	0.184±0.110	– 4.1	0.008
*Z*8-12:OAc + Citral (1:1)	0.603±0.222	0.253±0.096	– 4.8	0.004
*Z*8-12:OAc + Citral (1:100)	0.470±0.219	0.182±0.092	– 3.7	0.01

Five antennae were exposed to each treatment. Antennae were exposed to 6.25 µL ethanol/mL air in the control 2 and pheromone/pheromone + citral treatments. Antennae were also exposed 1.0 x 10^{-6} ng *Z*8-12:OAc/mL air in the *Z*8-12:OAc treatment, 1.0 x 10^{-6} ng citral/mL air in the citral treatment, 1.0 x 10^{-6} ng *Z*8-12:OAc + 1.0 x 10^{-6} ng citral/mL air in the *Z*8-12:OAc + citral (1:1) treatment, and to 1.0 x 10^{-6} ng *Z*8-12:OAc/mL air + 1.0 x 10^{-3} ng citral/mL air in the *Z*8-12:OAc + citral (1:100) treatment.

3.5 *Effect of pre-exposure of antennae to citral and citral combined with pheromone on male response to females.*

Fifteen minutes of exposure to the air, air + ethanol, (*Z*)-8-12:OAc, citral, and two (*Z*)-8-12:OAc + citral treatments had no effect on the number of male *G. molesta* initiating the activation, take-off, lock-on, close-in and touchdown phases of upwind flight behavior in response to a virgin, calling *G. molesta* female (Tab. 14). These treatments also had no effect on the number of males initiating these behaviors in response to the first of the three females that were used to test male responsiveness (Tab. 15). The time required to initiate each of the six upwind flight behaviors was not affected by treatment (Tab. 16).

Table 14: Number of male *Grapholita molesta* initiating successive phases of upwind flight to a virgin calling *G. molesta* female in a flight tunnel after 15 min of exposure to one of six treatments and results of *Logistic Regression Analyses.*

Treatment	Activation	Take-off	Lock-on	Close-in	Touchdown
Control 1 (Air)	15	15	15[a]	15[a]	15[a]
Control 2 (Air + Ethanol)	15	15	15[a]	15[a]	15[a]
*Z*8-12:OAc	15	15	14	14	14
Citral	15	15	15[a]	14	14
*Z*8-12:OAc + Citral (1:1)	15	15	11	11	11
*Z*8-12:OAc + Citral (1:100)	15	15	14	14	14
χ^2	-	-	3.3	3.8	3.8
df	-	-	2	3	3
P	-	-	0.2	0.3	0.3

[a] Exluded from analysis

Fifteen males were tested with each treatment. Antennae were exposed to 6.25 μL ethanol/mL air in the control 2 and pheromone/pheromone + citral treatments. Antennae were also exposed 1.0 x 10 $^{-6}$ ng *Z*8-12:OAc/mL air in the *Z*8-12:OAc treatment, 1.0 x 10 $^{-6}$ ng citral/mL air in the citral treatment, 1.0 x 10 $^{-6}$ ng *Z*8-12:OAc + 1.0 x 10 $^{-6}$ ng citral/mL air in the *Z*8-12:OAc + citral (1:1) treatment, and to 1.0 x 10 $^{-6}$ ng *Z*8-12:OAc/mL air + 1.0 x 10 $^{-3}$ ng citral/mL air in the *Z*8-12:OAc + citral (1:100) treatment.

Table 15: Number of males *Grapholita molesta* initiating successive phases of upwind flight to a virgin calling *G. molesta* female in a flight tunnel on the first of three attempts after 15 min of exposure to one of six treatments and results of *Logistic Regression Analyses*.

Treatment	Activation	Take-off	Lock-on	Close-in	Touchdown
Control 1 (Air)	15[a]	14	11	11	11
Control 2 (Air + Ethanol)	15[a]	15[a]	11	11	11
*Z*8-12:OAc	13	13	9	9	9
Citral	15[a]	14	11	10	10
*Z*8-12:OAc + Citral (1:1)	15[a]	14	10	10	10
*Z*8-12:OAc + Citral (1:100)	14	13	12	12	12
χ^2	0.4	0.9	1.7	1.8	1.8
df	1	4	5	5	5
P	0.5	0.9	0.9	0.9	0.9

[a] Excluded from analysis

Fifteen males were tested with each treatment. Antennae were exposed to 6.25 μL ethanol/mL air in the control 2 and pheromone/pheromone + citral treatments. Antennae were also exposed 1.0×10^{-6} ng *Z*8-12:OAc/mL air in the *Z*8-12:OAc treatment, 1.0×10^{-6} ng citral/mL air in the citral treatment, 1.0×10^{-6} ng *Z*8-12:OAc + 1.0×10^{-6} ng citral/mL air in the *Z*8-12:OAc + citral (1:1) treatment, and to 1.0×10^{-6} ng *Z*8-12:OAc/mL air + 1.0×10^{-3} ng citral/mL air in the *Z*8-12:OAc + citral (1:100) treatment.

Table 16: Mean±SD time (sec) required for male *Grapholita molesta* to initiate successive phases of upwind flight to a virgin calling *G. molesta* female in a flight tunnel after 15 min of exposure to one of six treatments and results of *Kruskal-Wallis* test (df = 5).

Treatment	Activation	Take-off	Lock-on	Close-in	Touchdown
Control 1 (Air)	5.9±8.2 (15)	41.2±36.5 (15)	3.7±2.5 (15)	4.0±2.5 (15)	1.4±1.3 (15)
Control 2 (Air + Ethanol)	5.6±3.3 (15)	54.1±51.6 (15)	4.3±3.0 (15)	3.3±1.5 (15)	2.2±1.9 (15)
*Z*8-12:OAc	6.7±7.7 (15)	105.3±115.4 (15)	5.1±2.6 (14)	3.3±1.7 (14)	2.4±3.1 (14)
Citral	7.7±13.9 (15)	54.0±56.7 (15)	4.7±6.4 (14)	3.3±0.9 (14)	1.4±1.0 (14)
*Z*8-12:OAc + Citral (1:1)	6.0±3.8 (15)	66.5±55.2 (15)	3.7±1.7 (11)	3.6±1.6 (11)	1.8±1.5 (11)
*Z*8-12:OAc + Citral (1:100)	13.7±17.9 (15)	142.9±129.7 (15)	3.5±1.6 (14)	4.1±1.9 (14)	1.7±1.8 (14)
χ^2	8.5	8.9	5.0	2.3	3.2
P	0.1	0.1	0.4	0.8	0.7

Number of males in parenthesis. Antennae were exposed to 6.25 μL ethanol/mL air in the Control 2 and pheromone/pheromone + citral treatments. Antennae were also exposed 1.0 x 10 $^{-6}$ ng *Z*8-12:OAc/mL air in the *Z*8-12:OAc treatment, 1.0 x 10 $^{-6}$ ng citral/mL air in the citral treatment, 1.0 x 10 $^{-6}$ ng *Z*8-12:OAc + 1.0 x 10 $^{-6}$ ng citral/mL air in the *Z*8-12:OAc + citral (1:1) treatment, and to 1.0 x 10 $^{-6}$ ng *Z*8-12:OAc/mL air + 1.0 x 10 $^{-3}$ ng citral/mL air in the *Z*8-12:OAc + citral (1:100) treatment.

3R *References*

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