Linalool fumigation improves mating competitiveness of males for population suppression of the global fruit pest *Cydia pomonella*

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Abstract

BACKGROUND: The implementation of sterile insect technique (SIT) has proven effective in the area-wide suppression of several significant agricultural and sanitary pests by employing traditional cobalt-60 (^{60}Co-γ) as a radiation source. Recently, X-ray has been validated as a feasible alternative to ^{60}Co-γ radiation sources. Nonetheless, higher doses of X-ray irradiation lead to insect sterility but diminish mating competitiveness, thereby impacting the effectiveness of SIT applications. Thus, it is crucial to ascertain the optimal irradiation dose and develop strategies to enhance the mating competitiveness of sterile insects to enhance SIT efficacy.

RESULTS: In this study, we determined the effect of various X-ray irradiation doses (ranging from 0 to 366 Gy) on the fecundity, fertility, and mating competitiveness of *Cydia pomonella*, a globally invasive fruit pest. Results demonstrated that the sterility rate of sterile males increased proportionally with irradiation dose up to 200 Gy, beyond which it plateaued. Notably, exposure to 200 Gy of irradiation notably decreased the mating competitiveness of male, as evidenced by a mating competitiveness index of 0.17 in laboratory and 0.096 in the orchard. This decline in mating competitiveness is likely linked to the down-regulation of genes associated with the recognition of sex pheromones, specifically *CpomOR3a*, *CpomOR3b*, and *CpomOR5*, following X-ray irradiation. Fumigation of the plant volatile, linalool at varying concentrations (70, 83, and 96 μL/m^3) resulted in

differential enhancements in male mating competitiveness, with the moderate concentration significantly improving the competitiveness of sterilized males, possibly by restoring their ability to recognize sex pheromones. Implementation of repeated releases of sterilized males on a pilot scale led to a notable reduction in the population of *C. pomonella* in the field.

CONCLUSION: These findings indicate that fumigation with plant volatiles has the potential to mitigate male sterility induced by X-ray irradiation, offering a promising approach to enhance the efficacy of SIT applications for the control of *C. pomonella*.

Keywords: Sterile insect technique; Codling moth; X-ray; Iirradiation; Mating competitiveness; Plant volatile

1. Introduction

The codling moth, *Cydia pomonella* (Linnaeus), is a notorious pest that infests pome fruits and walnuts worldwide[1]. Various methods, including agricultural, mechanical, chemical, and biological controls, have been employed to manage the population of *C. pomonella*.[2] Despite efforts to utilize sustainable practices such as agricultural techniques, pheromone-based mating disruption, and attract-kill strategies, effectively controlling dense populations of this pest remains a challenge.[2] Traditionally, the predominant approach to combat *C. pomonella* has been through the use of conventional insecticides[3]. However, the efficacy of chemical insecticides in controlling this

68 pest is limited due to factors like overlapping generations and the larvae's
69 habit of tunneling into fruit.[4,5] Furthermore, the widespread application of
70 insecticides has led to the development of resistance in *C. pomonella*.[5]

71 Insect sterility technique (SIT) is recognized as an eco-friendly, efficient,
72 and sustainable approach incorporated within the broader framework of
73 Integrated Pest Management (IPM).[6] The utilization of irradiated males in
74 conjunction with non-irradiated females results in sterile offspring.[7] However,
75 the slower progress in developing sterile insect control methods for
76 Lepidoptera in comparison to Diptera is linked to the former's resistance to
77 radiation.[8] This resistance in Lepidopteran insects is likely due to their
78 necessity for higher radiation doses for sterilization, which can compromise
79 their mating competitiveness and impede the success of sterile insect
80 initiatives. Nevertheless, the effective implementation of the Okanagan-
81 Kootenay Sterile Insect Release (OK SIR) program in British Columbia,
82 Canada led to the successful eradication of *C. pomonella* populations by
83 strategically employing conventional cobalt-60 (^{60}Co-γ) source in area-wide
84 pest management activities.[9] Ongoing endeavors in insect SIT focus on
85 assessing the effects of radiation on insect fertility, longevity, flight capacity,
86 and mating competitiveness,[10] with the aim of establishing standardized
87 procedures for consistent and reproducible induction of sterility.[11]

88 The performance of irradiated insects, particularly their mating
89 competitiveness and flight capacity, is of significant importance in the SIT

program, as it directly impacts the dispersal range and mating effectiveness of sterile insects in natural environments.[12] Previous research has indicated that male pupae irradiated with X-rays at a dose of 366 Gy resulted in complete sterility in *C. pomonella*, albeit with a low mating competitiveness index of 0.01.[13] The observed side effects of X-ray irradiation, such as reduced mating competitiveness, are likely attributed to its negative impact on flight ability.[4] Consequently, it is imperative to optimize irradiation doses and devise strategies to improve both mating competitiveness and flight capacity of sterile insects to ensure the effective implementation of the SIT program for pest management.

Host plant volatiles have been documented as influential factors in various behaviors exhibited by phytophagous insects, encompassing mating, feeding, fertilization, directed flight, and pheromone production.[14] Linalool, a volatile compound categorized as a light-flavored terpene alcohol,[15] has exhibited a significant attraction towards adult *C. pomonella*, leading to a considerable electrophysiological reaction from their antennae.[16] Studies conducted on *Ceratitis capitata* have shown an improvement in mating competitiveness following linalool fumigation, while not impacting the survival rate of sterile males.[15] Nevertheless, the influence of linalool fumigation on the mating competitiveness of male *C. pomonella* remains unexplored.

The olfactory system plays a vital role in various insect behaviors like feeding, orientation, host-seeking, mating, and oviposition.[17,18]

112 Chemoreception in insects is a complex process involving various
113 chemosensory genes, such as odorant receptors (ORs), gustatory receptors
114 (GRs), ionotropic receptors (IRs), odorant-binding proteins (OBPs),
115 chemosensory proteins (CSPs), and sensory neuron membrane proteins
116 (SNMPs).[19] In particular, the importance of chemosensory genes in
117 recognizing pheromones in *C. pomonella* has been extensively researched.
118 Tian *et al*.[20] have identified male pheromone receptor genes in *C. pomonella*,
119 including *CpomOR1*, *CpomOR2a*, *CpomOR3*, *CpomOR5*, *CpomOR6a*, and
120 *CpomOR7*, through transcriptome analysis of the antenna. Knock-out the
121 *CpomOR1* gene using CRISPR/Cas9 technology has been shown to impact
122 the fertility and fecundity of *C. pomonella*.[21] Moreover, *CpomOR3a* and
123 *CpomOR3b* genes in *C. pomonella* are involved in recognizing plant volatiles
124 and the sex pheromone codlemone, thereby enhancing the moths' response
125 to codlemone.[22] However, the underlying cause of the diminished mating
126 competitiveness observed in irradiated *C. pomonella* is yet to be determined,
127 specifically regarding its potential interference with the expression of sex
128 pheromone-related genes. Furthermore, the efficacy of linalool fumigation in
129 mitigating these effects remains to be elucidated.

130 The objective of this research is to: 1) ascertain the most effective
131 irradiation dosage to achieve a harmonious equilibrium between sterility and
132 mating competitiveness; 2) develop strategies to enhance the mating
133 competitiveness of sterile insects; 3) elucidate the potential mechanism of the

134 decline in mating competitiveness caused by irradiation and improvement
135 following linalool fumigation; 4) investigate the effect of pilot-release of sterile
136 males on suppression of the *C. pomonella* field population.

137 **2. Materials and methods**

138 **2.1 Insects**

139 The *C. pomonella* strain utilized in the investigation was initially obtained
140 from the Laboratory of Agricultural Invasive Biological Prevention and
141 Monitoring at the Institute of Plant Protection, Chinese Academy of
142 Agricultural Sciences. This strain was reared for more than 70 generations
143 within a growth chamber (MLR-352H-PC; Panasonic, Osaka, Japan) under
144 controlled conditions devoid of irradiation. The rearing was maintained a
145 temperature of 26 ± 1 °C, relative humidity ranging from 60% ± 5%, and a
146 photoperiod set at 16 hours of light followed by 8 hours of darkness.[23]

147 **2.2 Irradiation**

148 An X-ray irradiator (SR 100, Changzhou Sairui Instrument Technology
149 Co., Ltd, Changzhou, China) that was furnished with a Radcal Accu-Dose+
150 digitizer featuring a 10 × 6–0.6 CT ion chamber for the purpose of measuring
151 the average dose rate, was utilized in this study. This instrument was
152 employed to expose 8-day-old male pupae (1 day prior to emergence),[13]
153 which were positioned within a cylindrical transparent tube (diameter: 2 cm;
154 length: 6 cm) located in the irradiation chamber (dimensions: length: 21 cm;
155 width: 24 cm; height: 16 – 40 cm) to X-ray radiation at a dose rate of 4.3

156 Gy/min.

157 **2.3 Effects of different doses of X-ray irradiation on the mating and**
158 **reproduction of *C. pomonella***

159 The 8-day-old male pupae were divided into five groups comprising 90
160 individuals each, and subjected to X-ray irradiation at doses of 0, 50, 100, 150,
161 200, 250, and 300 Gy, respectively. Subsequently, the pupae within each
162 treatment were randomly divided into three groups consisting of 30 pupae
163 each, and placed in an incubator for emergency situations, with the
164 subsequent emergence count being documented. The matured males and
165 females from the same day were introduced into a mating box (25*12*15 cm)
166 at a ratio of 10 females to 10 males, and each treatment was repeated seven
167 times. Following the laying of eggs by the females and subsequent collection
168 of the egg papers, they were placed in individual disposable transparent
169 plastic bags (15*26 cm) alongside damp wipes on a daily basis, and then
170 transferred to the incubator for hatching. The eggs laid was recorded and the
171 hatching rate was calculated upon the demise of all females. The mortality of
172 the adult was monitored per day, with deceased individuals promptly removed.
173 The lifespan of males was documented, and expired females were gathered
174 in centrifuge tubes (1.5 mL) for dissection under a dissecting microscope to
175 observe mating sac morphology, define mating status, and calculate female
176 mating rates according to Zhang et al.[13]

177 Different proportions of "irradiated male: non-irradiated male: non-

178 irradiated female" at ratios of 0:10:10, 10:0:10, and 10:10:10 were established,
179 respectively, to calculate the mating competition index. These male individuals,
180 both irradiated and non-irradiated, along with the female counterparts, were
181 matched in mating age(25*12*15 cm). The eggs laid (fecundity) and hatched
182 (fertility) were meticulously documented for each grouping, with every
183 treatment being replicated thrice for statistical robustness. The competitive
184 mating index (C) of sterile males was then determined utilizing the
185 methodology outlined by Fried.[24]

186 $$E = \frac{N(Ha) + S(Ha)}{N + S}$$

187 $$C = \frac{Ha - Ee}{Ee - Hs} \div \frac{S}{N}$$

188 *N* is the number of normal male moths and *S* is the number of irradiated
189 male moths. *Ha* is the hatching rate of normal male moths paired with normal
190 female moths. *Hs* is the hatching rate of sterile male moths paired with normal
191 female moths. *Ee* is the hatching rate of mixed male moths of a certain S/N
192 ratio paired with normal female moths. *E* is the expected hatching rate of a
193 mixed S/N ratio male moth paired with a normal female moth.

194 **2.4 Effect of linalool fumigation on mating competitiveness of *C.***
195 ***pomonella***

196 Three disposable transparent plastic boxes (21*14*7 cm) with a volume
197 of 1500 mL each were assembled for experimentation. A strip of filter paper (1
198 cm in wide and 5 cm long) was suspended in the center of the lid of each box.
199 Male moths that newly emerged were introduced into the boxes.

Subsequently, linalool was evenly distributed onto the filter paper strips within the boxes to achieve concentrations of 70, 83, and 96 μL/m^3, which were clearly labeled on the lids of the respective boxes. After a 3-hour fumigation period, the moths were removed and the males and females from the same day were segregated into adult mating boxes at a 1:1 ratio. The assessment procedures remained consistent with those described in section 2.3.

