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Horticultural capture patterns of two fruit moth species in traps variously baited with both pheromones

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Abstract

Specificity of synthetic pheromone lures to trap plum and apple moth males is standard agricultural practice to predict timely application of insecticides to fruit trees. Compatibility of both lures in the same trap has long been accepted for capture of both in several two-moth combinations, but any specificity for their relative positioning in a trap has remained untested. Experimentally-distanced lures in a trap during a month of fruit moth activity in an English horticultural context in 2000 resulted in apparently random capture but with some pattern distortion. However, in subsequent years, when lures were deliberately distanced diagonally across the trap surface, a distorted capture pattern involving avoidance of males from their corresponding female's pheromone suggested some disturbance in olfactory sensory perception and flying response within the trap. Further application of identifying individual captured moth carcases as of plum or apple moth by dissection of abdominal genitalia apparently revealed measurable disinclination to land near their corresponding female's pheromone. Another experiment confirmed that two adjacent lures did not disturb normal moth flying within the trap, but distanced positions caused statistically-disturbed flight patterns. The report encourages further experimental testing of male moth targeting towards, or subtle confusion by, pheromone lure location in traps for both *Grapholita funebrana* and *Cydia pomonella* in horticultural settings, to define optimum distancing of lures for maximum response and to appreciate their function in fruit moth sensory capacity. Further study could extend to the other pairs of moths, claimed a third of a century ago to be trapped by both lures in the same trap. Male fruit moth sensory perception may be more complex than generally assumed.

Keywords: pheromone; *Cydia pomonella; Cydia funebrana* (*Grapholita funebrana*): plum moth; apple (Codling) moth; olfactory physiology; genital dissection

1. Introduction

The apple moth (Codling moth) was first described by Linnaeus as *Cydia pomonella* in the 18th Century. The plum moth was later described by Treitschke as *Grapholita funebrana*; it was also sometimes referred to as *Cydia funebrana* but always within the Tortricidae. Eventually, recognition of volatile female pheromones as key to efficient mating within species added fundamental characteristics to moth behaviour and defined chemical structures of specific pheromones as closely related linear hydrocarbons [1]. The 20th century saw widespread exploitation of volatile synthetic pheromones to trap newly emerged males as indicators for timely application of insecticides in commercial orchards.

A notable brief mention 30 years ago cited ten pairs of moth combinations [2] in which the lure for each can be used with that of the other in the same trap to attract both. The key descriptor is 'compatible' and a cited pair was *C. pomonella* and *C. funebrana.* While this is hardly relevant in agriculture, trap economy could apply in mixed fruit horticulture. Thus, to make a pilot test of the precise meaning of 'compatible' in terms of moth sensory physiology, experiments have been conducted over four years in a standard commercial trap in an urban garden with a history of plum and apple moth parasitism. The key feature was to locate the lures on the sticky trap surface, separated nearly as far away as possible (~15 cm), to test for evidence of any pattern of spatial capture on the sticky surface for the two moth species during the key seasonal period of male emergence. Noting a capture grid pattern of contrasting groups of five or six moths near lubes, but twice as many across a diagonal apparently avoiding them, stimulated the present report concerning also the further experiments over a total of four years.

2. Materials and Methods

The experimental context was a London suburban garden containing plum and apple trees with a history of Tortrix moth damage to fruit and occasional use of Agralan moth pheromone traps for monitoring pest occurrence. The present study used Plum moth and Codling moth traps marketed for small horticultural application, using pheromone lures, based on classical scientific discovery [3] and development of synthetic forms [4]. For the present context they provided 8-dodecenyl acetates for *G. funebrana* and 8,10-dodecadienols for *C. pomonella*, possibly formulated with some proprietary fruit esters. Commencing mid-May 2020, a single Agralan trap was positioned in open garden aspect, 1.7 metres above ground and with E-W access orientation at least 3 metres from the nearest fruit tree (gage plum var. early transparent). The unorthodox features were location of a plum moth lure at the eastern aspect of the trap and a codling moth lure diagonally opposed facing west (Figure 1A). Distanced positioning of chemically contrasting lures 15 cm apart was purely for experimental curiosity. There could be no other trap as a 'control' because of risk of disturbing the indigenous moth population flight paths. The trap was undisturbed during the following six weeks, while moth capture could readily be observed. Subsequent high prevalence of both types of moth pest in plum and apple fruit proved natural abundance of both during that early summer season.

