

Plant volatile compounds shorten reaction time and enhance attraction of the codling moth (*Cydia pomonella*) to codlemone

Dénes Schmera and Patrick M Guerin*

Abstract

BACKGROUND: The codling moth is the most serious pest of deciduous tree fruit (apples, pears, crabapples, walnuts, quince) worldwide. The high frequency of insecticide treatments per season has resulted in breakdown of codling moth control owing to insecticide resistance. As an alternative, integrated pest management includes mating disruption to achieve population suppression in orchards. Under this scheme, the sex pheromone of the codling moth, (*E, E*)-8,10-dodecadien-1-ol (codlemone), is released from dispensers in crops to hinder mating by luring males. Increasing the attractiveness of codlemone formulations to codling moth males can be regarded as a key to increasing the efficacy of mating disruption. With this aim, the effects of adding plant volatiles on the behavioural responses of codling moth males to codlemone were tested.

RESULTS: Adding *R*(+)-limonene, linalool, (*E*)- β -farnesene or ethyl (*E, Z*)-2,4-decadienoate to codlemone significantly increases the proportion of males flying to the pheromone source in a wind tunnel. The response level is equivalent to that of males responding to females releasing codlemone. Using real-time recordings, it is shown how these four plant products also shorten the response time of males to codlemone under the behavioural criteria time to activation, time till upwind flight is induced and time to pheromone source contact.

CONCLUSION: Shortening the response time and increasing source location by males of dispensers releasing codlemone with *R*(+)-limonene, linalool, (*E*)- β -farnesene or ethyl (*E, Z*)-2,4-decadienoate added would enhance mating disruption through better engagement of males with dispensers, to the detriment of females.

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Keywords: codling moth; *Cydia pomonella*; codlemone; pheromone; plant volatile; mating disruption; orchard; orchard pest; pest management; wind tunnel; behaviour

1 INTRODUCTION

Codling moth, *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae), is a worldwide pest of apple, pear and walnut. Public concern about the use of insecticides in fruit production and increasing resistance to insecticides in codling moth^{1–3} demand the development of novel and safe pest control techniques.^{4–6} Mating disruption with insect sex pheromones is one such technique that is based on confusing olfactory communication between the sexes of an insect species by releasing synthetic sex pheromone from dispensers within crops.^{7,8} Although several mechanisms have been proposed as to how mating disruption works,⁹ a mode of action based on competitive attraction to point sources of synthetic pheromone has received well-founded support,¹⁰ but see Stelinski *et al.*¹¹ for alternative explanations. Under such a scheme, a synthetic pheromone source engages males at a distance where its dose is in the range of that released by calling females (in the case of codling moth, about 100 pg min⁻¹).^{12,13} Consequently, if the efficiency of mating disruption depends on the extent of the area over which the pheromone released from a dispenser is at the optimal dose to engage males, it follows that extending this range is a way to increase the efficiency of mating disruption.

The identification of the main sex pheromone component of the codling moth, (*E, E*)-8,10-dodecadien-1-ol or codlemone,¹⁴

promoted mating disruption as a promising tool for environmentally safe control of this important pest.^{8–11,15} Although mating disruption of codling moth has been in use for over 20 years,¹⁶ it does not work well as a stand-alone technology when population densities are high.¹⁷ Increasing the attractiveness and effective range of codlemone dispensers provides a possibility of augmenting the efficiency of mating disruption.

Arn *et al.*¹² showed in wind tunnel experiments that, by adding the secondary pheromone component dodecan-1-ol, identified in the sex gland of female codling moths, to codlemone, the attractiveness of the latter to males could be enhanced, especially at under- and over dosed levels of codlemone. This shows that, by adding a biologically active component at an appropriate dose to codlemone, the dose range over which codling moth males are attracted can be widened. Later, other secondary sex pheromone components in codling moth female sex glands

* Correspondence to: Patrick M Guerin, Institute of Biology, Faculty of Science, University of Neuchâtel, Rue Emile-Argand 11, 2000 Neuchâtel, Switzerland. E-mail: patrick.guerin@unine.ch

Institute of Biology, Faculty of Sciences, University of Neuchâtel, Neuchâtel, Switzerland

were identified,^{18–20} and, depending on the dose applied, these products were also found to enhance attraction of codling moth males to codlemone. For instance, the proportion of males landing at the pheromone source in a wind tunnel increased from 55 to about 85% when (*E,E*)-8,10-dodecadienyl acetate released at 0.1 pg min⁻¹ was added to codlemone released at 10 pg min⁻¹.²⁰ Currently, the secondary sex pheromone components dodecan-1-ol and tetradecan-1-ol are widely used in mating disruption of codling moth, along with codlemone, to increase efficiency of dispensers.^{8,15}