2.5 Effect of linalool fumigation on mating competitiveness of sterile *C. pomonella*

To investigate the effect of linalool fumigation on mating competitiveness of sterile *C. pomonella* in laboratory, the 8-day-old male pupae were irradiated with X-rays at a dose of 200 Gy, subsequently maintained in a growth chamber until emergence. The freshly emerged male adults underwent a 3-hour fumigation with linalool. The procedures for fumigation, assessment of average survival time, fecundity per female, egg hatchability, mating frequency, and mating competitiveness followed the protocols described above-mentioned.

To investigate the effect of linalool fumigation on mating competitiveness of sterile *C. pomonella* in orchard, a field trial was conducted at Wumeifang Village, Jiuzhai Town, Gaizhou City, Yingkou City, Liaoning Province (40°3′52 "N, 122°5′44 "E) from August 1 to September 2, 2023. Small insect rearing nets (60 cm*60 cm*90 cm) with 40 mesh were affixed to tree trunks, and adult mating age were placed inside these nets for fumigation purposes. The

222 experimental procedures for evaluating mean longevity, single female
223 fecundity, hatching rate, mating rate, and mating competitiveness were
224 consistent with those described above.

225 **2.6 Effects of linalool fumigation on the flight ability of sterile *C.***
226 ***pomonella* moths**

227 The flight performance of moths was assessed using the FXMD-24-USB
228 insect flight information system (Henan Hebi Jiaduoke Industry and Trade Co.,
229 Ltd, Hebi, Henan, China), according to the method described in Huang et al.[4]
230 Normal, sterile, and fumigated sterile male moths were segregated into
231 distinct adult rearing boxes and provided with a daily diet of 10% honey water.
232 Then, 25 healthy male moths with intact wings that were 3 days post-
233 emergence were selected for the study, and the flight capability of each group
234 was evaluated. The insects were initially immobilized on a chilled surface
235 before their abdomens were exposed. A copper wire was fashioned into a
236 loop, folded at a 90° angle, and affixed to the moth's abdomen using a small
237 amount of adhesive, ensuring proper fixation by inflation and rapid
238 solidification of the glue. The other end of the wire was secured to the arm of
239 the flight mill, positioning the moth parallel to the ground and aligning its flight
240 direction with the mill. Subsequently, the insect flight information system was
241 activated to input data on the test subjects. Flight ability assessments were
242 conducted from 22:00 to 6:00 in a darkened environment, maintaining a
243 constant ambient temperature of 25 ± 1 ℃ and relative humidity of 70 ± 5%

244 throughout the evaluation process.

245 **2.7 RNA isolation and cDNA synthesis**

246 Total RNA was isolated from a collective of ten 3-day-old male adults of *C.*
247 *pomonell*a, who were subjected to either 200 Gy X-ray irradiation or not,
248 utilizing the EAStep Super Total RNA Extraction Kit (Promega, Shanghai,
249 China) in accordance with the manufacturer's guidelines. The concentration
250 and quality of the RNA samples were assessed employing a NanoDrop2000
251 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).
252 Subsequently, 1 μg of RNA from each treatment was utilized in the synthesis
253 of complementary DNA (cDNA) using the GoScript™ Reverse Transcription
254 Mix (Promega, Madison, WI, USA). The resulting cDNA was preserved at -
255 20 °C until further analysis.

256 **2.8 Reverse-transcription quantitative polymerase chain reaction**
257 **analysis**

258 The mRNA levels of pheromone recognition-related genes (Table S1,
259 Figure S1-S8) were measured using reverse transcription quantitative
260 polymerase chain reaction (RT-qPCR) with Bio-Rad CFX96 (Bio-Rad,
261 Hercules, CA, USA) in a reaction of 20 μL, containing of 1 μL of cDNA
262 template, 10 μL of TB Green Premix Ex Taq 2 (TliRNaseH Plus) (TaKaRa,
263 Dalian, China), 1 μL of each primer (10 μmol/L), and 7 μL double-distilled
264 water (ddH2O). The RT-qPCR cycled at 95 °C for 30 s, followed by 40 cycles
265 of 95 °C for 5 s and 60 °C for 30 s. Afterward, a melting curve was conducted

266 starting at 55–95 °C to identify the specificity of PCR products. The internal
267 control genes used were *EF-1α* (MN037793) and *β-Actin* (KC832921) of *C.*
268 *pomonella*.[25] For each three biological replicates and three technical
269 replicates were conducted. Relative expression levels for each gene were
270 calculated using the $2^{-\triangle\triangle CT}$ method.[26]

271 **2.9 Suppression of field population of *C. pomonella* by pilot release of**
272 **sterile males**

273 Pilot release of sterile males were conducted between July 8 and
274 September 2, 2023, in Wumeifang Village, Jiuzhai Town, Gaizhou City,
275 Yingkou City, Liaoning Province, China (40°3′52 "N, 122°5′44 "E). Prior to
276 release, a central tree within the orchard was designated and marked, with
277 sex traps positioned at 5-meter intervals in the cardinal directions of east,
278 south, west, and north from the central tree. Sterile males were gently coated
279 with fluorescent powder, distinguishing fumigated and unfumigated specimens
280 by color before being released at 18:00. The control orchard, devoid of sterile
281 male releases, was equipped with 5 trap sets, with trapped moth counts
282 recorded at 10-day intervals post-release. Additionally, daily temperature and
283 precipitation data were collected throughout the release period.

284 The five-point sampling method was employed in both the release
285 orchard and control orchard to designate five points within the central and
286 perimeter areas of the orchard. Around each of these points, five trees were
287 meticulously selected and distinctly labeled. Prior to the release, a

288 comprehensive examination of the total fruit count and the specific number of
289 fruits was conducted. Subsequently, at intervals of 10 days, the quantity of
290 infested fruits was meticulously documented, allowing for the calculation of
291 the fruit infestation rate. This rate was determined by dividing the total number
292 of infested fruits by the overall number of fruits examined, expressed as a
293 percentage.

294 **2.10 Data analysis**

295 The statistical significance of various biological parameters of *C.*
296 *pomonella*, encompassing metrics such as average emergence rate, longevity,
297 fecundity, sterility rate, mating rate, as well as the flight characteristics of
298 males including flight time, distance, and speed, along with gene expression
299 in controlled laboratory settings and dispersal distance within the orchard,
300 were meticulously analyzed utilizing the one-way analysis of variance
301 (ANOVA) coupled with Duncan's test ($P \leqslant 0.05$) through SPSS 19 software
302 (IBM Corporation, Armonk, NY, USA). Furthermore, significant difference in
303 total number of moths trapped per trap, amount of insects trapped and fruit
304 decay rate was analyzed through ANOVA with independent samples t-tests
305 (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$) using SPSS 19. The results were
306 graphically represented using GraphPad Prism 5 (GraphPad Software, San
307 Diego, CA, USA), with all values being expressed as mean ± standard error.
308 Additionally, the sterility rate of *C. pomonella* under varying X-ray dosage
309 levels was modeled using a logistic curve in Origin 2019 software (Origin Lab

310 Corporation, Northampton, MA 02115, USA).

311 **3.RESULTS**

312 **3.1 Effect of different doses of X-ray irradiation on the biological**
313 **parameters of *C. pomonella***

314 The effects of different doses of X-ray irradiation on the physiological
315 traits of *C. pomonella* were explored in a series of experiments that examined
316 the emergence rate, lifespan, reproductive capacity, and sterility of 8-day-old
317 pupae exposed to varying levels of X-ray radiation. The results indicated that
318 there was no statistically significant variance in the emergence rate of male
319 moths *C. pomonella* within the range of 50-250 Gy compared to the control
320 group. Notably, a reduction in the emergence rate of male *C. pomonella*
321 moths was evident at 300 Gy (Figure 1a). However, the survival rate of moths
322 arising from male pupae irradiated with various doses of radiation did not
323 exhibit a significant difference compared to the control group (Figure 1b).
324 Furthermore, a decrease in lifespan was evident with X-ray exposure
325 surpassing 200 Gy when compared to the control group (Figure 1c).
326 Fecundity levels remained steady across different radiation levels compared
327 to the control group (Figure 1d). Additionally, an escalation in sterility rate was
328 observed at doses below 250 Gy (Figure 1e), while a reduction in mating rate
329 was noted at doses exceeding 200 Gy (Figure 1f).

330 **3. 2 Effect of 200 Gy X-ray irradiation on mating competitiveness of**
331 **male *C. pomonella***

332 3.2.1 Laboratory test

333 The hatching rate of eggs for SM:NM:NF at the 1:0:1 ratio was found to
334 be 12.57±0.51%, a value significantly lower compared to the ratios of 0:1:1
335 (72.96±7.31%) and 1:1:1 (64.18±3.95%). When male adults were combined
336 with different ratios and matched with normal female adults, the expected
337 hatching rate (E) was 42.77%. The competitive mating index (C) was
338 determined to be 0.17, indicating that the mating competitiveness of male
339 moths was reduced by 200 Gy of irradiation (Table 1).

340 3.2.2 Orchard trial

341 The hatching rate of eggs for the SM:NM:NF group at the 1:0:1 ratio was
342 observed to be 10.82±4.46%, a significantly lower rate compared to the 0:1:1
343 ratio (64.16±6.11%) and the 1:1:1 ratio (59.49±2.47%). When male adults
344 were introduced in various ratios and paired with normal female adults, the
345 expected hatching value (E) was calculated to be 37.49%. Additionally, the
346 competitive mating index (C) was determined to be 0.096, suggesting that a
347 radiation dosage of 200 Gy diminished the mating competitiveness of male
348 moths (Table 1).

349 **3.3 Effects of different concentrations of linalool fumigation on**
350 **biological parameters of *C. pomonella***

351 To elucidate the impact of varying concentrations of linalool fumigation
352 treatments on the biological parameters of *C. pomonella*, experimental
353 investigations were conducted to assess the survival duration, oviposition,

354 egg hatching efficacy, and mating activity of male moths. The results revealed
355 no notable disparities in the aforementioned parameters among male moths
356 subjected to distinct levels of linalool fumigation in comparison to the control
357 cohort, indicating that the application of linalool fumigation did not adversely
358 affect the male moths (Figure 2).

359 **3.4 Effects of different concentrations of linalool fumigation on mating**
360 **competitiveness of *C. pomonella***

361 The hatching rate of eggs resulting from the pairing of fumigation-treated
362 normal males with normal females were determined to be 73.63±3.86 percent,
363 while the pairing of untreated normal males with normal females yielded a
364 hatching rate of 71.42 ± 1.74%. When comparing the hatching rate of
365 fumigation-treated normal males to the overall hatching rate, the value was
366 found to be 72.55±0.33%. There was no significant difference observed in the
367 hatchability of fecundity across three different mating combinations, with an
368 average rate of 72.52%. Furthermore, the mating competitiveness index of *C.*
369 *pomonella* normal males that were exposed to linalool fumigation at a
370 concentration of 70 μ L/m ^{3} was calculated to be 1.06, suggesting an
371 enhancement in the mating competitiveness of the males (Table 2).

372 The percentage of eggs hatched when 83 μL/m^{3} of linalool was used to
373 fumigate normal males paired with normal females was recorded at 78.47 ±
374 3.28%. For normal males paired with normal females, the hatching rate was
375 slightly lower at 71.42 ± 1.74%. When fumigated normal males were paired

376 with normal females, the hatching rate was 75.34 ± 0.43%. There was no
377 significant difference in the hatching rate of eggs produced by the three
378 different mating combinations, all averaging around the expected value of
379 74.94. The mating competitiveness index of males treated with linalool at 83
380 μL/m ^{3} was 1.25, indicating an improvement in mating competitiveness
381 compared to those treated at 70 μL/m^3 (Table 2).

