

Suppression of oriental fruit moth *{Grapholita molesta,* Lepidoptera: Tortricidae) populations using the sterile insect technique

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Abstract. The Oriental fruit moth (OFM) is a major insect pest of peaches in Bulgaria. Its control usually requires several insecticide treatments per season. This, however, gives rise to serious toxic residue problems. A program for suppression of OFM populations involving the use of sterile-insect technique (SIT) has been developed as an alternative to the chemical methods for OFM. Relevant information regarding laboratory rearing, radiation and basic biology are presented here. Expected effects of some release programs are modelled using appropriate mathematical simulations. Results obtained in a small field experiment showed high efficacy of a program integrating F_1 male sterility technique and classic SIT.

1. INTRODUCTION

The Oriental fruit moth (OFM), *Grapholita molesta* Busck (Lepidoptera: Tortricidae), is an economically important pest in Bulgaria. The insect occurs throughout the country but develops more numerous populations in the southern regions, where most commercial orchards are located. Peaches are the most important host of OFM in Bulgaria, while apples, quince and plum are secondary hosts. OFM larvae cause stem wilting by penetrating the tips of young twigs and burrowing downward. In addition, their feeding on green and ripening fruit results in damaged fruit with holes often filled with viscous sap and excrement. Feeding injuries cause premature fruit drop, decreased fruit marketability and compromise fruit exports. Larvae of the summer generations may cause serious damage on fruits of the middleearly and late peach varieties.

At present, control of OFM is usually achieved by several insecticide treatments directed mainly against the late spring and summer generation larvae. This practice, however, often results in toxic pesticide residues on fruits. Pesticide residue is a major concern because peaches are largely used for production of baby food on which no toxic contamination is allowed. The negative consequences of the intensive application of insecticides against OFM necessitated a re-evaluation of such control programs. Accordingly, efforts were turned to search for alternative methods which do not cause negative side-effects. So far, however, no effective biological control agents have been established and used successfully in OFM control programs. For this reason, the sterile insect technique (SIT), as a genetic approach to OFM population suppression, was investigated as an alternative to the chemical methods. SIT is based on the idea that successive releases of fully sterilized moths (classical SIT) during the overwintering generation (OWG) of OFM and continuing through the next spring generation (NSG) may reduce the density of the summer population to an acceptable (low) level. As a consequence, insecticide treatments could reduced or eliminated. The F_1 sterility technique, involving releases of partially sterilized male moths, is expected to produce a similar effect after one application against the OWG of OFM, resulting in inherited male sterility in the successive filial generations.

2. MATERIALS AND METHODS

2.1. Mass rearing of OFM

A semi-synthetic diet had been previously evaluated and found to be effective for rearing OFM (Table 1, Diet 1) [1]. This diet was slightly modified and tested on our colony (Table 1, Diet 2). Vicomplex (Pharmachim, Bulgaria), which contains vitamins A , B , C , D_2 and NPP was used in Diet 1, while Vitamino (Virbac Laboratories, France), a multivitamin mixture containing 9 vitamins, amino acids and minerals was used in Diet 2. Phytin (Pharmachim, Bulgaria) a calcium-magnesium salt of inosite-hexaphosphoric acid containing 22% organically bound phosphorus was used. It is considered a tonic that may have an invigorative effect on the organism. Nipagin (methyl-p-hydroxybenzoate) and benzoic acid were used as fungicides. Tetracycline dissolved in distilled water (1 g in 50 ml) was used as a bactericide. Diet 2 was found to be more effective and is less expensive to rear OFM. As such, it was selected for routine OFM rearing.

Diet spheres, made of approximately 38 g medium $(\sim$ 3 cm in diameter) are most suitable for larval development. They provided appropriate moisture and good texture and remain suitable for a sufficiently long period of time. Five larvae can normally develop in a single sphere. Strips of corrugated paper (width 10-15 mm) were placed in the rearing containers as pupation sites for mature larvae. Moth emergence, mating, oviposition and egg hatch take place inside glass jars. Cotton pads soaked in 2% sucrose solution provide adult nutrition. A ratio of 1 male:3 females is optimal for effective laboratory mating [2]. Infestation of the nutritive spheres is achieved by placing the spheres inside the jars during egg hatch for 24 h. A constant temperature of 26°C is optimal for larval development and pupation. A photoperiod of 16L:8D prevents diapause induction [3]. Temperatures between 23-25°C are required for moth eclosion, oviposition and egg hatch.