It was hypothesised that, similarly to secondary sex pheromone components, plant volatiles could also be regarded as candidate products to enhance the efficacy of mating disruption of codling moth. This assumption was based on the following facts. Firstly, basiconic sensilla of male moths such as those of the silkworm, *Antheraea polyphemus*, i.e. those sensilla that do not house the pheromone receptor cells, contain general odorant-binding proteins similar to those that occur in basiconic sensilla of females.²¹ These proteins surround the dendrites of receptor cells within olfactory sensilla on the insect antenna that are presumed to bind and ferry plant odours. It is therefore likely that the situation is the same in the codling moth. Secondly, odour components of plants elicit antennal receptor cell responses in male codling moths,^{22–24} suggesting that antennal receptor cells in male moths are tuned to the perception of plant compounds and not just to pheromones. Thirdly, early studies emphasised the importance of host plant volatiles on the behaviour of codling moth, where it was shown that α -farnesene is an attractant and oviposition stimulant for this species.^{25,26} Fourthly, codling moth males are attracted, like females, to the plant volatiles ethyl (*E,Z*)-2,4-decadienoate, or pear ester,^{22,24,27–29} *E*- β -farnesene,^{27,28} *E,E*- α -farnesene and (*Z*)-jasmane.²⁷ Fifthly, male codling moths respond to fruit-bated traps in orchards.³⁰ Finally, codling moth males were better attracted in a wind tunnel to a mixture of codlemone with any of the plant volatiles linalool, *E*- β -farnesene or (*Z*)-3-hexen-1-ol than to codlemone alone.³¹ For example, linalool released at 100 pg min⁻¹ mixed to codlemone released at 1 pg min⁻¹ increased the proportion of males that contacted the source from 37 to 60%.³¹ In summary, all of these observations suggest a fundamental role of plant-derived semiochemicals in the sensory ecology of codling moth males.

According to Witzgall *et al.*,⁹ behavioural response of male codling moth to plant volatiles is an adaptive mechanism to accelerate mate-finding because, under natural conditions, the time available each evening for mating is restricted. However, it is not clear whether plant volatiles enhance merely the activity of moths (influencing only early behavioural steps from activation to take-off³¹) or aid orientation (influencing behavioural steps from onset of upwind flight to source contact^{12,20}). The aim of the present study is to examine whether and how plant volatiles can influence the different steps in the behavioural responses of codling moth males to codlemone from activation to source contact in a wind tunnel. To this end, 11 plant volatile compounds^{22,30,32} were selected that could influence, or have been shown in previous studies to influence, the behavioural response of male *C. pomonella* to codlemone. In contrast to earlier studies, in addition to recording the different behavioural steps characterising the attractiveness of test products to males, the time elapsed from exposure to a treatment until a particular behavioural step was observed was also recorded. Using these real-time recordings, it is shown how plant volatiles can both shorten response time and enhance attraction of codling moth males to codlemone.

2 EXPERIMENTAL METHODS

2.1 Insects

Pupae of *C. pomonella* originating from Andermatt Biocontrol AG (Grossdietwil, Switzerland) were removed from the corrugated cardboard strips in which they had pupated, and the sexes were separated. Adult males and females emerged into plastic cages (30 cm × 30 cm × 30 cm, mesh size 24, type BUGDORM-I; Megaview Science Education Services Co., Ltd, Taichung, Taiwan) in a climate chamber (Rosemund AG, Liestal, Switzerland) at 25 °C and 65% RH in the photophase (18 h) and 18 °C and 85% RH in the scotophase (6 h).

2.2 Chemicals

Plant secondary products tested were hexan-1-ol (hereafter hexanol, >99% purity), (*Z*)-3-hexen-1-ol (>98%), (*E*)-2-hexen-1-ol (>99%), ethyl (*E,Z*)-2,4-decadienoate (pear ester, >97%), (*Z*)-3-hexenyl acetate (>98%), *R*(+)-limonene (limonene, >98%), (\pm)-linalool (linalool, >97%), β -caryophyllene (caryophyllene, >98.5%) and methyl salicylate (>99%) supplied by Sigma-Aldrich Chemie (Buchs, Switzerland), (*E*)- β -farnesene (beta-farnesene, >90%) from Bedoukian Research, Inc. (Danbury, CT), 4,8-dimethyl-1,3(*E*),7-nonatriene (dimethyl-nonatriene, ~94%) from Givaudan (Dubendorf, Switzerland) and (*E,E*)-8,10-dodecadien-1-ol (codlemone, >99.8%) from Siegfried Ltd (Zofingen, Switzerland). Ethanol was used as solvent (analysis grade, >99.8%; Merck AG, Dietikon, Switzerland).