382 The egg hatching rate of 96 μL/m^3 linalool-exposed normal male moths
383 when paired with normal females was determined to be 76.52 ± 1.36%, while
384 that of normal males paired with normal females was 71.42 ± 1.74%, and the
385 hatching rate of fumigated normal males when paired with other males and
386 females was 74.24 ± 3.83%. These results indicate that there were no
387 significant differences in hatching rates among the three mating combinations
388 studied, all of which were close to the anticipated value of 73.97%.
389 Furthermore, the mating competitiveness index of males exposed to linalool at
390 96 μL/m ^{3} was calculated to be 1.24, suggesting a reduced mating
391 competitiveness compared to males exposed to linalool at 83 μL/m^3 (Table 2).

392 **3.5 Effect of linalool fumigation on mating competitiveness of sterilized**
393 **male *C. pomonella* moths**

394 3.5.1 Laboratory test

395 The results revealed that male moths exposed to 200 Gy X-rays exhibited
396 notably reduced hatching and mating frequencies in comparison to the
397 untreated group. Notably, there were no substantial variations in any of the

398 outcomes between the fumigated and non-fumigated sterilized males,
399 indicating that the linalool fumigation did not impact the reproductive
400 characteristics of sterilized males (Figure 3).

401 Specifically, the hatching rate of linalool-fumigated irradiated sterilized
402 males that were paired with normal females was 13.44±2.33%, while that of
403 irradiated sterilized males paired with normal females stood at 10.97±2.41%.
404 Moreover, the hatching rate of fumigation-treated irradiated sterilized males
405 when paired with females was 12.27 ± 1.98%, suggesting no significant
406 variance in the hatching rate of eggs produced by the three different pairings
407 (F = 0.305, df = 8, P = 0.748), with an expected value of 12.21%. The mating
408 competition index of 1.11 for the irradiated sterilized males of moths fumigated
409 with linalool indicated an enhancement in the mating competitiveness of the
410 irradiated sterilized males due to linalool fumigation (Table 3).

411 3.5.2 Orchard trial

412 The results of the field experiment revealed a significant decrease in the
413 longevity and fecundity of sterile males of *C. pomonella* compared to the
414 control group. Interestingly, there was no notable variance between the
415 biological parameters of linalool-fumigated sterile males and non-fumigated
416 ones, suggesting that the fumigation treatments did not impact these
417 parameters within the field environment (Figure 3).

418 When linalool-fumigated irradiated sterile males were paired with normal
419 females, the hatchability was observed to be 14.98±3.89%, while it was 10.82

420 ±4.46% for *C. pomonella* irradiated sterile males mated with normal females.
421 Moreover, the hatchability of fumigation-treated irradiated sterile males when
422 paired with females stood at 12.84±1.98%. These findings indicate that there
423 was no significant difference in hatchability and fecundity among the three
424 different combinations (F = 0.249, df = 8, P = 0.756), with an average value of
425 12.90%. The mating competition index of 1.06 for the linalool-fumigated
426 irradiated sterile males of *C. pomonella* suggests that linalool fumigation
427 enhanced the mating competitiveness of the irradiated sterile males (Table 3).

428 **3.6 Effects of linalool fumigation on the flight ability of sterile *C.***
429 ***pomonella* moths**

430 The flight duration of 3-day-old male adults, sterile males, and fumigation
431 treated males was 53.82±7.17, 5.61±2.36, and 10.18±3.46 min, respectively.
432 While no significant disparity was observed in the flight duration between
433 sterile males and fumigation treated males, both groups exhibited significantly
434 shorter flight times compared to the control group (F = 30.820, df = 44, P <
435 0.001). Moreover, there were no significant differences in flight distance or
436 flight speed among the treatment groups when compared to the control group,
437 (Figure 5).

438 **3.7 Effects of linalool fumigation on the expression levels of sex**
439 **pheromone receptor-related genes in sterile *C. pomonella* moths**

440 RT-qPCR results showed that *CpomOR1, CpomOR2a, CpomOR3a,*
441 *CpomOR3b, CpomOR5, CpomOR6a, CpomOR7, CpomGOBP1,* and

442 *CpomGOBP2* genes were significantly reduced in *C. pomonella* male moths
443 that emerged from pupae irradiated with 200 Gy X-ray (Figure 6).
444 The expression levels of *CpomOR3a, CpomOR3b,* and *CpomOR5* were
445 significantly rebounded after linalool fumigation (Figure 6).

446 **3.8 Suppression of field population of *C. pomonella* by pilot release of**
447 **sterile males**

448 A total of 5342 sterile males were released, with 2735 of them being
449 released from linalool fumigated moths. Among these, 60.44±4.48% of males
450 flew out normally, while 6.83±1.51% fell to the ground post take-off, leaving
451 39.56±4.48% unable to take off normally (Table S2). The percentage of males
452 falling to the ground was 59.84±6.86%. Additionally, 2607 sterile males of *C.*
453 *pomonella* without linalool fumigation were also released, where 62.17±4.25%
454 flew out normally, 17.07±2.86% fell to the ground after take-off, and 37.83±
455 4.25% couldn't take off normally. Notably, 78.19±6.36% of males picked with
456 tweezers failed to take off and fell to the ground (Table S3).
457 Throughout the experiment, the ambient temperature at the experimental
458 site exhibited minimal variations, with a singular precipitation event exceeding
459 100 mm (Figure S9). Results revealed that, at a 5-meter distance, the
460 recapture of sterile male moths in traps averaged 12.25±3.07, whereas the
461 recapture of fumigation-treated sterile males was 8.75 ± 3.16. Similarly, at
462 distances of 10 m, the recaptures were 10.00 ± 1.78 and 9.75 ± 1.18,
463 respectively. At 15 m, the retrievals were 8.50 ± 1.55 and 6.50 ± 1.19,

464 respectively. Finally, at a distance of 20 m, the retrievals were 0±0 and 1.50±
465 0.65, respectively. The results indicated that there were no statistically
466 significant variations in the recaptures of sterile males and fumigation-treated
467 sterile males of *C. pomonella* at the 10 and 20 m distances at a significance
468 level of 0.05 (P > 0.05). However, notable differences were noted in
469 recaptures at 5 and 15 meters, with statistically significant variances observed
470 at the 0.05 level (P < 0.05) (Figure S10).

471 The deployment of *C. pomonella* sterile males over a period of two
472 months resulted in a notable decline in fruit infestation within the treated
473 orchards, registering at 24.87±2.17%. This rate was significantly lower (*P* <
474 0.05) in contrast to the control orchard where infestation levels reached 34.01
475 ±2.87%. These findings provide evidence that the introduction of sterile *C.*
476 *pomonella* males effectively mitigated fruit infestation occurrences, thereby
477 diminishing both the incidence of infested fruits and the overall infestation rate
478 within the orchard (Figure 7C).

479 The captures of wild male moths did not exhibit a notable variance
480 between the release area and the control from July 7 to July 14, 2023.
481 Nonetheless, subsequent to July 31, 2023, a noteworthy decline in captures
482 within the release area was observed, presenting a significant contrast with
483 the control group (P < 0.05). Based on these findings, it is evident that the
484 introduction of sterile male *C. pomonella* moths in the natural environment
485 substantially reduces the population of wild male moths (Figure 7d).

486 **4 DISCUSSION**

487 The radiation dose is a critical factor in achieving the desired sterility level
488 of insects in the SIT program.[8, 27] In this study, we demonstrated that the
489 biological traits of male *C. pomonella* varied in their decline with increasing
490 levels of irradiation. This observation is consistent with a previous study on
491 *Glossina palpalis* tsetse flies, which showed that higher radiation doses
492 reduced the fecundity of females.[28] Additionally, we observed a significant
493 decrease in the mating rate of sterile male *C. pomonella* with normal females
494 when exposed to radiation above 200 Gy, mirroring the findings in *G.*
495 *palpalis*.[28] The OK SIR program, designed to control *C. pomonella* populations,
496 initially utilized 250 Gy of ^{60}Co-γ irradiation, resulting in male sterility rates of
497 85% to 92%.[29] Since 2014, a consistent dose of 200 Gy has been employed
498 to balance sterility levels and mating competitiveness.[30] To optimize the trade-
499 off between male sterility and minimize the negative effects of radiation on the
500 insects, it is recommended to use 200 Gy as the optimal dose for inducing
501 sterility in males *C. pomonella*.

502 The mating competitiveness of sterile insects is crucial for their ability to
503 effectively compete with wild males for mating opportunities with females.[31,32]
504 Previous research has shown that inadequate dosages may result in
505 incomplete sterilization, while excessive irradiation can reduce the mating
506 competitiveness of released insects compared to wild counterparts, thus
507 affecting the success of SIT programs.[33] Therefore, maintaining a delicate

508 balance between mating competitiveness and sterility is essential during
509 copulatory interactions with wild female insects.[34] Evaluating the optimal
510 irradiation dose is critical to assessing the mating competitiveness of male
511 insects bred for this purpose.[33] However, reduced mating competitiveness is
512 commonly observed in irradiated *C. pomonella* populations.[7] Jiang et al.[35, 36]
513 also reported a significant decrease in the mating competitiveness of male
514 *Spodoptera frugiperda* following exposure to 250 Gy of X-ray irradiation. In
515 our recent studies, male *C. pomonella* pupae irradiated with 366 Gy of X-rays
516 exhibited mating competitiveness indices of 0.001 in laboratory[13] and 0.0088
517 in orchards.[37] In this study, when subjected to 200 Gy of X-ray irradiation, the
518 mating competitiveness index of sterilized male *C. pomonella* decreased,
519 indicating a decline in mating competitiveness post-irradiation. Nevertheless,
520 compared to the impact of 366 Gy irradiation, the mating competitiveness
521 index of *C. pomonella* males following 200 Gy irradiation increased to 0.17 in
522 laboratory conditions and 0.096 in orchards, suggesting that an optimized
523 irradiation dose can enhance the mating competitiveness of sterile insects to
524 a certain degree. However, the mating competitiveness of *C. pomonella* males
525 post-exposure to 200 Gy irradiation persists at a sub-optimal level. Therefore,
526 it is imperative to prioritize efforts aimed at improving the mating
527 competitiveness of sterile males in future studies.

528 The diminished mating competitiveness evident in irradiated insects may
529 stem from the compromised olfactory system in male insects, leading to a

530 reduced ability to perceive female sex pheromones [38]. To investigate this
531 hypothesis, linalool, a volatile compound found in host plants known to
532 enhance the attraction of *C. capitata* moths[16], was utilized to fumigate male *C.*
533 *pomonella* moths. Although linalool fumigation did not affect longevity,
534 fecundity, hatching rates, or overall mating performance, it did enhance the
535 mating competitiveness of male *C. pomonella* moths. Another investigation on
536 *C. capitata* demonstrated that the mating competitiveness of normal male
537 adults and mass-reared irradiated males increased subsequent to fumigation
538 with ginger root oil (GRO), compared to non-fumigated adults. Notably, GRO
539 fumigation did not have any notable impacts on the body weight or longevity
540 of irradiated mass-reared males.[39] These results suggest that linalool
541 fumigation might potentially mitigate male sterility induced by X-ray irradiation,
542 possibly due to enhanced male sex pheromone communication in the
543 antennae of male *C. pomonella* moths, as observed in *C. capitata* post ginger
544 root oil fumigation.[40] Furthermore, we revealed a decrease in gene expression
545 in *C. pomonella* males' antenna following 200 Gy of X-ray irradiation,
546 suggesting a reduced sex pheromones response in male moths. Conversely,
547 an increase in the expression of *CpomOR3a*, *CpomOR3b*, and *CpomOR5*
548 genes was observed after linalool fumigation, enhancing the recognition of
549 sex pheromones. These findings support the efficacy of linalool fumigation in
550 mitigating male sterility induced by X-ray irradiation, likely through the
551 upregulation of essential chemosensory genes and the activation of

552 pheromone recognition mechanisms in *C. pomonella*. Further confirmation
553 through behavioral experiments, comparative genomic and transcriptomic
554 analyses, and molecular biology experiments is required to determine if the
555 reduced mating competitiveness of irradiated insects is due to dysfunction of
556 the olfactory system and the involvement of additional chemosensory genes
557 in the recognition of pheromones in male adults.