Similarly, in mid-May 2021 the same trap frame was re-positioned but with lures closely adjacent at the centre. The climate was exceptionally cold and wet until 25 May when conditions changed to favour moth emergence and night flying; abundant capture occurred during the subsequent week (Figure 1B).

Again, in May 2022, another trap was used for 2 weeks during capture of available flying moths. The primary purpose was to repeat the experiment of 2020 but with maximum diagonal distancing of pheromone lures. An additional challenge to differentiate between *C. pomonella and G. funebrana* males was undertaken, for this and the previous capture experiments, by application of the illustrated dissection protocols for male genitalia [5] with identification temporarily blinded concerning sample context. A small portion of sticky trap bearing a carcass was excised and transferred to an Eppendorf tube containing lemon oil to release the carcass. After several hours, the carcass was transferred to 10% potassium hydroxide solution overnight to release abdominal scales. Dissection in water under a Leitz dissecting microscope with very small brushes could ideally reveal the genital cucullus to allow recognition of plum or apple moth characteristics.

In 2023 the experimental objective was to repeat the 2021 design but, by two adjacent traps, to separate the capture environments by a double wall. This would allow both pheromones into the general capture environment but to avoid direct atmospheric mixing. The objective was to test normal moth behaviour in traps with the lures distanced apart.

Application of statistics to measured distances between a pheromone lure and the trap position of relevant individual moths used two-tailed T-test methodology to define the degree of behaviour significance of groups of the two types of fruit moth.

3. Results

2020

Opportunity for the horticultural study in a London garden commenced in 2020 in response to safeguarding in the global Covid epidemic, focusing on a single moth trap designed to capture both plum and apple fruit pests. Confident use of both pheromone lures was based on scientific literature [2]. The natural capture distribution (Figure 1A) appeared to imply that most moths landed away from the lures; this pattern raised a question about definition of the satisfactory implications of the 1988 principle. Thus, in the following year (2021), close central location of the lures was tested for its immediate atmospheric homogenization of pheromones emitted within the centre of the trap. Fairly random capture ensued, enough to allow zonally focal landing of moths on the surface in another experimental circumstance (Figure 1B) to be a meaningful deviation. Thus, in the third-year, a maximum lure distancing was employed (Figure 1C) to test any repeat of the principles curiously expressed in the first year's findings.

Meanwhile, discovery of an extensive literature item [5], physically differentiating between males of the two fruit moths (*C. pomonella* and *G. funebrana*) by a detail of the genitalia, introduced a further analytical tool to distinguish between carcasses of the present two moths. Availability of a Leitz dissecting microscope offered opportunity to differentiate between the responses of both moths captured in the lured trap context.

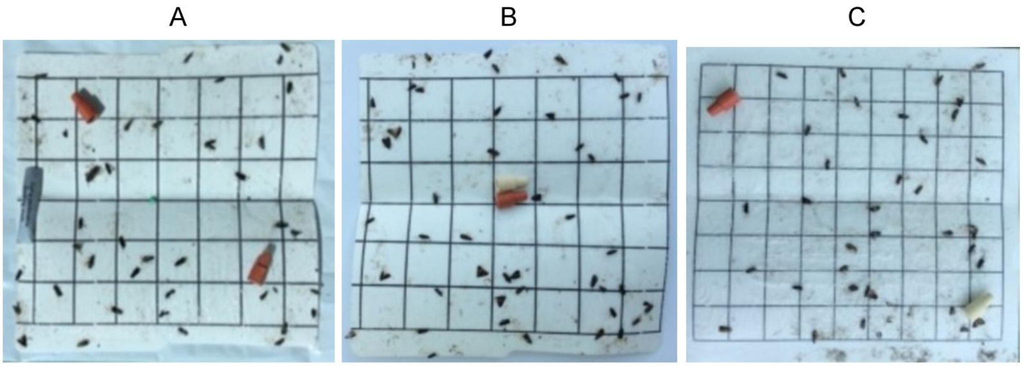

Figure 1 Moth capture traps at the end of experiments in 2020 (A), 2021 (B) and 2022 (C), photographed before attempt at species identification. In the 2020 picture the plum lure is top left and apple lure is bottom right. In 2021, lures were adjacent; in 2022 orientation was as in 2020.

Returning to the 2020 illustration, apart from capture of a wasp and two ants, the pattern of carcasses on the trap (Figure 1A) was notable on account of distribution towards the non-lure corners, but also apparently avoiding the central part of the grid. Although the pheromone lures were distanced by 15 cm there was rather little capture near them. Retrospective attempt two years later to identify the moths by their genitalia was compromised for this experimental trap by their abdominal emaciation, but the characteristic shape of the plum moth valva's cucullus was clear in seven cases, notably clustered far away from their pheromone lure. Nevertheless, the general clustering away from the lures, in a roughly two-fold ratio, raised questions about qualitative interpretation of the original literature statement about multiple lure acceptability for capturing some pairs of moth species [2].