2.3 Wind tunnel

A wind tunnel (195 cm long, 60 × 60 cm) of non-reflecting glass was equipped with centrifugal ventilators at both ends that operated simultaneously to move air across its length at 30 cms⁻¹ through active charcoal filters at the up- and downwind ends. Overhead illumination was provided by 36 W fluorescent tubes (Philips type TLD36W/830) at 36 kHz. Two groups of six tubes 120 cm long (total illuminated length 190 cm) hung 12 cm above the top of the wind tunnel. The light intensity was regulated with a potentiometer to produce ca 10 lux along the wind tunnel floor. Light was dispersed using a Perspex Prisma[®] crystal-clear plastic sheet under the fluorescent tubes and by placing crepe paper on the roof of the wind tunnel. The wind tunnel was housed in a walk-in climate chamber (Schaller Uto AG, Bern, Switzerland) that allowed the air stream to be maintained at 18 ± 0.5 °C and 85 ± 2% RH during experiments. These conditions were adequate for males to fly and make contact with calling females in the wind tunnel.

2.4 Release of test stimuli in the wind tunnel

Stock solutions of synthetic chemicals were made up in ethanol and were dispensed from an ultrasound evaporator³³ at the head of the wind tunnel, as already described.³⁴ Briefly, the ultrasound evaporator consisted of a syringe pump that pumped the test solution at 10 μ L min⁻¹ from a 5 mL gas-tight syringe into PTFE microtubing connected to a borosilicate glass capillary with a drawn-out tip (10–20 mm tip length, 30–40 μ m i.d. tip opening). A frequency generator producing a 80 kHz signal was applied to a piezoceramic disc that hung from the glass capillary. This caused the capillary tip to oscillate and produce an aerosol of the test solution that broadened to ca 10 mm at 50 mm from the source. The oscillating glass capillary was installed in the middle of the upwind end of the wind tunnel at 30 cm from the floor. A metal grid cylinder (15 mm long, 30 mm o.d.) placed

over and along the axis of the capillary protected the latter against damage by moths attempting to contact the chemical source. Oscillations of the capillary tip dispersed the solution as microdroplets that evaporated within a few cm downwind of the release point to create an invisible plume at 10–15 cm from the source. The ratio of codlemone and plant volatiles was 1 : 10 in the present experiments. When the responses of males to calling females were tested, a single calling female *C. pomonella* was presented in a glass tube (25 mm o.d., 21 mm i.d., 125 mm long) closed at either end with curtain netting (1.5 mm mesh). The tube was placed on a platform 30 cm high at the upwind end of the wind tunnel with its long axis parallel to the wind direction.

2.5 Experimental protocol

Experiments were performed with 2–4-day-old males within 0.5–3 h of the onset of the scotophase, when females called. Males were transferred from the rearing cabinet to the environmental cabinet housing the wind tunnel (both at the same environmental conditions) 20 min before the experiments. A single male was presented in a glass tube (125 mm long, 25 mm o.d., 21 mm i.d.) on a stand (30 cm high) at the downwind end of the wind tunnel (the upwind end of the glass tube was 35 cm from the downwind end of the wind tunnel) in each test, i.e. at the same height as the oscillating capillary. Sixty males were tested per treatment, with the exception of tests with different release rates of codlemone, when on average 24 males were tested for each dose. After the glass tube with the male had been put on the stand, first the downwind cap and then the upwind plastic cap on the glass tube were removed, and the door of the wind tunnel was closed. Real-time recording started immediately by starting the OBSERVER v.5.0 software package (Nodus Information Technology, Wageningen, The Netherlands), which ran on a portable PC. The same person put the glass tube on the stand, removed the caps, closed the door and started the software. Two minutes were allowed for the male to respond to each test treatment, during which the following behaviours were recorded by eye and the time at which each behavioural criterion began was recorded by pressing the appropriate key of the software protocol: moth *activation* (movement in the release tube), *wing fanning*, *take-off*, *upwind flight* (upwind flight of at least 15 cm towards the odour source), passing the *midline* of the flight zone in the wind tunnel (158.5 cm in total from the upwind end of the release tube to the protection grid over the glass capillary tip), *close-in* (approaching to within 15 cm of the metal grid) and pheromone source *contact* (contact to the metal grid by the moth).

2.6 Statistical analysis

The proportion of males showing each behavioural element per treatment was calculated (number of males responding under each behavioural category over the total number of males tested). These treatment proportions were then compared using generalised linear models (GLMs) with binomial distribution and logit link function. Kaplan–Meier curves³⁵ were used for time-based representation of responses of males to different test solutions. These response–time relationships were compared using the Cox proportional hazard model.³⁶ All statistical analyses were performed using the statistical software R,³⁷ with a type I error rate of 0.05.