558 Previous studies have demonstrated that the release of *C. pomonella*
559 males into the pear orchard following exposure to 366 Gy X-ray did not affect
560 the wild populations or the fruit decay rate.[13] In this study, we carried out a
561 small-scale trial in different fields utilizing the SIT to manage *C. pomonella*
562 populations. Our study demonstrated that by releasing three successive
563 batches of sterile male *C. pomonella* moths irradiated at 200 Gy, along with
564 linalool fumigation, in the specified orchard area, there was a substantial
565 decrease in *C. pomonella* infestations in the apple crop. This reduction was
566 evident through significantly lower infestation rates compared to the control
567 orchard. Additionally, field observations indicated a notable decrease in the
568 recapture rate of wild *C. pomonella* moths, aligning closely with the findings of
569 Huang *et al*.[4], in comparison to the control group. These results suggest that
570 the utilization of sterile males effectively suppressed the wild *C. pomonella*
571 population in the orchards, consequently reducing fruit damage. The findings
572 highlight an enhancement in the field application of the SIT for *C. pomonella*
573 control after optimizing the irradiation dose.

In summary, we have identified the optimal sterilizing dose of X-ray irradiation for *C. pomonella* and introduced a plant volatiles fumigation approach to enhance the mating competitiveness of sterilized males. Additionally, we investigated the effects of irradiation and fumigation on the expression of genes related to pheromone recognition in the male antennal. Notably, our study demonstrated the successful reduction of the *C. pomonella* population in the orchards through repeated releases of sterilized males in conjunction with linalool fumigation on a pilot scale. These findings offer valuable insights for the implementation of the SIT as a strategy of controlling *C. pomonella*.

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Author Contributions Statement

Sheng-Wang Huang: software, methodology, investigation, formal analysis, writing original draft, Peng-Cheng Wang: software, methodology, investigation, formal analysis Yan Wang: software, review and editing. Jie-Qiong: formal analysis, conceptualization. Ping Gao: writing, review, and editing, resources. Xue-Qing Yang: supervision, resources, project administration, funding acquisition, conceptualization, writing, review, and editing.

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723 **Table 1** 200Gy X-ray irradiation on the mating competitiveness of *C. pomonella* adults

Experimental site	Matching ratio (S♂: N♂: N♀)	Fecundity (eggs laid per female)	Hatching rate/%		Competitive capacity
			Observed value	Expected value	
Indoor experiment	0 : 10 : 10	116.20±19.97 a	72.96±7.31 a		
	10 : 0 : 10	103.75±4.42 a	12.57±0.51 b		
	10 : 10 : 10	106.07±5.80 a	64.18±3.95 a	42.77	0.17
Field experiment	0 : 10 : 10	57.45±4.09 a	64.16±6.11 a		
	10 : 0 : 10	60.13±10.60 a	10.82±4.46 b		
	10 : 10 : 10	54.26±6.81 a	59.49±2.47 a	37.49	0.096

724 Data are mean± SE. N: Number of unirradiated males; S: Number of irradiated males.

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739 **Table 2.** Effect of different concentrations of linalool fumigation on the mating competitiveness of *C. pomonella* males

Fumigation concentration (μL/m³)	Matching ratio (F♂: N♂: N♀)	Fecundity (eggs laid per female)	Hatching rate/%		Competitive capacity
			Observed value	Expected value	
	0 : 10 : 10	95.41±1.98 a	71.42±1.74 a		
70	10 : 0 : 10	96.46±9.51 a	73.63±3.86 a		
	10 : 10 : 10	98.74±9.33 a	72.55±0.33 a	72.52	1.06
	0 : 10 : 10	95.41±1.98 a	71.42±1.74 a		
83	10 : 0 : 10	94.75±13.79 a	78.47±3.28 a		
	10 : 10 : 10	95.83±2.05 a	75.34±0.43 a	74.94	1.25
	0 : 10 : 10	95.41±1.98 a	71.42±1.74 a		
96	10 : 0 : 10	89.55±3.98 a	76.52±1.36 a		
	10 : 10 : 10	95.02±0.89 a	74.24±3.83 a	73.97	1.24

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749 **Table 3.** Effect of different concentrations of linalool fumigation on the mating competitiveness of 200 Gy X-ray sterilized *C. pomonella* males

Experimental site	Matching ratio (F♂: N♂: N♀)	Fecundity(eggs laid per female)	Hatching rate/%		Competitive capacity
			Observed value	Expected value	
Indoor experiment	0 : 10 : 10	106.54 ±5.71 a	10.97±2.41 a		
	10 : 0 : 10	103.30± 12.00 a	13.44±2.33 a		
	10 : 10 : 10	94.35± 11.43 a	12.27±1.98 a	12.21	1.11
Field experiment	0 : 10 : 10	60.13±10.60 a	10.82±4.46 a		
	10 : 0 : 10	55.40±5.28 a	14.98±3.89a		
	10 : 10 : 10	61.66±4.09 a	12.84±1.98 a	12.90	1.06

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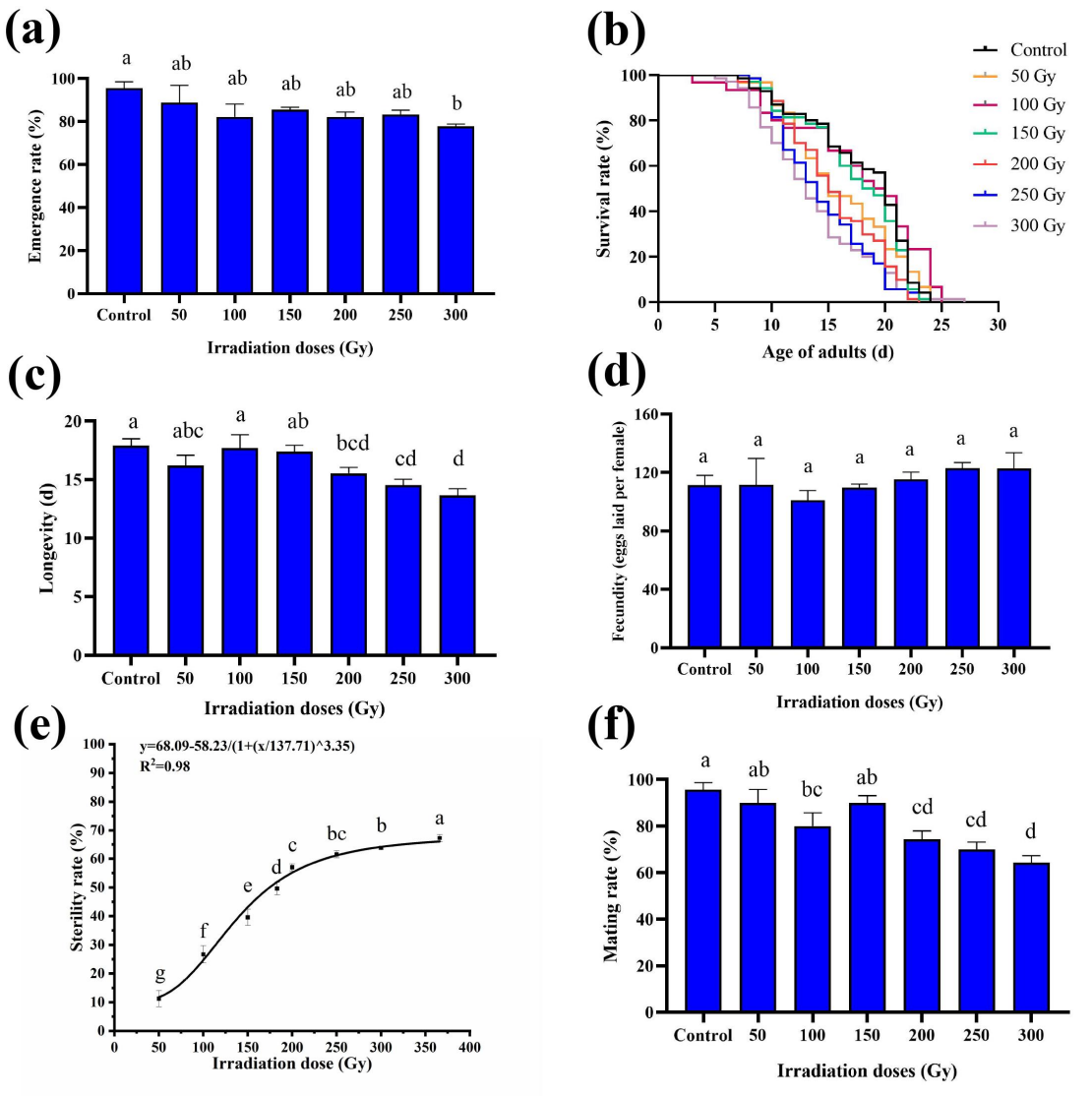


Figure 1. Effect of different doses of X-ray irradiation on the biological parameters of male *C. pomonella*. (a) Emergence rate; (b) Survival rate; (c) Longevity; (d) Fecundity (eggs laid per female); (e) Sterility rate; (f) Mating rate. Letters on the error bars indicate significant differences analyzed by the one-way analysis of variance (ANOVA) with Duncan's test.

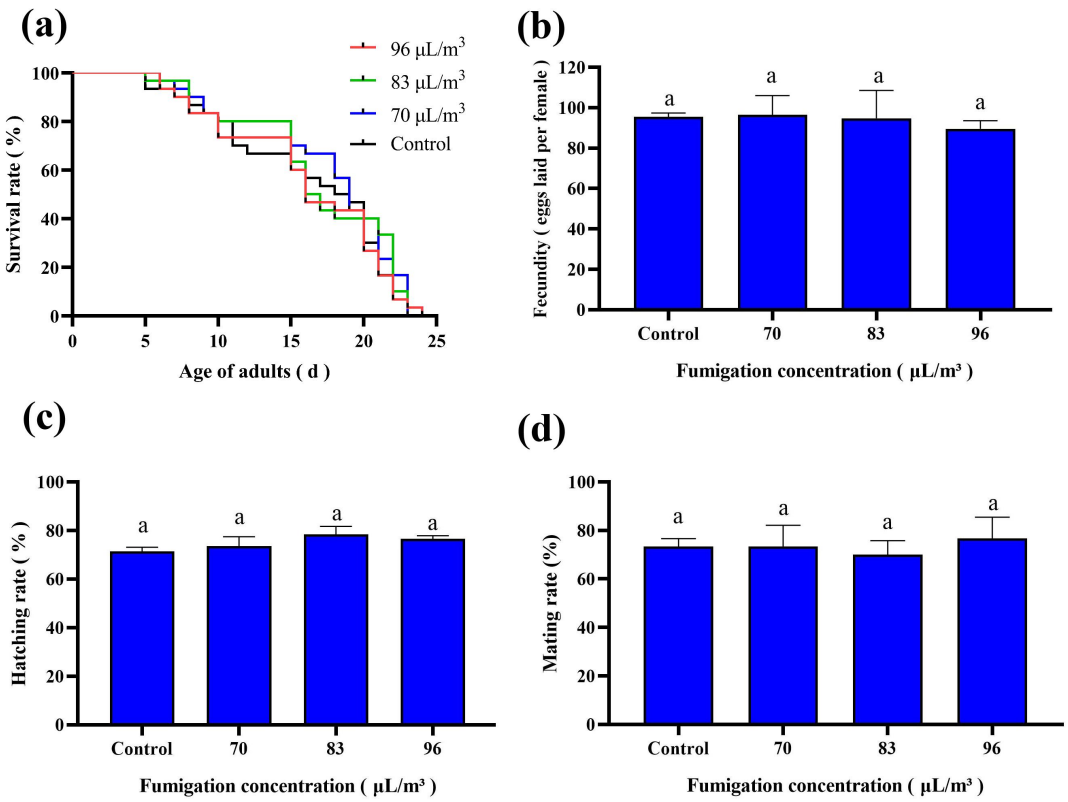


Figure 2. Effects of different concentrations of linalool fumigation on the biological parameters of male *C. pomonella*. (a) Survival rate; (b) Longevity; (c) Fecundity (eggs laid per female); (d) Hatching rate; (e) Mating rate.Letters on the error bars indicate significant differences analyzed by the one-way analysis of variance (ANOVA) with Duncan's test.