All corrugated paper strips were fumigated with formaldehyde to prevent mould contamination of the diet. We isolated a granulosis virus, belonging to the family Baculoviridae subgroup B from our OFM colony [4]. Virus management requires frequent total disinfection of the rearing room, rearing boxes and other equipment with 1% KOH solution.

2.2. Radiation biology

Irradiation was conducted in a Co^{60} irradiator and the insects were treated with doses ranging from 50 to 500 Gy [5].

2.3. Field experiments

A typical peach-growing region in South Bulgaria was used for this experiment. Mature pupae were treated with partially or completely sterilizing doses of radiation and allowed to emerge from strips of corrugated paper in the field. The following treatments were released into different 1 ha blocks: (1) F_1 Sterility + SIT: successive releases of partially and fully sterile males and completely sterile females were made into populations of the OWG and the NSG of OFM; (2) Chemical control: 3 insecticide treatments were applied against populations of OFM in the OWG, NSG and first summer generation; (3) Untreated control: the area was left untreated for OFM. All blocks contained early, middle early and late peach varieties. Fruit injury by OFM larvae was recorded from samples of <1000 fruits for each group of varieties in each treatment.

2.4. Field biology and population dynamics

OFM pheromone traps (Pherocon-OFM), C^{14} -labeled male OFM moths [6] and the releaserecapture method [7] were used to determine the dispersal of released insects and to evaluate trap efficiency. All irradiated moths needed for the field trial were additionally marked with Calco-Red dye added to the larval diet.

2.5. Simulations

Effects of different SIT programs were evaluated by modelling several scenarios using mathematical models [8].

3. RESULTS

3.1. Mass rearing of OFM

Some biological characteristics of OFM reared on immature apples and Diet 1 are summarized in Table 2 [1]. These parameters were used to develop quality control standards for OFM laboratory populations.

Data collected from 5 selected (of 39 total consecutive) generations of OFM reared on Diet 1 are shown in Table 3. Data for 5 selected (of 13 consecutive) generations of OFM reared on Diet 2 are summarized in Table 4. There is a slight increase in both male and female pupal weights over successive generations. Significant increases in egg production over time were also evident. In general, all data indicates that long term rearing on a suitable diet and under favourable laboratory conditions does not have a negative effect on the reproductive and developmental biology of OFM.

Table 2. Some biological characteristics of *Grapholita molesta* reared on immature apples and on diet 1.

¹ Average for the first five generations.

Table 3. Developmental and reproductive parameters of five selected generations of *Grapholita molesta* reared on diet 1.

Table 4. Developmental and reproductive parameters of five selected generations of *Grapholita molesta* reared on diet 2.

Parameters	Sex	OC	Storage period (days)						
		Parameters	30	45	60	90	120	150	180
% Emergence		≥ 90	83.3	89.5	87.6	69.9	18.8	20.1	5.4
Sex ratio $(M:F)$		$0.8 - 1.2$	1.12	1.04	0.96	1.10	1.46	1.68	4.9
Adult Longevity (days)	M	\geq 17	16.7	17.2	16.9	14.0	10.0	11.0	4.8
	F	\geq 19	18.7	17.0	18.6	18.2	13.4	10.8	8.3
Fecundity $#$		\geq 25	26	27	25	26	22	18	17
eggs/female)									
% Egg hatch		>70	76.8	86.6	77.3	78.6	75.7	87.0	78.0

Table 5. Some developmental parameters of *Grapholita molesta* after pupae were stored at 4— 5°c for different periods of time

Dose(Gy)	F_1 embryonic mortality (% \pm SE)					
	P crosses ¹ : IM x NF			P crosses ¹ : IF x NM		
	А	В	Α	В		
0 ²	15.4 ± 2.8	14.3 ± 1.6	17.0 ± 2.0	13.8 ± 1.9		
50			79.8 ± 3.1	77.5 ± 2.8		
100	37.5 ± 2.2	34.8 ± 1.8	98.5 ± 2.3	99.0 ± 0.6		
150	42.8 ± 3.1	36.9 ± 2.6	100	100		
400	92.5 ± 2.1	94.1 ± 2.0				
450	94.8 ± 1.2	95.6 ± 1.9				
500	96.2 ± 1.6	97.0 ± 1.5				

Table 6. Radiation induced sterility in *Grapholita molesta.*

¹ I, irradiated; N, non-irradiated; A, pupae irradiated; B, adults irradiated.