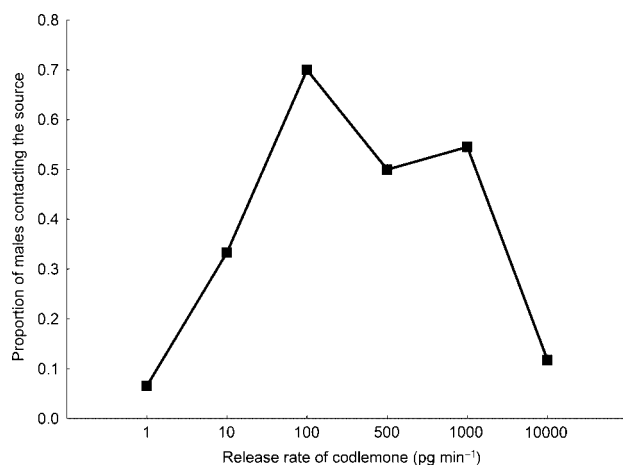


Figure 1. The proportion of male codling moth contacting the source at different release rates of codlemone.

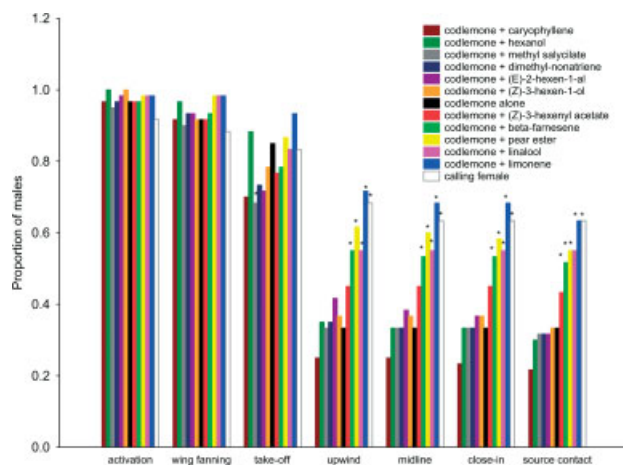


Figure 2. Wind tunnel responses of male *C. pomonella* to calling females, to codlemone alone and to two-component blends of codlemone (released at 100 pg min⁻¹) and plant volatiles (each released at 100 pg min⁻¹). Asterisks indicate significant differences between the responses of males to test solutions containing codlemone alone compared to codlemone admixed with a plant volatile and to calling females by the generalised linear model at a 0.05 type I error rate ($n = 60$ for each treatment).

3 RESULTS

3.1 Dose-dependent response of male codling moths to codlemone

Firstly, the responses of male *C. pomonella* to codlemone were examined at different release rates in order to establish a suboptimal attractive dose that could be amenable to improvement by adding plant volatile products. Under the present wind tunnel conditions, codlemone released by 100 pg min⁻¹ was the optimal dose, with 70% of the males contacting the source (Fig. 1). This proportion of source contact was very close to that observed with calling females (63%) (Fig. 2). Release rates between 1 and 100 pg min⁻¹ were suboptimal and attracted fewer moths, whereas release rates higher than 100 pg min⁻¹ reduced the number of males that succeeded in contacting the source. At release rates below 10 pg min⁻¹ and above 1000 pg min⁻¹, males contacted the source in less than 15% of cases (Fig. 1). At a release rate of 10 pg min⁻¹ of codlemone, a high proportion of male *C. pomonella* showed activation (97.7%), wing fanning (91.6%) and take-off (85.0%), but a significantly lower

Table 1. Summary of results (estimate, standard error, z-value and P-value) from the generalised linear model analysis of the behavioural responses of male *C. pomonella* to plant volatiles (released at 100 pg min⁻¹) admixed to codlemone (released at 10 pg min⁻¹). Only compounds influencing the behaviour of *C. pomonella* significantly different to codlemone are listed

Behavioural step	Compound	Effect	Estimate	Standard error	z-value	P-value
Take-off	Methyl salicylate	Negative	0.965	0.456	2.118	0.034
Upwind flight	Beta-farnesene	Positive	-0.893	0.377	-2.369	0.018
Upwind flight	Pear ester	Positive	-1.169	0.382	-3.063	0.002
Upwind flight	Linalool	Positive	-0.894	0.377	-2.369	0.018
Upwind flight	Limonene	Positive	-1.621	0.396	-4.090	<0.001
Passing midline	Beta-farnesene	Positive	-0.827	0.377	-2.194	0.028
Passing midline	Pear ester	Positive	-1.099	0.380	-2.891	0.004
Passing midline	Linalool	Positive	-0.894	0.377	-2.369	0.018
Passing midline	Limonene	Positive	-1.462	0.389	-3.750	<0.001
Close-in	Beta-farnesene	Positive	-0.827	0.377	-2.194	0.028
Close-in	Pear ester	Positive	-1.030	0.379	-2.717	0.007
Close-in	Linalool	Positive	-0.894	0.377	-2.369	0.018
Close-in	Limonene	Positive	-1.462	0.389	-3.750	<0.001
Contact	Beta-farnesene	Positive	-0.759	0.377	-2.018	0.043
Contact	Pear ester	Positive	-0.893	0.377	-2.369	0.017
Contact	Linalool	Positive	-0.894	0.377	-2.369	0.018
Contact	Limonene	Positive	-1.240	0.383	-3.236	0.001