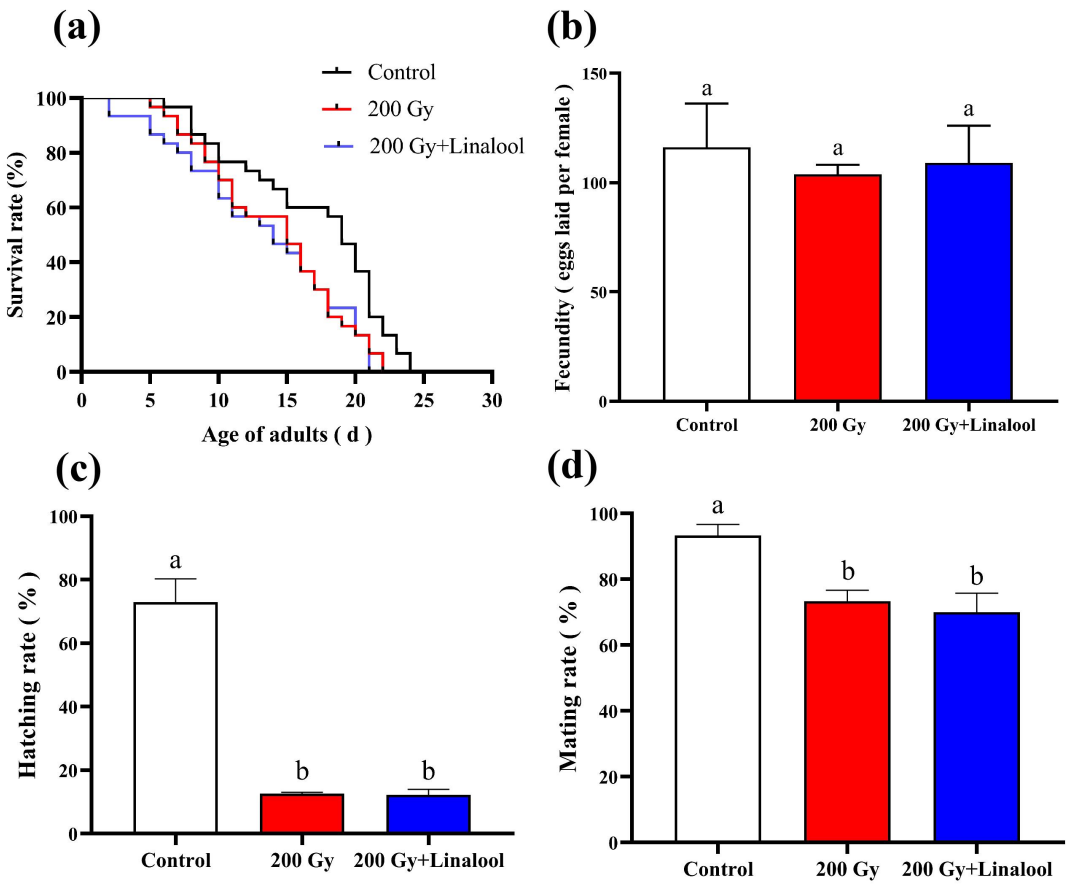


Figure 3. Effects of different concentrations of linalool fumigation on the biological parameters of 200 Gy X-ray sterilized male *C. pomonella* (laboratory experiment). (a) Survival rate; (b) Longevity; (c) Fecundity (eggs laid per female); (d) Hatching rate; (e) Mating rate. Letters on the error bars indicate significant differences analyzed by the one-way analysis of variance (ANOVA) with Duncan's test.

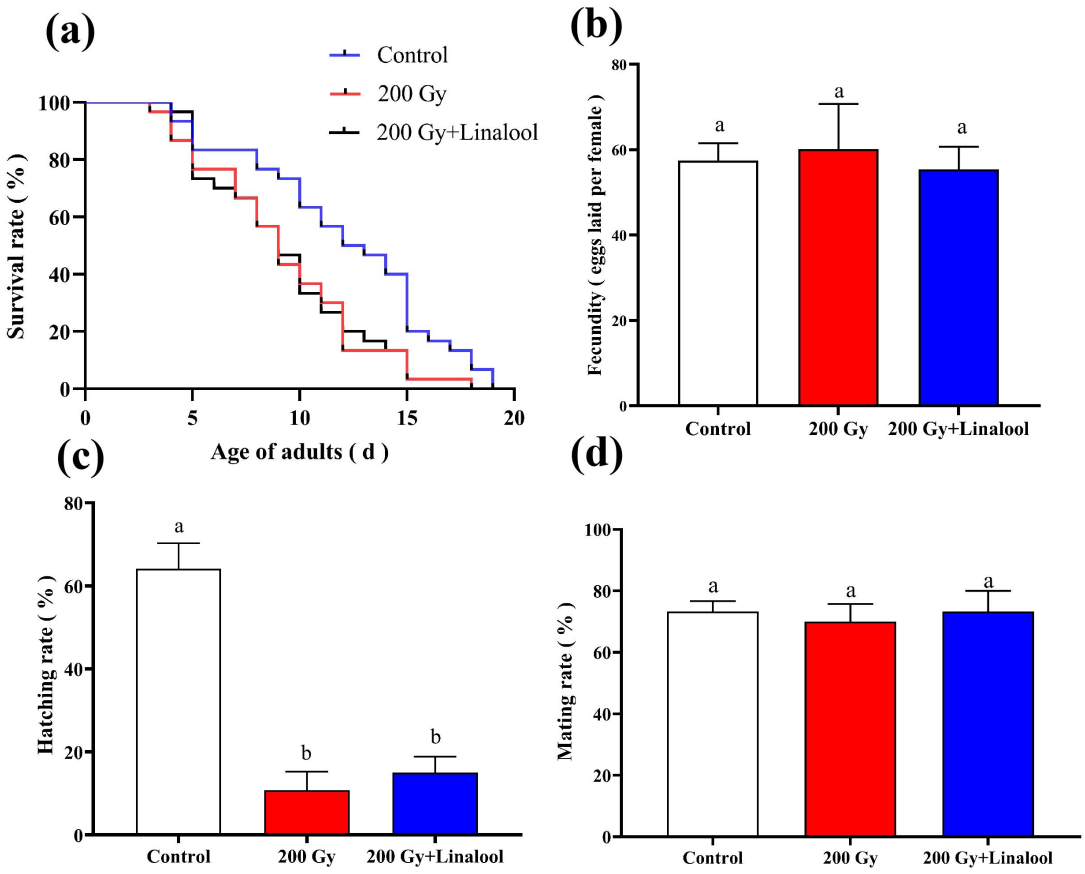


Figure 4. Effect of different concentrations of linalool fumigation on the biological parameters of 200 Gy X-ray sterilized male *C. pomonella* (field experiment). (a) Survival rate; (b) Longevity; (c) Fecundity (eggs laid per female); (d) Hatching rate; (e) Mating rate. Letters on the error bars indicate significant differences analyzed by the one-way analysis of variance (ANOVA) with Duncan's test.

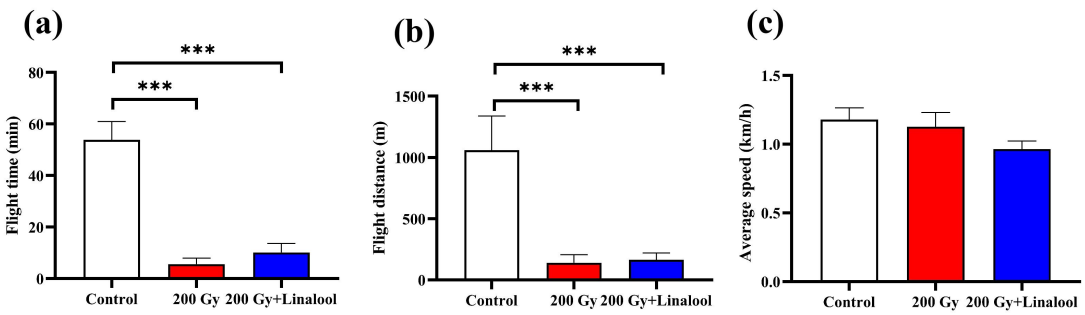


Figure 5. Effects of X-ray irradiation on the flight ability of male adults of *C. pomonella.* (a) Flight duration; (b) Flight distance; (c) Average flight speed. Asterisk (*) on the error bars indicate significant differences analyzed by the one-way analysis of variance (ANOVA) with independent samples t-tests (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

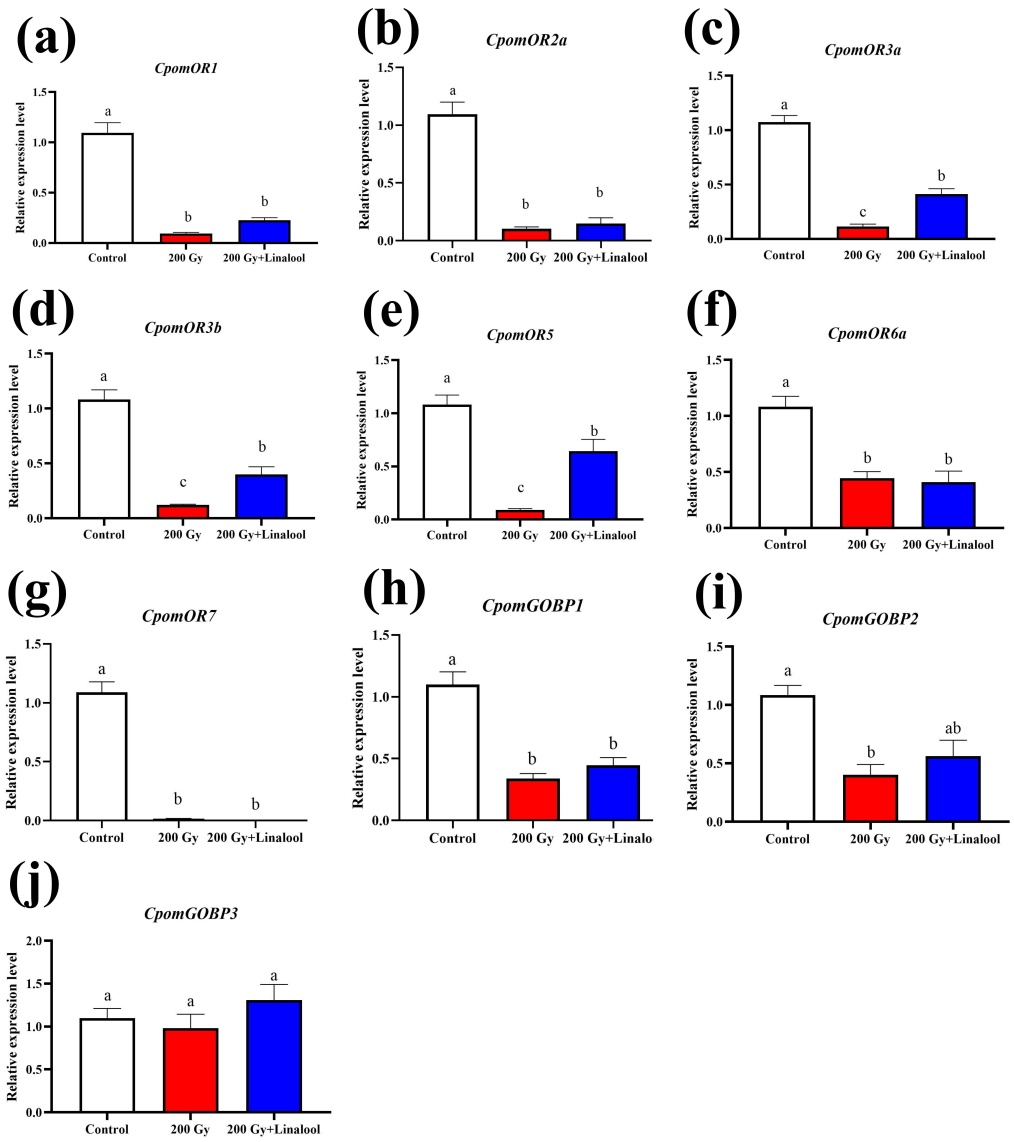


Figure 6. Effect of X-ray irradiation on the relative expression levels of the pheromone recognition-related genes in male adults of *C. pomonell*a. (a) *CpomOR1*; (b) *CpomOR2*a; (c) *CpomOR3*a; (d) *CpomOR3b*; (e) *CpomOR5*; (f) *CpomOR6a*; (g) *CpomOR*7; (h) *CpomGOBP*1; (i) *CpomGOBP2*; (j) *CpomGOBP3*. The 8-day-old male pupae (1 day before emergence) were exposed to 200 Gy of X-ray, and the expression levels of pheromone receptor-related genes in 3-day-old male adults were analyzed. Letters on the error bars indicate significant differences analyzed by the one-way analysis of

variance (ANOVA) with Duncan’s test.