2021

Specifically designed to seek confirmation of the avoidances perceived in 2020, moth capture was seen to be widely distributed across the trap (Figure 2). Otherwise, carcass distribution appeared to be roughly random across the trap surface. Species identity of 66% of carcasses was determined in a plum to apple ratio of 1:3, but notably no plum moth was near its lure and one had even landed beside the apple lure. Apple moths largely avoided the plum lure and were widely distributed across the non-lure diagonal of the trap, within which also the unidentified carcasses mostly occurred. Providing maximum 18 cm distancing of pheromone lures across a trap diagonal still appeared to disturb a near-random capture across the trap, although not as much as the 15 cm used in the 2020 experiment.

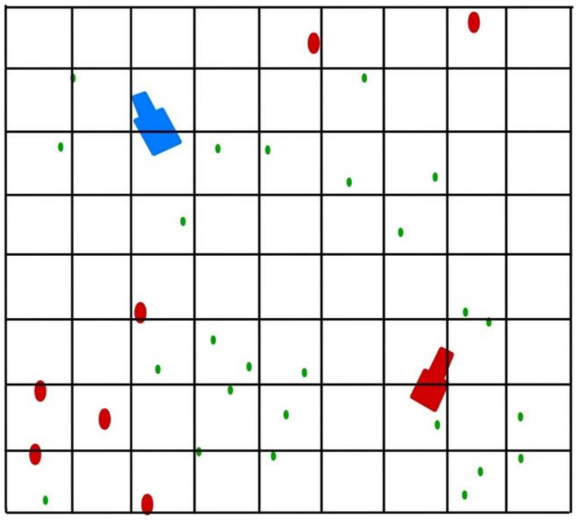

Figure 2. Fruit moth distribution in capture associated with distanced pheromone lures for plum moth (red) and apple moth (blue) in 2020. Figure orientated as in Figure 1A. Plum moth carcasses marked in red. Small green symbols represent fruit moths whose species identity could not be determined. Identification data was obtained only in 2021 after anatomical identification methodology was discovered for these very similar male moths.

2021

A new experiment in 2021 needed to verify whether two adjacent centrally-located lures in a trap caused no disturbance in a random trap pattern. Figure 3 illustrates the design, outcome and analysis which confirmed this as far as moth carcass identification allowed.

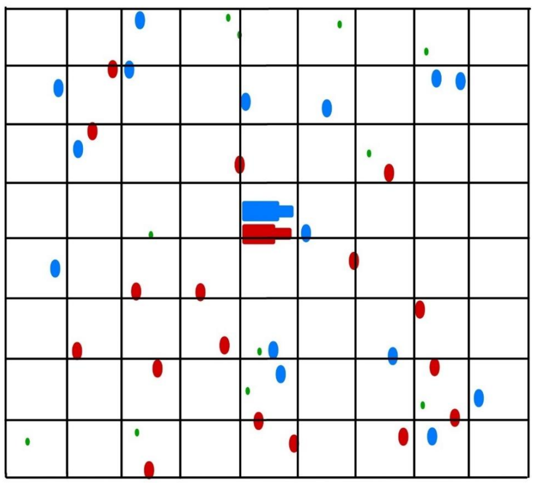

Figure 3. Fruit moth distribution in capture associated with centrally-adjacent pheromone lures for plum moth (red) and apple moth (blue) in 2021. Figure orientated as in Figure 1B. Carcasses identified as plum moth (red) and apple moth (blue). Small green symbols represent fruit moths whose identity could not be determined.

Specifically designed to seek confirmation of their avoidances perceived in 2020, moth capture was seen to be widely distributed across the trap (Figure 3). Otherwise, carcass distribution appeared to be roughly random across the trap surface. Moth identity of 66% of carcasses was determined in a plum to apple ratio of 1:3, but notably no plum moth was near its lure and one had even landed beside the apple lure. Apple moths largely avoided the plum lure and were widely distributed across the non-lure diagonal of the trap, within which also the unidentified carcasses mostly occurred. Providing maximum 18 cm distancing of pheromone lures across a trap diagonal still appeared to disturb a near-random capture across the trap, although not as much as the 15 cm used in the 2020 experiment. Subsequent revelation of natural moth infestation of fruits in adjacent apple and plum trees showed that the capture experiment had been performed within natural epidemic circumstances for both pests.