proportion (33.3%, GLM, estimate -2.443, standard error 0.454, z-value -5.381, P-value <0.001) showed upwind flight, passed the midline and approached and contacted the source (Fig. 2). This suboptimal release rate of 10 pg min⁻¹ of codlemone was chosen as the level at which to test the effects of plant volatiles. The latter were released at 100 pg min⁻¹ according to Yang *et al.*³¹ and admixed with codlemone.

3.2 Effect of plant volatile compounds on the attractiveness of codlemone

The proportion of males showing activation (95–100%) and wing fanning (90–98.3%) responses to codlemone admixed with any of the plant volatiles was no different to the response to codlemone alone or to calling females (Fig. 2). Except for methyl salicylate, none of the plant volatiles added to codlemone affected the proportion of males taking off compared with codlemone alone or with calling females (Fig. 2). Methyl salicylate added to codlemone resulted in a reduction in the proportion of males taking off to 68.3% (Table 1, Fig. 2). Adding β -farnesene, pear ester, linalool and limonene to codlemone significantly increased the proportion of male *C. pomonella* undertaking upwind flight, passing the midline, closing-in and making source contact compared with the proportion responding to codlemone alone (Table 1, Fig. 2). The levels of responses under these four behavioural criteria to these treatments were not different to that recorded for calling females (Table 2, Fig. 2). The other plant volatiles had no effect on the proportion of male *C. pomonella* undertaking upwind flight, passing the midline, closing-in and making source contact (Fig. 2).

3.3 Time-based comparisons of responses to treatments

The time-based reactions of male *C. pomonella* to different test solutions were analysed under the behavioural steps of activation, upwind flight and source contact. Codlemone released at 10 pg min⁻¹ activated 81% of the males within the first 10 s of experiments and 87% within the first 20 s (Fig. 3). Three plant compounds, hexanol, limonene and methyl salicylate, influenced

Table 2. Summary of results (estimate, standard error, z-value and P-value) from the generalised linear model analysis of the behavioural responses of male *C. pomonella* to plant volatiles (released at 100 pg min⁻¹) admixed to codlemone (released at 10 pg min⁻¹) compared with calling females. Only plant compounds causing the same effects as calling females are listed

Behavioural step	Compound	Estimate	Standard error	z-value	P-value
Upwind flight	Beta-farnesene	-0.569	0.379	-1.496	0.135
Upwind flight	Pear ester	-0.294	0.389	-0.765	0.444
Upwind flight	Linalool	-0.569	0.379	-1.496	0.135
Upwind flight	Limonene	0.159	0.398	0.398	0.690
Passing midline	Beta-farnesene	-0.413	0.373	-1.109	0.267
Passing midline	Pear ester	-0.141	0.376	-0.375	0.707
Passing midline	Linalool	-0.346	0.373	-0.927	0.354
Passing midline	Limonene	0.223	0.386	0.577	0.564
Close-in	Beta-farnesene	-0.413	0.732	-1.109	0.267
Close-in	Pear ester	-0.210	0.375	-0.561	0.575
Close-in	Linalool	-0.346	0.373	-0.927	0.354
Close-in	Limonene	0.223	0.386	0.577	0.564
Contact	Beta-farnesene	-0.479	0.372	-1.289	0.197
Contact	Pear ester	-0.346	0.373	-0.927	0.353
Contact	Linalool	-0.346	0.373	-0.927	0.354
Contact	Limonene	<-0.001	0.379	<-0.001	1.000

the reaction time of male *C. pomonella* to codlemone (Table 3). Admixing hexanol or limonene to codlemone decreased the reaction time of male *C. pomonella*: in the first 10 s of experiments, the proportion of activated males increased to 95 and 92%