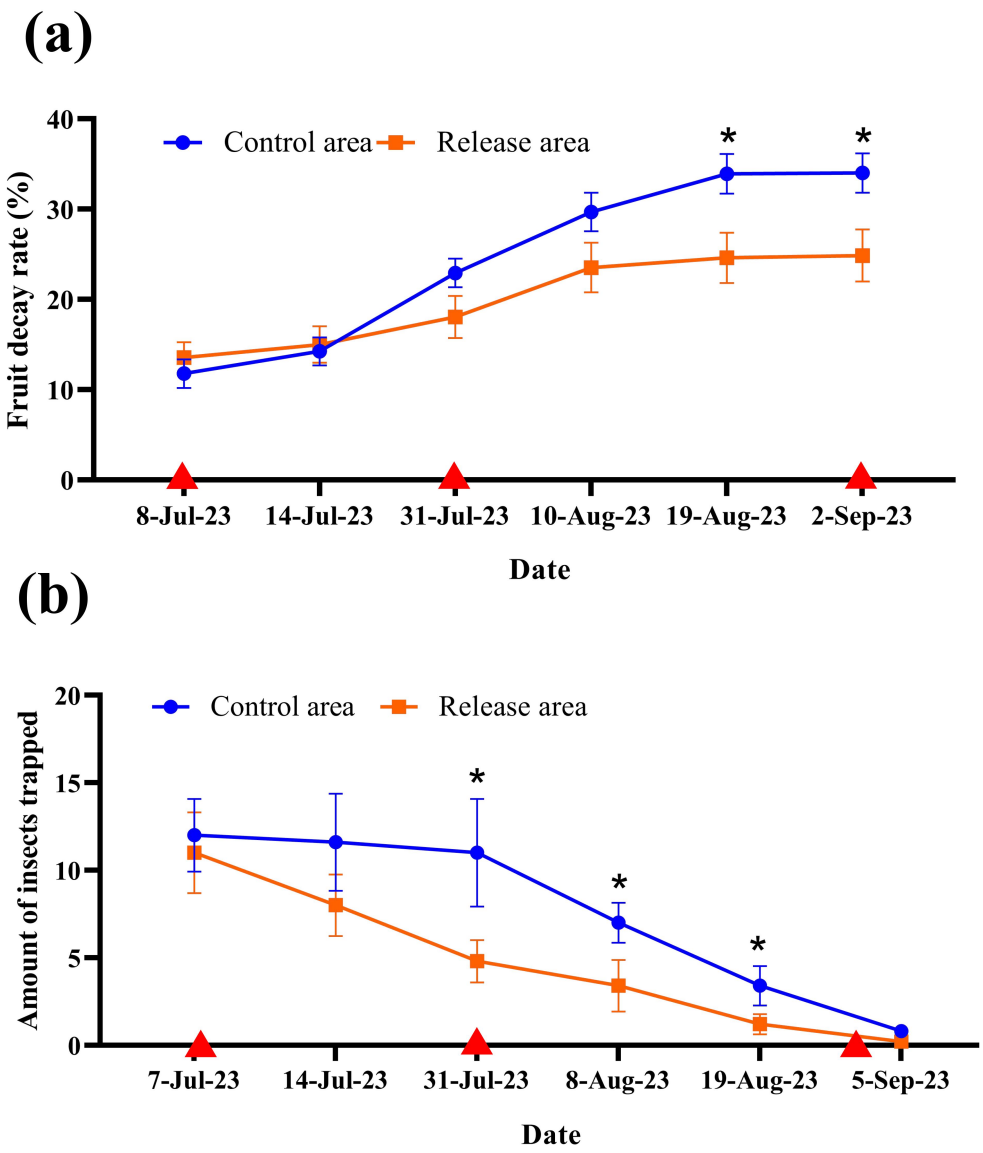
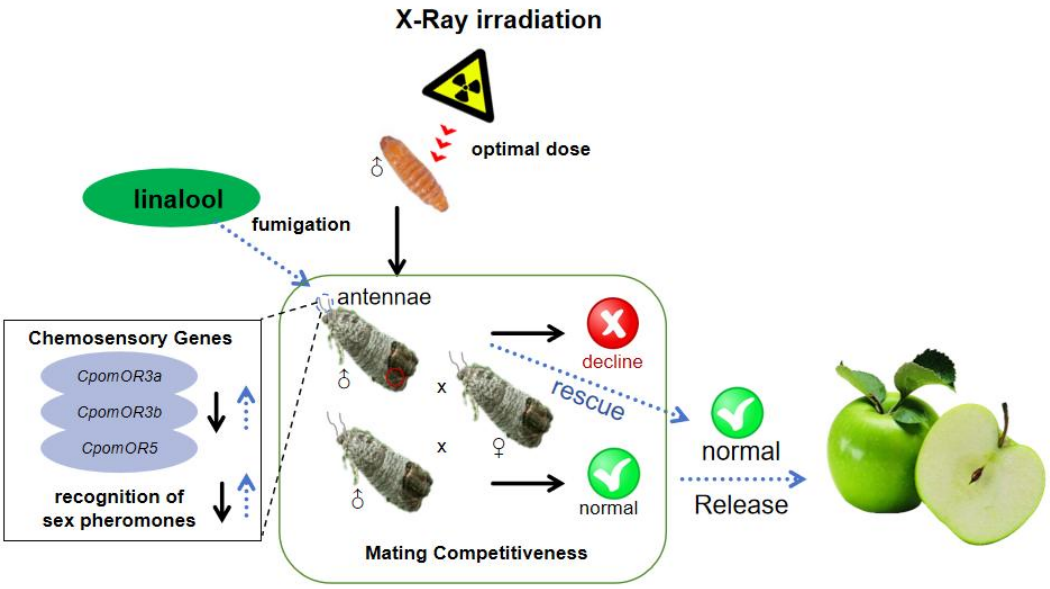


Figure 7. Suppression of field population of *C. pomonella* by pilot release of sterile male moths in an apple orchard. (a) Survey of fruit decay rate in control and release areas. The red triangles represent the date of release. (b) Number of sterile males recaptured by traps at different distances. (*) on the error bars indicate significant differences analyzed by the one-way analysis of variance (ANOVA) with independent samples t-tests (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).



Graphic Abstract

We determined the optimal X-ray irradiation dose and developed a linalool fumigation approach to improve the mating competitiveness of sterilized insects, thereby bolstering the efficacy of against *Cydia pomonella*.

Supporting information

Linalool fumigation improves mating competitiveness of males for population suppression of the global fruit pest *Cydia pomonella*

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Table S1. The primers used in this study.

Primer name	Primer sequences	Length (bp)	Reference
EF-1α-F	GGTCCCCTCCAAGCCTCTGT	157	Wei et al., 2020
EF-1α-R	CTCGGCAGCTTTGGTGACCT		
RPL12-F	CGACTGGAAGGGCCTCAAGA	156	Wei et al., 2020
RPL12-R	TTGCCGCTGTGCTTGATGTT		
CpomOR1-F	AGGTGCTATTCTCAGGCCTG	104	Tian, 2020
CpomOR1-R	CATATGTCAGATGGCCCACG		
CpomOR2a-F	CAAAGGTACAGGCGCAAAC	194	Tian, 2020
CpomOR2a-R	AACGGCATCAGGCAGATAA		
CpomOR3a-F	TGCTCTACATTGGACACCGAAG	156	Huang, 2019
CpomOR3a-R	CCATACACTCCCAGGGCAAAT		
CpomOR3b-F	GTAAGTTTTATGGGCTGGTTTTT	142	Huang, 2019
CpomOR3b-R	GCAGGTTTAGGGAAATTGTATAT		
CpomOR5-F	ATACGGACCTCTGACGATCG	143	Tian, 2020
CpomOR5-R	TCCTGCGGTTACTCGTATCC		
CpomOR6a-F	TCAGACTTCTGTGGCGTTCG	164	Tian, 2020
CpomOR6a-R	CCATCCCACCTGCTTTTAGAC		
CpomOR7-F	GCATGGTTTTGGGTCCGAAT	100	Tian, 2020
CpomOR7-R	AGGGACCGTGCATTAGACTT		
CpomGOBP1-F	ATTCATCAAGTCTTTCCCTAACG	186	Garczynski et al., 2013
CpomGOBP1-R	TCCGCCATCATCATTTCC		
CpomGOBP2-F	TGCGAGAAGCAATACGACG	176	Garczynski et al., 2013
CpomGOBP2-R	CCAGTATCATTTTCAATTCAACG		
CpomGOBP3-F	GCCATCATCTGTATGTCCCA	160	Garczynski et al., 2013
CpomGOBP3-R	TGTCCTCGTATTGCTTCTCG		

Table S2. Flight status of released sterile males in the field.

	Total released	Normal flight out (%)	Abnormal flight out (%)
Sterile males exposed to linalool	2735	60.44±4.48	39.56±4.48
Sterile males	2607	62.17±4.25	37.83±4.25*
Total released	5342		

Table S3. Percentage of sterile males that can normal flight out but landed and abnormal flight out with landed after pick offs in the field.

	Normal flight out but landed(%)	Abnormal flight out with landed after pick offs (%)
Sterile males exposed to linalool	6.83±1.51	59.84±6.86
Sterile males	17.07±2.86*	78.19±6.36

Figure S1. The cDNA sequence of CpomGOBP1.The location of the primer sequence is indicated by an arrow.

>CpomGOBP1:

CACAACCAAACATGTCGCACATAGCCCGGCTGGTGCTCTCGTTGGCGGCGGTTGCGCT

CGCGCACGCCACCGTGGAGGTGATGAAGGACGTCACCCTTGGCTTCGGAGAGGCGCT

CGAGCACTGCAGAGAGAGTAGCGGCCTAACAGAAGAGAAAATGGAGGAGTTCTTCC

ACTTCTGGCACGACGACTTCAAGTTCGAACACCGGGAGCTGGGCTGCGCAATCCAGT

GCATGTCCAGGCACTTCAACCTGCTGACTGATGGACAGAGGATGCACCATGAAAACA

***CpomGOBP1* F** →

CGGATAAATTCATCAAGTCTTTCCCTAACGGTGAGGTGTTATCCAAAACAATGGTAAC

CCTGATCCACTCCTGCGAGCAGAAGTTCGACGACATGGAAGACCACTGCTGGCGAAT

CCTGCGCATCGCCGAGTGCTTCAAGAGCGGCTGCCAGGAGCGCGGGTTAGCGCCGTC

← ***CpomGOBP1 R***

TATGGAAATGATGATGGCGGAGTTTATTATGGAGTCCGAGGTTTAAAGCTTTGGAATG

TGAAATGACATTTCTCGACAAATAACAAATTTAATGTCACCGATTACCGCGATAGTTG

CTCATGAAATACAATTGTTTTAATGTGTTACATGTATCGCATATTATACATTTAAAATA

CGGCCCGTCATTTGGAACCCTTTGCAGCGCAGCGTTCGTGTGGAACGGGAGATTGGA

GAAAAATATATGCGAAATGTAAAAA

Figure S2. The cDNA sequence of CpomGOBP1.The location of the primer sequence is indicated by an arrow.