2022

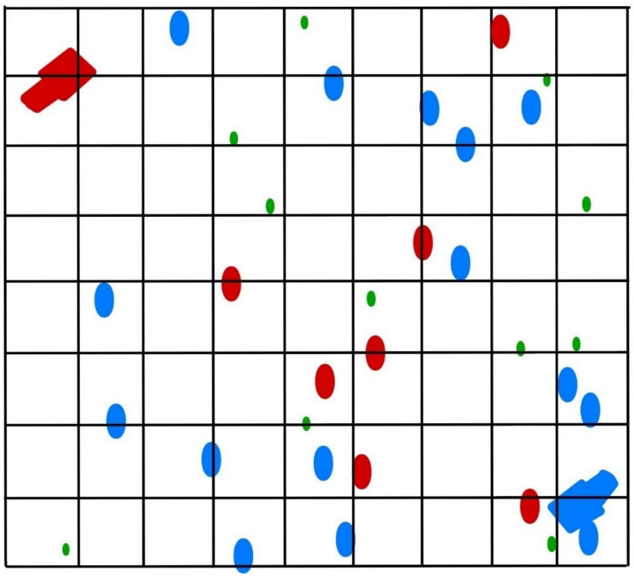

Figure 4. Repeated capture of moths with distanced lures with identification of individual carcasses and trap location for measuring individual lure distance for statistical evaluation.

The initial experiment of 2020 was repeated with more confidence, particularly with the additional confidence of identifying sufficient moths in each group for subsequent use statistically.

2023

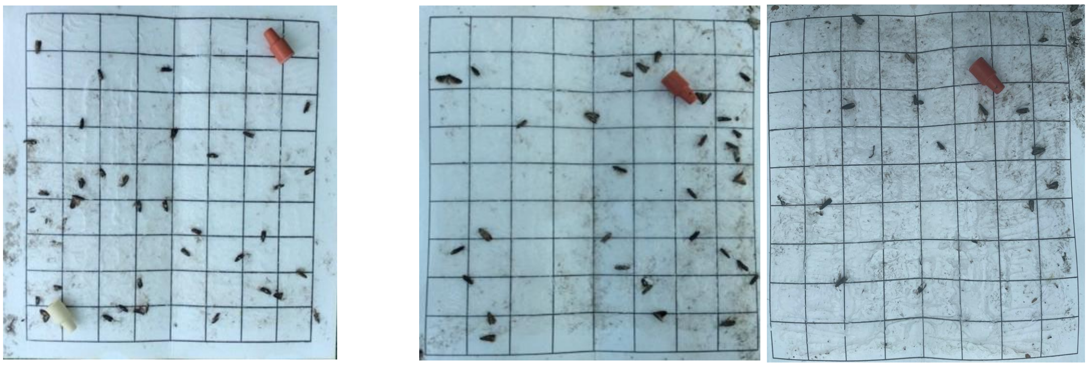

Figure 5. Moth trap from 2022 (left; competition between plum and apple lures) and (right) the two traps illustrating the combined plum moth capture for 2023.

Observations in previous years required application of statistics to substantiate verification of sensory disturbance caused by distanced lures. Further experimentation was thus designed both to acquire replicatory data and partially to exclude at least most of any competitive influence of nearby lures in the same experimental atmosphere. Two traps were secured adjacently in the usual location, one with an apple moth lure and the other with a plum lure arranged in the general conformation illustrated in Figure 5 but with internal atmospheres limited by a double wall between traps. The design thus protected moths entering their relevant trap with their lure from direct influence of the lure in the other trap.

Throughout May to July only 2 moths were trapped by the apple lure, interpreted partly as the consequence of the extreme summer heat of the previous year eliminating their fruit locally as host to caterpillars for the current year's adults. In addition, the main apple tree, nearing a century old, died without yielding mature apples as hosts for apple moths' overwintering. In contrast, plum moths were abundant to justify eventually substituting an idtools.org/id/leps/tortai/Grapholita_funebrana.htm second trap surface using the same lure (Figure 5). Cumulative capture was 51, providing a distribution pattern close to expected normality for statistical comparison, while still having an apple moth lure in the vicinity.