2 Control crosses: NM x NF.

Mature pupae collected in the laboratory were kept at $4-5^{\circ}$ C for different periods of time ranging from 30 to 180 days. The results of routine quality control tests on the stored pupae showed that under such conditions pupae could survive successfully for 60 days without any deleterious effects on the emerging adults (Table 5). However, when pupae were stored for more than 60 days, progressive reduction in moth emergence, longevity and fecundity were recorded. Sex ratio was also skewed in favour of the males. Only egg hatch remained unaffected.

3.2. Radiation biology

Data related to the development of SIT are given in Table 6 (Genchev and Gencheva [5]). For males and females, irradiated as mature pupae or as adults, the sterilizing effects are expressed as dominant lethal mutations in spermatozoa or in oocytes. A dose of gamma between 400-500 Gy, administered to pupae or adults resulted in almost complete male sterility (93-97%), but had less of an effect on adult longevity (Table 7, after [5]). In general, these results suggest that a dose of 400-500 Gy induces a level of sterility that satisfies the requirements of SIT. The dose that causes complete female sterility is 150 Gy. This dose is much lower than the dose needed for male sterilization, and provides a good opportunity for releasing both sexes without any risk that the irradiated females will contribute larvae to the population density.

Dose	Mean adult longevity \pm SE (days)			
(Gy)	Irradiated pupae	Irradiated imago		
	21.5 ± 1.2	20.0 ± 1.0		
100	20.8 ± 1.6	19.0 ± 0.8		
150	21.2 ± 1.2	21.8 ± 1.8		
400	20.1 ± 1.0	18.5 ± 1.1		
450	18.5 ± 1.5	17.5 ± 0.8		
500	18.3 ± 1.0	14.8 ± 0.9		

Table 7. Longevity of *Grapholita molesta* males exposed to various radiation doses.

¹ Untreated control.

Table 8. Embryonic mortality and sex ratio in f₁, f₂ and f₃ generations of *Grapholita molesta* when male pupae were irradiated with 125 gy

¹U= untreated; I= irradiated; M=male; F= female

Doses between 100 and 150 Gy, causing partial sterility in male OFM (37-43%), are considered as appropriate to be tested for induction of F_1 sterility. These doses are currently being tested. However, 125 Gy (Table 8) is being used in an experimental release program.

Results from laboratory experiments with fully sterile moths (pupae treated with 450 Gy) are presented in Table 9. All the release ratios provided high mortality of the F_1 eggs and may be suitable in a release program.

3.3. Basic biology

OFM completes 3 full and 1 partial generation each year. The insect hibernates as a mature larva. Pupation in the overwintered generation usually occurs in April, and about 30 days are required for the development of a generation in the field. Table 10 presents data for moth population dynamics, summarized from historical observations and from using pheromone traps at regional stations in North and South Bulgaria. This information is essential for scheduling the timing of the OFM release program. If the intended targets are the populations of OWG and NSG in South Bulgaria, then releases of sterilized moths should be made between 5-20 April and 25 May-5 June, respectively. As such, the sterile insects needed should be available no later than the beginning of April or the middle of May, respectively.

Studies where released radio-labelled male moths were used indicated that the maximum distance where OFM was trapped was 80 m. As such, the distance between traps should be less than 160 m. The average trap efficiency recorded in our studies was 6.1%. This value was derived from data of 12 field tests where traps were placed in a circle with radius of 50 m

Only males irradiated	Both sexes irradiated			
Parental Ratio IM:NM:NF ¹	F_1 egg mortality $(\% \pm SE)$	Parental Ratio IM:IF:NM:NF ¹	F_1 egg mortality $(\% \pm SE)$	
$0:1:1^2$	15.4 ± 2.8	0:0:1:1 ¹	13.8 ± 2.0	
10:1:1	88.5 ± 4.5	10:10:1:1	89.2 ± 6.0	
12:1:1	$90.0 + 4.2$	12:12:1:1	91.3 ± 5.4	
15:1:1	92.6 ± 4.5	15:15:1:1	91.9 ± 4.0	

Table 9. Mating competitiveness of sterile *Grapholita molesta* males irradiated as pupae with 450 Gy.