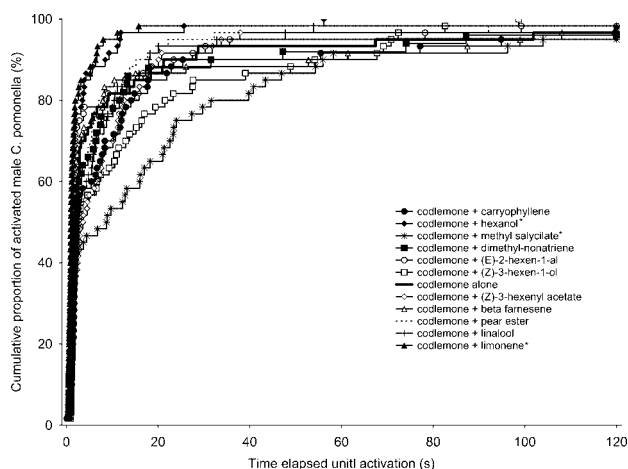


Figure 3. Cumulative proportions of activated male *C. pomonella* by test solutions containing codlemone alone (black line) and with single plant volatiles added, plotted against the time elapsed until activation was observed ($n = 60$ for each treatment). Plant volatiles that reduced the time until activation of males are above the black line, and those that increased the time until activation are below the black line. Compounds significantly influencing the responses of males by the Cox proportional hazard model at a type I error rate of 0.05 are marked with an asterisk.

respectively (Fig. 3). Admixing methyl salicylate to codlemone increased the reaction time: only 53% of males were activated in the first 10 s of experiments, and only 65% in the first 20 s (Fig. 3).

Considering the upwind flight behavioural step, limonene, pear ester and β -farnesene admixed to codlemone shortened significantly the time till the upwind flight response of male *C. pomonella* to codlemone commenced, and the other plant volatiles tested had no effect on this behavioural step (Table 3, Fig. 4). Whereas 33% of *C. pomonella* males showed upwind flight to codlemone within the 120 s experimental period, following admixture of limonene, pear ester or β -farnesene to codlemone this proportion was reached within 26, 23 and 25 s, respectively (Fig. 4).

When codlemone was released at 10 pg min^{-1} , the first source contact happened only after 20 s of exposure (Fig. 5). After 60 s the proportion reaching the source increased to 23%, and to 33% for the whole experimental period (120 s) (Fig. 5). Admixing limonene, pear ester, β -farnesene or linalool to codlemone significantly decreased the time required by male *C. pomonella* to contact the

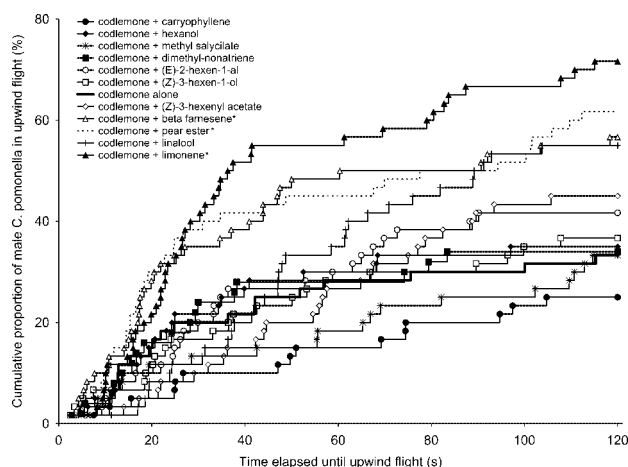


Figure 4. Cumulative proportions of male *C. pomonella* showing upwind flight to test solutions containing codlemone alone (black line) and with single plant volatiles added, plotted against the time elapsed until upwind flight was observed ($n = 60$ for each treatment). Plant volatiles that reduced the time until upwind flight of males are above the black line, and those that increased the time until upwind flight are below the black line. Compounds significantly influencing the responses of males by the Cox proportional hazard model at a type I error rate of 0.05 are marked with an asterisk.

odour source: the 33% source contact level observed to codlemone within 120 s was reached within 37 s when limonene was admixed to codlemone, and within 41 s when pear ester was added to the pheromone (Table 3). Similar source contact levels were observed for linalool and β -farnesene added to codlemone within 68 and 62 s, respectively (Fig. 5).

The effect of time elapsed until activation and upwind flight to a treatment on the probability of an individual reaching the treatment source was analysed. The GLM revealed a highly significant ($P < 0.001$) correlation in both cases, i.e. treatments that induced males to accomplish these behaviours more quickly were also more likely to attract these males to the source successfully.