4. Discussion

The study has confirmed in principle that mixed pheromone lures in traps can capture males of both moths [2] as shown by their randomized carcass distribution, but that could require adjacent lures to enable immediate mixing of the emitted pheromones. However, it appears from experiment that pheromone lure distancing can disrupt moth landing patterns, suggestive of sudden olfactory stimulus of a relatively high atmospheric pheromone content challenging their sensory physiology. Already, it is remarkable that the present small male moths are programmed to respond naturally to extremely dilute atmospheric concentration of the relevant female pheromone and fly in the dark up a concentration gradient to their target. Olfactory detection and neurochemical response to follow a flight path is already a notable achievement for the little insect. It is thus not surprising that the experience of flying into a trap with a high atmospheric concentration of familiar pheromone, and then meeting another one, is a completely foreign factor for the moth's aero-flight experience. Such can reasonably result in some confusion, with no solution but to land somewhere and reassess the situation and find the outcome is death. Thus, findings in the present very preliminary exploration of moth response may have no practical impact on fruit moth biology, but they can offer fundamental challenge not only to marvel at such a small creature but also to consider its molecular sensory detection of, and flying response to, structurally-related volatiles.

Concerning the 2023 study, to accumulate a firm basis for uncomplicated male behaviour amongst the combined 51 plum moths captured, its use to assess the behaviour of identified plum moths in the 2020 experiment at least gave a T test P value of 0.0030. Its interpretation as 'very statistically different' subsequently confirmed the initial tentative encouragement of the 2020 study. Further to the 2023 study, also using its data of plum moth capture distancing from the lure, the plum moth capture distances in the two-moth experiment of 2022 were also 'very statistically different' according to internet-sourced calculations.

The list of pairs of moths shown to be congenial partners in trapping [2] offers opportunity to explore the present topic further and might even become a factor of taxonomic significance if it has fundamental implication for sensory physiology mechanism within moth reproduction; for *C. funebrana* a comprehensive monograph is cited [6]. For the present two moths, differentiation is particularly difficult among trapped carcasses. Applying modern genomic differentiation would require sophisticated techniques [7] and specific resources to undertake them. However, an advantage of the present experimentation is that it has provided a minimal un-natural circumstance for exploring male moth species' specificity to the pheromone of its female. Both Tortrix moths in the present study responded somewhat similarly, which might be consistent with some common heritage.

An intention here is not just to present an apparently new exploratory finding, but to encourage further attempts to verify whether exploitation of two lures to economise capture of males of two similar moths could, by modification, open new insight into sensory physiology.

If the present unorthodox experiment is pointing to some differential olfactory-neural physiology it would be interesting to explore a little further to refine the critical lure distancing value for these two moths, and to explore with other examples in the list of 10 candidates for such. For the present study, distancing of lures across the sticky trap surface was initially made across the trap diagonal to allow optimum surrounding space for moth landing. From the 2022 experiment it appears that any further distancing is not helpful for maximum expression of moth confusion. Thus, any refinement might allow alignment of the lures along the main trap axis.

The present study design applies conveniently to a single trap in a reasonably large garden with several fruit trees. Thus, targeting amateur involvement in collating data is particularly encouraged through promotion through gardening/horticultural magazines, although stimulating interest in mechanisms of olfactory perception and response also among scientists can only be welcomed. It is recognised that commercial formulation of pheromone products includes other plant-based odorants, for which there is already attention in the literature concerning insect sensory perception. Nevertheless, in making this communication, a prospective objective is encouraged to accumulate experimental horticultural data sufficient for statistical verification concerning expression of cross-reactivity to pheromones within close taxonomic groups of Tortrix moths. Exploration of the other nine moth species combinations [2], reported to accept combined pheromone strategies for capture, is also desirable.

Statistical analysis of the relative capture distribution of identified plum and apple moths recorded in the 2022 experiment gave P = 0.2240, interpreted as of a not-significantly-different pattern in their reaction to both lures. A similar pattern was suspected for the initial experiment in 2020 in which several plum moths were identified and the other captured fruit moths were expected to be mainly apple moths, behaving similarly, since their lure was also used.

Concerning plum moths, based on the presumed non-competitive cumulative capture pattern in the 2023 study, their pattern distortion in 2022 was shown statistically via the two-tailed T test to have been ‘very significantly different’ (P = 0.003). This is attributed to the influence of apple moth pheromone influence nearby in the trap. Notably also, at the end of July 2023, caterpillars were found in ripening fruits of the nearby transparent gage fruit tree, reminding that the little plum moth is also a horticultural pest.

It is now over 35 years since combined application of lures was encouraged in the USA for agricultural capture of several pairs of native fruit moth species [2]. Only near the end of the present study was that found, but no obvious application. Perhaps interest in fruit moth sensory perception might encourage simple experiments in horticulture where some lures are readily available.

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