¹ I= irradiated; N= non-irradiated; M= male; F= female.

² Control cross of non-irradiated moths.

Table 10. Population dynamics of *Grapholita molesta* in Bulgaria.

OWG, overwintered generation.

A, the date of earliest beginning of the flight.

B, the date of most frequent beginning of the flight.

C, the date of most frequent flight peaks.

D, time interval (days) from the beginning of the flight and the flight peak.

around the release site. Confirming tests carried out in 1997 showed that trap efficiency varied between 6.4 and 5.7%. We used this data to calculate the population density and we found that it ranges between 400 and 600 moths per hectare (males $+$ females, sex ratio of 1:1). Because these data indicate a relatively low population density, the OWG should be the most appropriate target for initiating the sterile moth release program.

3.4. Simulations of SIT programs for OFM population suppression

The effects of two successive releases of fully sterile moths into wild populations of different sizes were modelled using the following model equations: $N = mc$ for the size of free developing population (f x f = 1) and N(F₁) = mc/(a + 1) for the size of F₁ population after SIT application (s x f = 0). In these equations $N =$ number of insects in the population (both males and females); m = number of females in the population (m = $N/2$); n = number of males in the population (n = N/2); b = sex ratio of males to females (b = n/m); a = ratio of sterile males to fertile males; and $c =$ fecundity (number of eggs per female). The following postulates were followed: $b = 1$ (constant); $c = 25$ (constant); $a = 15 : 1 (= 15)$ or $20 : 1 (= 20)$, respectively (variable); N for initial population $= 2000, 1600, 1200, 800$ and 400 moths per

Population	Initial ratio 2	Population size (no. of moths per generation)					
Treatment	IM:NM:NF	\mathbf{P}	F ₁	F ₂	F ₃		
Non-treated	0:1:1	2000	17500	153125	1339844		
Treated	15:1:1	2000	1094	598	5233		
Treated	20:1:1	2000	833	347	3038		
Non-treated	0:1:1	1600	14000	122500	1071875		
Treated	15:1:1	1600	875	479	4191		
Treated	20:1:1	1600	667	278	2430		
Non-treated	0:1:1	1200	10500	91875	803906		
Treated	15:1:1	1200	656	359	3141		
Treated	20:1:1	1200	500	208	1823		
Non-treated	0:1:1	800	7000	61250	535938		
Treated	15:1:1	800	438	240	2100		
Treated	20:1:1	800	333	139	1216		
Non-treated	0:1:1	400	3500	30625	267969		
Treated	15:1:1	400	219	120	1050		
Treated	20:1:1	400	167	70	613		

Table 11. Simulated population changes in three *Grapholita molesta* generations after application of SIT^1 .

¹ Two successive releases of fully sterile moths in the P and F_1 generations.

² Initial ratio of released irradiated (I) males to non-irradiated (N) moths of the wild population.

hectare; f x f = 1 for the cross of fertile male with fertile female producing normal offspring; s $x f = 0$ for the cross of sterile male with fertile female producing no offspring; a total correction of 30% for natural embryonic, larval and pupal mortality was introduced for every simulated population. The postulate for variable value of the ratio of released sterile males to fertile wild males was accepted in order to simulate the interactions between the moths depending on the ratio in the population. The constants, i.e. sex ratio, fecundity and total correction for natural mortality, were supported by data obtained in laboratory experiments.

The expected population reduction after the application of SIT is summarized in Table 11. Although proposed situations are simulated and the postulate s $x f = 0$ (i.e., an absolute male sterility) is fixed, the data clearly indicate the downward trend in the OFM population occurring when fully sterilized moths are introduced. Because the number of sterilized individuals for each release is the same, the second treatment in an already reduced population will result in a significantly increased ratio sterile to fertile males (27:1 and 48:1 from an initial ratio of 15:1 and 20:1, respectively). This increase in ratios is a very important factor governing the process of OFM population suppression by SIT. Population reductions predicted by the model for two successive releases in populations of 800 and 400 moths per hectare are of special interest because these are similar to the densities found for the OWG generation in Bulgaria.