4 DISCUSSION

This study focuses on the effects of plant volatiles on the attractiveness of codlemone to codling moth males in a wind tunnel, with the supposition that these products could contribute

Table 3. Summary of results (estimate, standard error, z-value and P -value) from the Cox proportional hazard analysis of the time-dependent behavioural responses of male *C. pomonella* to plant volatiles (released at 100 pg min^{-1}) admixed to codlemone (released at 10 pg min^{-1}). Only compounds influencing the reaction time of *C. pomonella* significantly different to codlemone are listed

Behavioural step	Compound	Effect on reaction time	Estimate	Standard error	z-value	P-value
Activation	Hexanol	Decreased	0.648	0.185	3.504	<0.001
Activation	Limonene	Decreased	0.765	0.185	4.123	<0.001
Activation	Methyl salicylate	Increased	-0.463	0.187	-2.479	0.013
Upwind flight	Limonene	Decreased	1.074	0.271	3.960	<0.001
Upwind flight	Pear ester	Decreased	0.843	0.278	3.038	0.002
Upwind flight	Beta-farnesene	Decreased	0.751	0.282	2.666	0.008
Contact	Limonene	Decreased	0.899	0.276	3.255	0.001
Contact	Pear ester	Decreased	0.686	0.283	2.421	0.015
Contact	Beta-farnesene	Decreased	0.568	0.2887	1.979	0.048
Contact	Linalool	Decreased	0.559	0.283	1.973	0.048

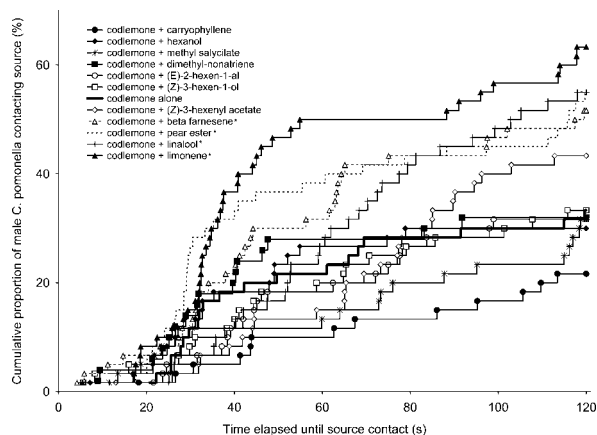


Figure 5. Cumulative proportions of male *C. pomonella* showing source contact to test solutions of codlemone alone (black line) and with single plant volatiles added, plotted against the time elapsed until source contact was observed ($n = 60$ for each treatment). Plant volatiles that reduced the time until source contact of males are above the black line, and those that increased the time until source contact are below the black line. Compounds significantly influencing the response of males by the Cox proportional hazard model at a type I error rate of 0.05 are marked with an asterisk.

to better attraction of codlemone for the codling moth males. It is demonstrated how the plant volatiles limonene, pear ester, β -farnesene or linalool enhance attraction of codling moth males to codlemone. Using real-time recordings, it is shown how these plant products not only enhance attractiveness but also shorten the reaction time of male *C. pomonella* to codlemone. Based on our observations, it is concluded that admixing limonene, pear ester, β -farnesene or linalool to codlemone aids source location by males such that host plant volatiles dispensed with codlemone should contribute to better mating disruption of *C. pomonella*. Under the present wind tunnel conditions, an under dosed level of codlemone (10 pg min^{-1}) was efficient in inducing the behavioural steps up to take-off (85% of males). However, a fall-off occurred, with only 33% of male *C. pomonella* responding with upwind flight, passing the midline, closing-in on the codlemone source and contacting it. In contrast, when any of the plant volatiles limonene, pear ester, β -farnesene or linalool were admixed at a release rate of 100 pg min^{-1} to codlemone, the proportion of males showing upwind flight, passing the midline, closing in and making contact with the odour source increased to match the level of the response to calling females (Fig. 2).

It is shown here for the first time that limonene, a major odour constituent of citrus but also present in apple,^{30,38} admixed to codlemone can enhance the attractiveness of male *C. pomonella* to codlemone. In agreement with the present findings, it has already been shown that attraction of male *C. pomonella* to codlemone can be enhanced by addition of either β -farnesene or linalool.³¹ In contrast to the present results, Yang *et al.*³¹ found that admixing (*Z*)-3-hexen-1-ol to codlemone enhanced the attraction of male *C. pomonella*. Although the present experiments do not support this finding, the proportion of male *C. pomonella* showing upwind flight, passing the midline, closing in and making contact with the odour source was always numerically higher when (*Z*)-3-hexen-1-ol was admixed to codlemone than with codlemone alone. The present report shows that pear ester can enhance the attractiveness of codlemone in a wind tunnel. The effect of pear ester on codlemone has already been demonstrated in the field, where this product caused an increased capture of codling moth

males to baits with codlemone.³⁹ It should be mentioned that, under the present wind tunnel conditions, some plant volatile compounds released at 100 pg min^{-1} admixed to codlemone showed no effects, and others showed a negative effect. It is assumed, in agreement with Yang *et al.*,³¹ that male *C. pomonella* are sensitive to the dose of the plant volatiles, and that the tested release rates are not necessarily the most attractive doses for codling moth either alone or in a mixture with codlemone.