>CpomGOBP2:

ATCACGATGGCTTTATACTGGCTGGTTGGTTTAGTGTTGGTGGGGGGCAAGATGGTGG

ACGGGACGGCGGAGGTCATGAGTCATGTGACTGCGCATTTTGGGAAGGCTTTAGAGC

AATGCCGTGAGGAGTCCCAGCTGTCCCCAGAAGTCCTGGACGAGTTCCACAACTTCT

GGCGCGAGGACTTTGAAGTGGTCCACCGCGAGCTCGGCTGCGCCATCATGTGCATGT

CCAACAAGTTCTCCCTACTGCAAGATGATGCCCGCATGCATCATGAGAACATGCATG

ACTACGTTAAGAGCTTCCCTCAAGGTGACGTCCTCTCGGCCAAGATGGTCGAACTGAT

CpomGOBP2 **F** →

CCACAACTGCGAGAAGCAATACGACGACATCCCTGACGACTGCAGCCGCGTTGTCAA

GGTCGCAGCCTGCTTCAAGGTCGACGCTAAGAAAGCTGGTATCGCACCCGAGGTCGC

← *CpomGOBP2 R*

CATGATTGAAGCGGTGCTGGAGAAGTATTAAGACCCATTGGCAGAACGTTGAATTGA

AAATGATACTGGTGGATAGATGAAGTGGGTTTTCATTCACTAGTTGTTATAGTCGGAC

ATCAGTAAGTGTAAGATCTGAGTGGATGCTCGAAGCGGAGCGTTCGGCGGAGCATGC

AGCGTGGCGTCGGGCTCACAAGTGATGTGAGCAGCATGCACTGAGGCCGCTCCTATA

CGTTTGCATTCGCATGCCCCGCCCTGCTGCACGCCCCACTCAAGCGTCCACTCAGGCC

ATACACAAATTTTGTGTTTCCATAAAAAAAAATCCTGATATAAATGGAAAAATGAAA

TTAGGAGTAGTAGTAGTAATCCCTTTTTTGCGTACAATATTTTGACCATCGGAATTTG

AGCTATGTAAAAATTTGAAGCTATTTTATTTTTCCCAGAATATGTATAAGTAGGATAA

TATATACCCTAGTAGAAGCAGATTTTTTCGACTTCCCGACCCTGACTATTGTATTGCGT

CCATAAATATATGAAAATAAGGTTTAGAACCCAGTCAGAATTTACATAAAATCTTTAC

TTAAAGAGTTTGTAACTAATTATGTAATTCATAGTGAAAATATCATTGGTCATCAAAA

AGTGTATTTTTTTTTCCAGTATTGTTTTTAATTTTTATATACAATCAAAAATTTATACAT

CGAACAATGTTTAAAGAGTGATTTTTAGTAGATAGTTTTGATTAAGTTCATTTCATCG

ACTATTTGGTTTAGATTAGAATTGTTGTGATTTAATAAATAGTTGTTTGAGGGAAAAA

Figure S3. The cDNA sequence of CpomGOBP3.The location of the primer sequence is indicated by an arrow.

>CpomGOBP3:

AGACGAATGGTCATTGTCTTTGAAATGGCGCTGTGCTGGATGGTTTTGACGCTGGTCC

TGGCTGCTGGAGAGATGGAGGCGGCATCGGAACATTCCATAACCATGAGTAATGTCA

ACGCGCATTATGGGAAGACGCTGGAGATGTGCCGGGAGGAGGCTCAAATATCTCCCG

AGATCATGCAAGAATGGCATCACATCTGGGATGATGACTTCGAGGTAGTACAGAGAG

CpomGOBP3 **F** →

AGATCGGCTGCGCCATCATCTGTATGTCCCACAAGTTCTCCTTACTGCAGGATGATAA

TCGAATGCATCATGAAAGCATGTTTGATTATATCAGAAGTTTCGATAATGGTGACGTT

← *CpomGOBP3 R*

CTCTCAGCAACAGTGGTAGAACTATTCCATAACTGCGAGAAGCAATACGAGGACATC

GAAGACGACTGCAGCCGTGTAACCAAGGTCATGGCCTGCTTCAAGGTTGAGGCCAAG

AAGGCTGGCATCGCGCCCGAGGTGGCCATGATTGAAGCTGTGCTGGAGAAATATTGA

AACCCGATGGCAGAATTTGGAAATCGCAACGATACAGGATTGACGTGTTTTAGCCAC

TTCCGATATTTTTGTCTGTAATACCTACATAAGTGAATATATGAACGAATAAATTATA

ACCTAGTTTTGTTGCATCGATAATAATTGATTTTGATTACTTGTGTTTAACTACTAACC

ATTAAAATTTGCCGTTGCAAAAA

Figure S4. The cDNA sequence of CpomOR1.The location of the primer sequence is indicated by an arrow.

>CpomOR1:

ATGTCTTTGAAAAGCCGTGTTTGGGAAAAGTTGAAGAAGAAGTTTGATGACGGTGAG

GTAGACTCCCCTCTGAAATACACGTACGTCAAACAAGTGTCATGCCTAATGAGTTCCG

TCGGCGCCTGGCCTCACAGGCAGTTTGGTCGCCAGAAACTCCACACTCTGCTATCAAT

CTATAATCTCGGGCTACATGGCGTGTGCGCAGGCATGTTTATTCTTGGGCTGTTGTATT

GGCGGCAGCGCAGACATAATATGTCGTTTTTCGATTCGGGGCACATATTTTTGTGTAT

GCTTTTTGACCTTTTGGTTTTGCTGAGATTCATGGTGACTCAGACAACGAAATATCAA

GAAACAATTAAAGCTTTTTTGCTTGAATTTCACCTGTTTTACTTCAAGGATCGATCGCC

GTATGCTGCAAAGGTCCACACGCAAGTTCATACCATATCGGGCATGTTCAGTTTTTAC

***CpomOR1* F** →

GTCATATGTCAGATGGCCCACGGCATGGCATTGTTCGTGCTAATGCCTTGCTACAGTA

← ***CpomOR1 R***

ACTTACGCAAAGGCATGTTTGGCAAAAACAGGCCTGAGAATAGCACCTTCGAGAATT

CAGCTTACTACTACCTACCGGACGCCTGTTATACTACTTTACGAGGATACTGGATATT

ACTCGCTTTCAATGCTTTCTCGTCTTACATTATAACTATTGGTCTCTTCGAGTTCGACC

TTATGATCAGTATGATGGTCTTCCAAATCTGGGGTCATCTAAAAATTTTGAAAAATAG

CTTGTTAACTATGCCTTTGCCGGTAAACAGCAAAGACGGCTTGTATTCACCAGAAGAG

AATATCAAAATCAAAGCATTGTTGAAGGAAATTATTGAACATCACACCCTGATTATA

AAATTCGTGGACGGATGTTCCAATACGTTTAGCGAGTACCTGTTCACGTTCTACCTGT

TCATGCAGTTCATCACCTGCATCTTACTGCTGGAAGTATCTTCGTTCACAGCAGACGC

TCTGGGTAAATACGGCCCCTTAACCATTGGGATGCATCAGCAGTTAATACAAGTGTCT

ATACTTTTTGAAGTCTTGAATACAAAGAGCAACGAACTGATCGACGCTATGACACAA

ATACCATGGGAACATATGAACACGAGCAACAGAAGAACAGTGTTGTTCTTGATATGC

AGGATTCAAATTCCTGTCTCTTTGAAGGCTGGTGGGATGGTGCCGGTCGGAGTCAATA

CAATGCAGGCGGTCTTGAAGGGTTCGGTGACGTACTATATGATGTTGAAGGCTTTCGC

TGCTGAGGGGTAA

Figure S5. The cDNA sequence of CpomOR2a.The location of the primer sequence is indicated by an arrow.

>CpomOR2a:

ATGATTTTAAAACAAGTGTTTGATGTTTTGAAGAAGAGATTTGATGATGGTTACGTCAA

CACGCCTCTGGATTTCAAATATGTGGCTCAACTGCAATTCGTGCTGACGACCGTAGGCT

CCTGGCCATATAAACAATTTGGACGAAATCGATTAGCGGCCATTTTGTCTACGTACAAC

GCCTTTCTAATACTCGTTAGCACCAATTTGTGCGTTTTGGATTTAATCTATATACGCGTGA

ATAGAGTAAAACTATCGTTTTTTGATTTGGGACATAATATATTGCGTTTGATTTTTACTTT

CTTGTATCTGCAAAGACTATTGACGGCAAGGACAAGTAAATATCAAGAGGTAATAAAG

GATTATTTACTTGACTTTAACTTGTTTTACTTCAAGGGCCGGTCACCATACGCTGCAAAG

CpomOR2a **F** →

GTACAGGCGCAAACACACATTATATCAGGAATGTTCACGATCTACGTGATGTGGCAGAT

GGTCATAGGAGTATCTCTGTTCATATTCATGCCGTGGTTCAACAATTATAACAGAGGCAT

← *CpomOR2a R*

GTTCAGTGAAAACCGGCCCCAAAACAGCACGTTCGAGCACTCTGTATACTATTATCTGC

CTGATGCCGTTTATACTACTGAAGAGGGATACTGGATATTATTCGTCTTCAATATTCCAAT

TTCCTACGTCACCACAATTGGTCTTTGCGTTTTTGATCTCTTACTGATCTTGATAGTGTT

CCAAATTTGGGGGCACCTGAGGATTCTGAAGCATAATTTGCAAAACATTCCACTGCCG

GAAAACAGTATTATGTACTCAGTTGAAGAGAATAATAACATACGAATGTTATTAAAGGA

GAATATATTACATCACAACATTATTATACAATTCGTGGACAGATGCTCGGACGCATTCAG

CGAATATTTGTTCGCGTTCTACCTGTTCATGCAGTTTATCACCTGCATCTTACTGCTGGA

AGTCACTACGTTCACCGCGGACTCGCTGGCGAAGTACGGCCCCCTAACCGTCGTCATG

CATCAGCAGTTAATACAAGTGTCCATATTGTTCGAGATGTTAAACACCAAGAGTGAGCA

GCTGATCGACACCGTATACGCCATCCCGTGGGAGCATATGGACACGAAGAACAGGAGA

ACTGTGTTGTTTTTCCTGCACAAGATTCAGACCCCAGTCAGTCTGAAGGCTGCCAAGG

TCGTACCTGTGGGAGTCAACACTATGTCTGCAATTCTGAAGACCACGTTCTCGTACTAC

ATGATGCTGAAGGCTTTAGCTGGGGAGCGATAA

Figure S6. The cDNA sequence of CpomOR5.The location of the primer sequence is indicated by an arrow.