When classic SIT is used initially and is followed by F_1 sterility, the suppressive effects will result from the following consequences. 1) Loading the parental (P) population with dominant lethal mutations; 2) introduction of various chromosomal aberrations in the already lowered F_1 population which further impacts the F_2 population and gives rise to high level of inherited male sterility; 3) a corresponding reduction in the F_3 generation accompanied with inheritance of male sterility, but at a lower rate; and 4) slow population increase in the F_4 generation.

Table 12. Simulated population changes in four *Grapholita molesta* generations after application of F_1 sterility and sterile-insect techniques consecutively.

¹ Treated generations: P (1st release) and F₁ (2nd release); both sexes were released and the initial ratio of released to wild moths were $a = 15:1$; data were calculated for two initial population sizes, 800 and 400 wild moths. 2 F₁ male sterility = 80%, F₂ male sterility = 60%, and sex ratio b = 3:1 (i.e., threefold reduction of m) in both F₁ and F_2 generations were used in calculations.

Table 13. Results of a small-scale field trial for evaluating the efficacy of an integrated sterileinsect release program for *Grapholita molesta* control.

Successive releases in overwintered generation (OWG) and next spring generation (NSG).

² Used against the populations of OWG, NSG and the summer generation I.

When F_1 sterility is used first and is followed by SIT the expected suppressive effects will result from: 1) introduction of chromosomal aberrations in the P generation, causing a reduction in the F_1 generation and a high level of inherited male sterility; 2) loading of F_1 individuals with dominant lethal mutations through the release of fully sterile moths, resulting in suppressed reproduction because of simultaneous effect of dominant lethals and chromosomal aberrations; 3) strong reduction of the F_2 generation and secondary inheritance of male sterility; and 4) corresponding reduction of the F_3 and a slow increase in the F_4 .

Results of simulated single application of F_1 sterility and simulations of both programs is presented in Table 12. The data suggest that the most effective program for OFM would integrate consecutive releases of F_1 sterile OFM followed by the classic SIT. Undoubtedly, the use of SIT plus F_1 sterility and even F_1 sterility by itself could also be applied, but to populations of lower initial densities.

3.5. Small scale field trial

Based on results from the model simulations, the integrated release program involving *F* sterility against the OWG generation plus SIT against the NSG generation was evaluated under field conditions. We assumed the following to be true: Sex ratio in OFM is 1:1; the prerelease population density was estimated to be $600 (300M + 300F)$ moths per hectare; the OFM do not fly long distances and are generally distributed uniformly in the peach orchard.

The release of partially sterile males for the OWG started on 29 April, 1997. In order to achieve the 20:1 sterile to wild male ratio, 12,000 irradiated pupae were dispersed in the field three times at intervals of 10-11 days. The release of fully sterilized moths against the NSG generation began on 10 June, 1997. A total of 11,900 irradiated pupae were introduced into the orchard at the same time intervals. Flight records showed a steady flooding of the OWG population with partially sterile male OFM. hi most cases, the number of trapped marked males was greater than the number of wild male moths. A similar situation was observed after introduction of completely sterile individuals into the population of NSG but without predominance of the sterile males. A probable reason for this might be the increased number of wild males because of the inherited F_1 sterility effects (see sex ratio in Table 8).

Percentages of fruit damage from the different experimental treatments are shown in Table 13. These data unequivocally indicate a very high effectiveness of the program for genetic control of OFM. Integrating F_1 sterility and classic SIT resulted in a strong population reduction, followed by a reduced increase in the OFM population density. As a consequence, additional insecticide treatments were not needed to control this pest.

4. CONCLUSIONS

General conclusions from our experiments are summarized below:

- (1) The technology developed for mass rearing OFM is efficient and technically feasible.
- (2) Appropriate radiation doses for complete and partial sterilization for OFM pupae and adults have been determined. The level of mating competitiveness of the sterilized male moths is adequate.
- (3) The basic biology and radiation biology make OFM suitable for development and application of SIT for population suppression.
- (4) Information on flight behaviour and population dynamics is essential for calculating the timing and number of OFM to be released in an SIT program.
- (5) The overwintering generation is the most suitable as a target for sterile moth releases because of the relatively low population level.
- (6) Data obtained from modelling simulation suggest a downward trend in OFM populations when sterile moths are introduced.
- (7) Field experiment suggested high effectiveness of a program integrating F_1 sterility and classic SIT.

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