Increasing the attractiveness of codlemone to codling moth males can be regarded as a key to increasing the efficiency of mating disruption of codling moth. In addition to the enhancing effects of secondary pheromone components,^{8,15,18–20} it is now known that plant compounds can also enhance the attractiveness of male *C. pomonella* to codlemone (Yang *et al.*,³¹ Buchbauer *et al.*³⁸ and the present study). According to Yang *et al.*,³¹ admixing either hexenylbutanoate, methyl salicylate or (*Z*)-3-hexenyl benzoate to an under dosed level of codlemone resulted only in the enhancement of the early behavioural steps of activation and wing fanning, but not the later steps from upwind flight to source contact. In contrast, admixing the female sex gland component dodecan-1-ol to an under dosed level of codlemone did not influence activation and wing fanning, but this secondary sex pheromone component strongly influenced the later behavioural steps from upwind flight to source contact in two separate studies.^{12,20} Witzgall *et al.*²⁰ also found that admixing codlemone ester, (*E,E*)-8,10-dodecadienyl acetate, to an under dosed level of codlemone only influenced the late behavioural steps. By contrast, Yang *et al.*³¹ did find that some compounds such as linalool, β -farnesene and (*Z*)-3-hexen-1-ol influenced the whole behavioural repertoire of male *C. pomonella* when admixed with an under dosed level of codlemone. In the present set of data, when codlemone alone was tested at a release rate of 10 pg min^{-1} , there was a drop between the proportion of males taking off and the proportion undertaking upwind flight. However, there was no such drop when β -farnesene, pear ester, linalool or limonene was admixed to codlemone or when a calling female was tested. As an enhancing effect was observed in the late behavioural steps for these four products, it can be concluded that admixing them to an under dosed level of codlemone aids orientation by codling moth males to the source.

For the first time, real-time recordings of the responses of male *C. pomonella* are used here in order to show that admixing plant volatiles to codlemone shortens the reaction time of male *C. pomonella* under the behavioural criteria of time to activation, time till the upwind flight response is induced and time to source contact. Similar effects have been recorded for plant compounds acting on the responses of the male grape berry moth, *Eupoecil-iaambiguella*, to its sex pheromone.³⁴ Adding β -farnesene, pear ester, linalool or limonene to codlemone shortens significantly the time required by males to make source contact, and the same four products significantly increase the proportion of males making contact with the pheromone source. Apparently, admixing those plant volatiles to codlemone that shorten the response time of the male also increases the probability of a better localisation of the test stimulus, as has already been shown for the grape berry moth.³⁴ This implies more oriented responses of males, a phenomenon already documented for the grapevine moth, *Lobesia botrana*.⁴⁰ In this species, 3D analysis of flight tracks showed that males made more directed flights to calling females than to a synthetic blend of pheromones in a wind tunnel. Unfortunately, no 3D recordings of *C. pomonella* flight to treatments were made in this study.

In contrast to several previous studies where the responses of moths to test stimuli were recorded in wind tunnels, the

generalised linear model (GLM) with binomial distribution and logit link function was used in the present study to analyse the behavioural responses of male *C. pomonella*. The behavioural steps generally quantified in wind tunnel experiments are activation, wing fanning, take-off, upwind flight and odour source contact. Such responses of males show a binomial distribution (for instance, activated or not). In contrast to ANOVA, where data are assumed to be measured at intervals or ratio scales,⁴¹ the GLM can handle such binomial datasets and can be used to compare the proportion of moths showing a specified behavioural step between treatments. A reanalysis of the data of Yang *et al.*³¹ using the GLM showed that adding either linalool, *E*- β -farnesene or (*Z*)-3-hexen-1-ol to the pheromone resulted in attraction of significantly more males than codlemone alone, a finding not accounted for using ANOVA in the original study. It is significant that these three products were identified in air samples from apple branches and were also found to elicit antennogram responses from *C. pomonella* females.³²

In summary, the present study has focused on how plant volatiles can influence the responses of male *C. pomonella* to codlemone. It has been found that, by adding either linalool, β -farnesene, pear ester or limonene to codlemone, the response time of male *C. pomonella* is shortened and the proportion of males attracted to the pheromone source is increased. Time is a critical factor for male *C. pomonella*, as their energy resources are limited and the time available each evening for mating is restricted. For mating disruption in orchards, shortening the response time of male *C. pomonella* to dispensers that release synthetic codlemone with plant volatiles would enhance the efficiency of this pest control method by ensuring engagement of males with dispensers, to the detriment of females.

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