>CpomOR5:

ATGGCTCCACCACGAGTGTTTCGTTCTTTGAAGAGATGGTTTGACGACAGCGACGCAA

AACACCCACTGGAGTTCAACTATGTCCGACATCTGATCTTCTTGCTAAGCTTCATAGGA

TCTTGGCCTCACAAACAATTCGGACGTGACCGATTACACTTCGTCCTGTCCATATTCAA

CCTGTTCCTGATCGTGGTGGGCATAACCATATCCATTGCGGCTGTGGGATACATATGGAG

TAAGAGGGAGACTATATCTTTCTATGATATGGGACATGTAATTTTGTGTATTTTGTTGGA

GACTTTATTTTTGCAAAGATTAATGACGGGCCGAACGGAAAAGTATGGTGAGATTGTAA

AGGATCTCCTGCTCAATTTTCATCTCTTTTATTTCCAGAGTCGATCGCAATACGCTTCTA

AGGTGTACAAGCAGGTTCAGTTCGCCTCAAAGATATTCACTATTTACGTCACCTGTCAC

CTTATAACTGGATTCACCCTGTTTTCTTTCATGCCTTGGTACAATAACTATAAGAACGGA

ATGTTCAGTCCTGACCGCCCACCGAATAGGACTTTCGAACACTCCCTCTATTTCTACTG

TTTCACTGACGAAGTCTATACAACATTAAAGGGCTATTGGATTCTCTTCTCATTCAACGT

CCCCACGTCCATCAACACCAGCAGCGGTATACTAACCTTCGATCTTCTGCTCAGCCTAA

TTGTGTTCCAAATTTTGGGACATCTTATGATTATGAAACACGATTTGTTGAGCATACCTC

CGACTACCGATAAATACTCGCCAGAGGAGAATATGCGAGTCAAAGAGACTTTGAAAGG

AATCATCGATCACCACAACATAATTATAAATTTCGTCGACAAGTGCTCCGACGCGTTTA

GTGAATACCTCTTCATGTTTTACTTGCTCATGCAACTCCTTACCATCGTGGTGACGGTGG

CpomOR5 F →

ATTTGTCCACCTTCACTGCGGACGCGTTGGCCAAATACGGACCTCTGACGATCGCTATA

TATCAACCGCTGATCCAGATATCGATTCTTTTCGAAATGATAAGCACACAAAGCGAGAA

CpomOR5 R ←

ACTTGTTGATGCCATATACGAGATTCCGTGGGAGTGCATGGATACGAGTAACCGCAGGA

CGGTGATGTTCTTTCTGCTTCGTGCGCAAACGCCGGTAACTCTGAAGGCCGCCAAGAT

GGTGCCCGTTGGCGTAATGACTATGACCGCTGTACTGAAGACAACATTCTCCTATTACA

TGTTATTGAACGCCGTAGCTGAATCTGCTGAGCAGTAG

139 **Figure S7.** The cDNA sequence of CpomOR6a.The location of the primer sequence is
140 indicated by an arrow.

>CpomOR6a:

ATGCAGACAAAAAGGCAAACCAGTGAGCCGAAAGTATTCTCCCTAGATTATATGAAAT

CGCTCCGCTTCACGCTCGAAACGATAGGACAATGGCCCAACCGATCACTAGGGGACCT

TTCACGGCGTGCAGTAATGTTGGCCACGTATCACAAATTCTTAATATGCACATTTTGTTT

TACGGAAATCCTAGCGTTTTCGTACATGATAAAGCACAGAAAAACGATCCGTTTTATTG

ATATGGGGCAAATTTACACGAATTTGTTTTTAACCGGGCTATTCCTTCAAAGAGCATCG

CTACCTTTTCAGAAGAACTACAAAAAGTGTGTGAAAAAGTTTGTTTTAGAATTTCATTT

AATGCACCATGAGCATTTAAGTGAATTTGCAGCGAAGGAACTACGCAAAGTCAACAAA

ATCTGCAAGATCGCGACCAAAGTCATCTATCTCCAGCTCGCCTGCGGAATGCTGGCGTA

CAACCTCTCCCCCCTTTTCCGGAACTATTACGAAGGAATGTTCGCAGGCGAACTCCCC

GAAAACAAATCGTTTGTACACTCCGTCGACTACCTTCTACCTTTTGACGCTTATCGCAG

TTTCAAGGGTTACCTAGTTATATTCATATGGAATTGGTTCCCTACATACAATATACCTACT

GCTATGGGTATTTATGATCTGTTGGTGTTCGTAATGGTTTTCCATATGGTCGGGCATATGA

ATATACTTCTTAATAGTTTGAAAGAATTTCCAAGGCCGCAAGAGGATGGTTTGCCGTCG

ACACGGGAGTATAATGAAGAGATATTTGGATTGTTGAAAAATGTGATTCGACATTATCA

GATAATCAAGGATTTCATGGGAGATATGACTGCAGCATTTGATCTGACGTTGTGTTGCTA

TCTAGCATTTCACCAAGTCATGTGTTGTCTGATGTTGCTAGAATGTTCTACATTGGAGCC

GGAGGCCTTGGTAAAATATGGGATGCTTGCCGCGGTCATATTCCAGCAATTAATTCAGA

***CpomOR6a* F** →

CTTCTGTGGCGTTCGAGTTAATTAAATCAAAGAGTAAACGTCTCGGTGATGAAGTATAT

GCAGTACCATGGGAATATATGAACGTGAAAAACAGAAGAATTTTCGTACTATTCCTTAG

← ***CpomOR6a R***

GAACGTGCAGTACCCATTAGGTCTAAAAGCAGGTGGGATGGTGCCAGTGGGAGTGATG

AGTATGTCTACGATCATAAGGACATCAATTTCATATTACATCATGCTAGCCACATTCGCA

141 GACTAA

142

143

144

Figure S8. The cDNA sequence of CpomOR7.The location of the primer sequence is indicated by an arrow.

>CpomOR7:

CpomOR7 **F** →

ATGACAACACAAACCAGGGACCGTGCATTAGACTTAGACTACATGCGTGTGATCCGTC

← *CpomOR7 R*

TATACCTCGACACGATCGCTCATTGGCCGAACGAAAAATTCGGACCCAAAACCATGCG

TACGCGCATCCTGTCCATATATCATATCTCAATATTGACAATGCTCGTAGCAGTAGTTGCT

GCTGAGATCTTGGCGTTGTTTTTCGTCCGAGGCAGAATGCCAACTCATGGTTTTATTGA

TTTGGGGCAGGATTACTTGTCTATTTTGATAGGCTGCGTAATGATTCCAAGACTGACACT

AATACTCCAAGAAAAATACTGTTCACATATAAAAACCTTCGTTTCAAAATTTCATTTACT

GGAACATAAACATGAATCTGGATTTGCCGCTAAGGAGTATCAGAAGGTCAACAAAATT

TGCAGAATTGCAACCATGATTATTTTGCTGGAGTGCCTCCTTGGGCAAATGATGTTCAA

CGTGGTCCCATTATATGTAAACATACAAGCAGGATTGTTCACGTCCCGTGACAATAGAC

CACAGAATGTAACCTTCGTTCACTCTTTAAACTATTATTTCATAATAGACCAATACAATG

ATGCGATTGGTTATGGCATAGCCTCTTTTATAAACGCTTACGTTTCATACATGTGTGGTG

TTGAGTTCTGTGGCATAGATTTATTAATTTATATAATGGTCTTCCACATATTGGGTCATTTT

AACATACTGGTGGATAAAATGAGAAACTTTCCGAGACCAGTAAACTTTCCTGATGAATC

TCAAAAATATTCTGAGCGACAGTATAATGAAGAAGCATTAAAAGTTTTGAAGAATTTAA

TACAACATGACCAGTTAATTAAAGAATTCATGAACAATACGTCTAAGACGTTTAGCATC

ACCCTTTGTATTTGCCTTTTGTTCCATCAGGTTTCTGGATGCATTTCGCTTCTAGAAATTT

CTCCGATGACTGCTGAGGCACTGACACGATATGGTCCCTTGATATTAGTGTTGTTCAATC

AGCTGATACAGATGTCGGTGATATTCGAACTCATAAGCTCCAAGAGTAACAAACTCTCG

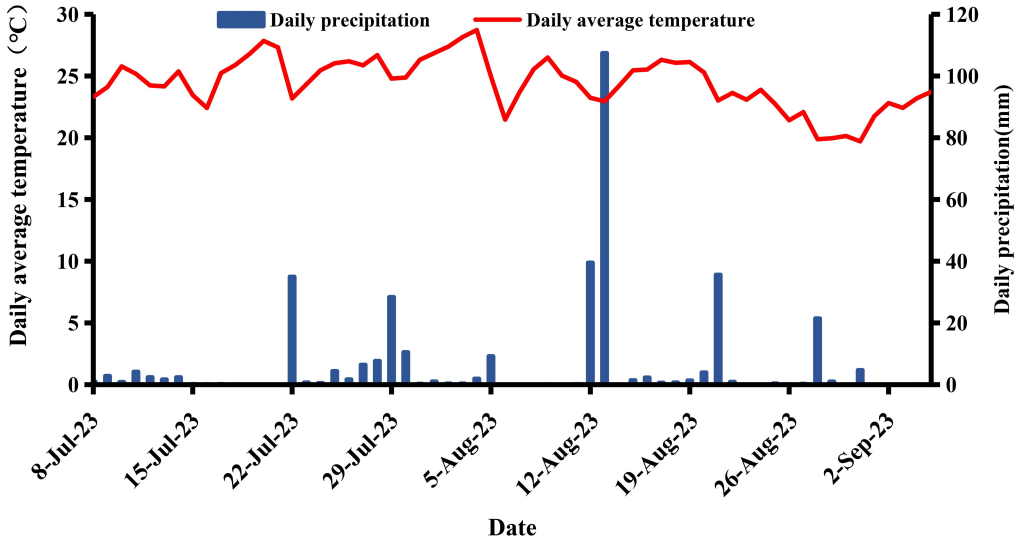
GATGAAGTATATGCGCTGCCGTGGGAGCTGATGGACGCCAAGAACAGGAAGACCATGC

TGCTCTTCCTCGTGAATGTGCAGCGGCCCAGGGGTCTGAAGGCTGGTGGTTTGGTTTC

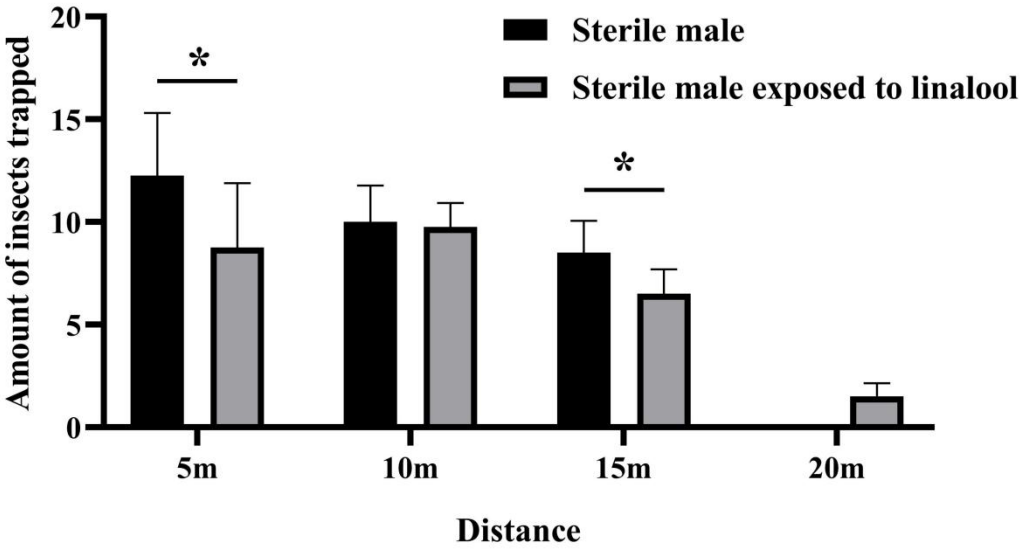
TGTGGGTGTGCTTACTATGGCTCAGATTATAAAAAACTCAGTTTCTTATTTCTTAATGTT

AAGGACGCTTGGAAACTTTTAA

Figure S9. Thee ambient temperature and precipitation event at the experimental site throughout the experiment.



180 **Figure S10.** The number of sterile males and fumigation-treated sterile males of *C.*
181 *pomonella* recaptured at different distance (m) far away from the release center. Asterisk
182 (*) on the error bars indicate significant differences analyzed by the one-way analysis of
183 variance (ANOVA) with independent samples t-tests (*, P < 0.05).



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